Effect of natural antioxidants on protein and lipid oxidation in fish (*Siganus sutor*) processed in a locally fabricated hybrid windmill-solar tunnel dryer

By

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A thesis submitted to the University of Surrey in partial fulfilment of the requirements for the Degree of Doctor of Philosophy in the Faculty of Health and Medical Science

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September 2018

ABSTRACT

The aim was to evaluate the drying characteristics, quality, safety, carbon footprint and physico-chemical properties of fresh and antioxidant-treated dried *Siganus sutor* fish fillets. An innovative hybrid windmill-solar tunnel dryer was designed and fabricated in Kenya to harness solar and wind energy, day and night and in damp weather conditions. The moisture content for both salted and unsalted *Siganid* fish reduced exponentially to 19.9 % over a 3-day drying period. The quality and yield of *Siganid* fish fillets after delayed icing for 0, 2 and 4 h that was determined using the Quality Index Method, was linearly related to storage time. Biochemical evaluation of solar dried fish, stored for up to 75 days, showed lowest levels of Peroxide Value (PV), Thiobarbituric Acid Reactive Substances (TBARS), Volatile -Total Basic Nitrogen (TVB-N), trimethylamine, pH and moisture in vacuum packaging, followed by polythene packs and highest levels in samples with no packaging. Microbial plate counts were significantly reduced after solar tunnel drying of the Siganids.

Fish fillets were treated with synthetic antioxidant BHA (control) and extracts from water hyacinth, seaweeds and turmeric as sources of natural antioxidants. The efficacy of antioxidants to reduce lipid oxidation products PV and TBARS was in the order BHA>tumeric>seaweed>water hyacinth and significant ($p<0.05$). Small deformation rheology of stored (up to 90 days) solar dried fish fillets treated with natural antioxidants had lower G' values compared to the control, reflecting desirable texture qualities. The thermodynamic properties (denaturation temperature (T_m) and heat enthalpy change (ΔH) altered significantly only after 60 days storage.

The carbon footprint was low because of low labour input, non-motorized fishing vessels and renewable energy-wind and solar used for drying. A descriptive generic Hazard Analysis

Critical Control Point tool for solar dried fish was obtained for the first time. The above findings can enhance the processing and preservation of fish and influence fish quality and fisheries policies.

DECLARATION OF ORIGINALITY

This thesis and the work to which it refers are the results of my own efforts. Any ideas, data, images or text resulting from the work of others (whether published or unpublished) are fully identified as such within the work and attributed to their originator in the text, bibliography or in footnotes. This thesis has not been submitted in whole or in part for any other academic degree or professional qualification. I agree that the University has the right to submit my work to the plagiarism detection service TurnitinUK for originality checks. Whether or not drafts have been so-assessed, the University reserves the right to require an electronic version of the final document (as submitted) for assessment as above.

Peter Michael Oduor-Odote

ABBREVIATIONS

BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
PG	Propyl gallate
TBHQ	Tert butyl hydroquinone
HACCP	Hazard Analysis Critical Control Point
CP	Critical Point
CCP	Critical Control Point
BMU	Beach Management Unit
AC	Alternating current
DC	Direct Current
PV	Peroxide Value
TBARS	Thiobarbituric Acid Reactive Substances
TMA	Trimethlyamine
TVB-N	Total Volatile Base Nitrogen
TPC	Total Plate Count
QIM	Quality Index Method
MDA	Malondialdehyde
DSC	Differential Scanning Calorimetry
Pa	Pascal
G’	Storage modulus
G’’	Elastic modulus
ΔH	Heat enthalpy change
LCA	Life Cycle Analysis
ISA	Iron Sulphate Agar
RM	Raw material
GHG	Green House Gases

ACKNOWLEDGEMENTS

This has been a long journey which started when I was not really feeling well but over time, I regained my health and there was Prof. Nazlin Howell who stood firm and helped me throughout this study with words of encouragement. The EU Securefish project, coordinated by my supervisor Professor Nazlin Howell, for the period it lasted was key to major funding of the project especially for work in specialized labs like at the University of Surrey where part of the practical work was carried out. The contribution by Dr. Farah Badii is recognized in a big way for her in-depth knowledge in lab analysis using the more advanced equipment. My fellow PhD colleagues and laboratory staff during my presence at the University of Surrey are acknowledged. Dr. Jorge Gutierrez-Merino joined in later in part supervision. I would like to thank also my co-supervisor Prof. Douglas Shitanda. May I take this opportunity to thank and acknowledge the financial considerations by Dr. Julius Francis of WIOMSA for enabling the short visits to the University of Surrey. The role played by the local team members in KMFRI and out of KMFRI led by Dr. Cyprian Odoli, Dr. Gareth Kituu, Josephine Marigu, Stanley Onyango, Maurice Obiero, Raymond Ruwa, Sam Ng'ete, Melvin Karuiki and the late Benard Ogongo is acknowledged. Dr. Marnix Poelman, Dr. Kees Goudswaard, Dr. Hans van de Vis, Dr. Paul Bartels, are acknowledged. Thanks to KMFRI and the directors of KMFRI in succession from Dr. Johnston Kazungu, Dr. Renison Ruwa and now Prof. James Njiru for an enabling environment and the staff of the Faculty of Health and Medical Sciences, University of Surrey, Kipini Beach Management Unit especially Bwanaher Hamadi ("Aboo"). My wife Florence, daughter Yvonne and son Fred for bearing with my occasional absence and now my grandchildren Hera Amani and Emanuel Chako, who may find more time with me after this are acknowledged.

May god bless you all

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ANNEXES

CHAPTER 1

CHAPTER 1

1.0 INTRODUCTION

1.1. Background

The UN Food and Agriculture Organization (FAO, 2009) stated that "Food security exists when all people, at all times, have physical, social and economic access to sufficient safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life". Just under one billion people in developing countries go hungry (www.fao.org, 2011**)** and 60% of infants die from not having enough protein, vitamins and minerals. Food insecurity is likely to increase because of (i) a rise in population from 7 billion to 9 billion by 2050; (ii) climate change; (iii) low energy and water availability and (iv) increased amount of food required (Defra, 2010).

Fish, an important food commodity is worth 148 billion dollars and is consumed world-wide; it is the main source of protein for nearly one billion people and affects the livelihood of 540 million people globally (FAO, 2007). Therefore, sustainable fisheries are necessary for nutrition, hunger mitigation and food security. However, fish supplies are reducing because of climate change, overfishing, pollution, bad management and losses postharvest. One of the aims of the UN Development goals and the SECUREFISH project, that this research was part of, was to address postharvest losses by building the local technology through sustainable processing, storage and quality control of fish and fish products.

The fisheries in Kenya is divided into freshwater and marine water fisheries with total landings of 160,000 mtonnes. The marine fisheries is now estimated to contribute about 23,000 mtonnes before exploitation of the 200 nautical mile EEZ stretch with an estimated capacity of 150,000 to 300,000 mtonnes (KMFRI State of Fisheries report 2018). Kenya has now focused on the blue economy for maximum utilization of aquatic resources. The current GDP from fisheries in Kenya is 0.5% and with sustainable exploitation of the resources under the Blue Economy, the GDP is expected to rise to 5%. (KMFRI State of Fisheries report 2018).

Approximately 14,000 fishermen are involved in the coastal fisheries, supporting about 60,000 people in the value chain. About 80% of production is by small scale artisanal fishers while the rest (20%) is landed by industrial and semi-industrial fishers. The small scale fishers operate

traditional fishing boats in the 190 landing sites without ice and proper preservation methods, and hence suffer post-harvest losses (KMFRI State of Fisheries Report, 2018). Most artisanal fish landings take place in the reef area where demersal fish account for 45% of the landings. *Siganids* are the most abundant demersal fish. The other fisheries include pelagic (35%), mollusks (9%) and crustaceans (3%) (Figure 1.1). Other specialized fisheries within inshore areas include sea cucumbers, cephalopods (octopus and squids), and elasmobranchs (sharks and rays).

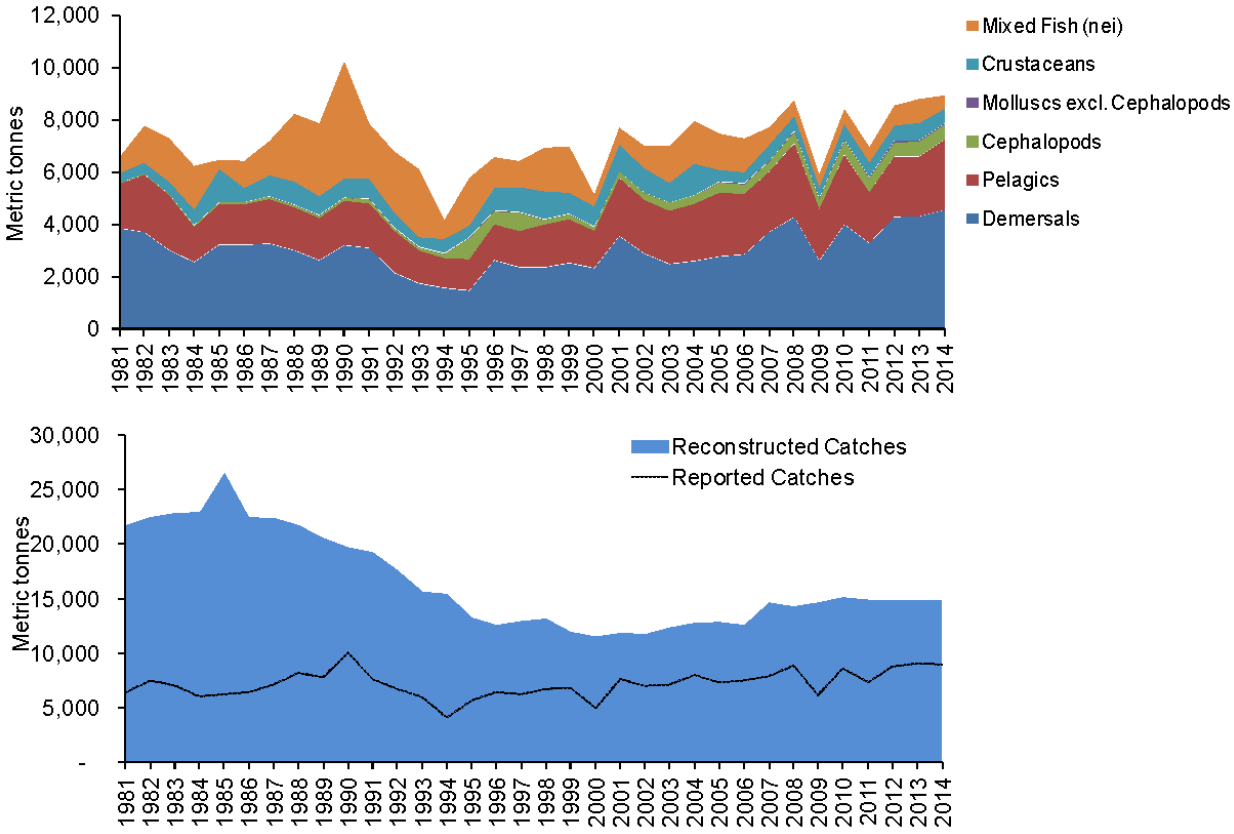


Figure 1.1: A comparison of long-term trends in marine fisheries production for Kenya based on (a) officially reported catches (Source: FAO unpublished data) and (b) reconstructed catches (Source: La Manach, 2005) (Source: KMFRI State of Fisheries Report, 2018).

Fish protein is important in the diet of local people and the Kenyan Government has targeted fisheries in their poverty reduction strategy (Harris, 1993, Kenya Vision 2030). Value addition and post-harvest control therefore become important areas of focus. Some value-added products have been made from *Lates niloticus* like fish fingers and fillets but there is a huge gap in the market for value-added products especially from waste products like bycatch and filleting waste (FAO, 2007).

One of the commonly used traditional fish preservation methods in Kenya is sun drying. This method or preservation is also reflected in the bulk of fish sold in the markets. The fish landings in the coastal region of Kenya are low between the months of April and September when it is raining, and the monsoon winds are strong. Between the months of October to March, there is glut of fish (Fisheries Report, 2000), sunshine is abundant, fishermen sell fish cheaply to middlemen and yet they can dry the fish using improved methods to lower moisture content and store them for use during the lean fishing season.

Most fish landing beaches at the Kenyan coast are far from cities, lack regular transport and there is no refrigeration as the areas lack power supply. Sustainable methods of fish preservation therefore need to be developed for such areas for longer shelf life of fish. The traditional drying methods employed by fishermen at the coast of Kenya include laying fish on rocks (Figure 1.2) or on the ground to dry (Fisheries Dept Report 2000; personal observation).

Figure 1.2: Traditional drying of fish in the open on rocks in the North coast of Kenya

These traditional methods result in fish drying for a longer time, with risks of lipid and protein oxidation (Gardner, 1979), poor hygiene and more labour incurred as fish must be brought home every time it rains and, in the evenings, to avoid overnight dew leading to delayed drying and higher chances of mould infestation. The fish are also exposed to contamination by dust and sand including insect infestation. There is also exposure to harmful hazards from improper human handling and from animals and birds. All these factors result in fish with low overall quality. The market circulation of such fish is low leading to low income (Bala and Mondol, 2001; Sablani *et al*., 2003; Sankat and Mujaffar, 2004; Mujaffar and Sankat, 2005).

1.2 Hybrid solar tunnel windmill dryer

The quality of cured fish can be improved through technological advances. This will help in reducing post-harvest losses and produce better quality fish with a wider market appeal. Drying at higher temperatures and with controlled humidity can make fish dry faster and to a lower moisture content with increased shelf life. As there is much sunlight available in Kenya, improved drying methods can help to improve quality. Sustainable supplies of energy which are cheap include solar and wind energy.

Improved drying was achieved by the introduction of tent-like enclosed solar dryers (Doe et al 1977; Ahmed et al, 1979; Curran and Trim, 1982; Rao et al, 1987; Bala, 1997, 1998; Bala, 2000), but these early models had problems of air movement inside the dryer (Bala and Woods, 1995; Bala and Mondol, 2001) and their drying capacity was also low. Improved dryers with forced air convection (Elshiatryl et al, 1991; Muhlbauer et al, 1993; Bala, 1997; Bala and Mondol, 2001) have been fabricated and a tunnel dryer was trialed in Kenya (Oduor-Odote et al, (2008a) and Shitanda et al, (2008). These forced convection dryers like the solar tunnel dryer have certain advantages because they have two DC fans at the entrance and exit ends of the dryer. These fans provide air movement necessary to remove moisture that has evaporated to the surface of the fish in the drying chamber and replace the evacuated air with fresh warm air in a continuous process. These two DC fans require very little energy to drive them and a single photovoltaic source of one deep cycle battery 100Ah and 40W solar panel is adequate. Drying trials carried out on the Kenyan coast have shown that this solar tunnel dryer is suitable for drying fish and other farm products (Oduor-Odote, 2009).

Solar drying is not possible on rainy days, damp weather and at night. It is important to have dryers that allow drying to proceed always. This has been made possible by the introduction of hybrid dryers that use biomass and solar systems (Elepano and Satairapan, 2001). These systems use gas or gasifier stoves and agro-waste like coconut husks (abundant in Kenya) and rice husks. Three disadvantages with this system were: 1) the biomass is obtained at a cost due to quantities required and 2) this type of hybrid system has fluctuations including quality and flow rate of drying air which may be due to changes in weather; 3) It is difficult to maintain the required quality of air and the flow rate when operating supplementary heat sources.
The introduction of a hybrid windmill-solar tunnel dryer is a more efficient method of ensuring drying is continuous using renewable energy (Figure 1.3). Apart from the advantages of the

solar tunnel dryer over the conventional solar dryers, integrating it with a windmill shall widen the scope of utilization. This is because the windmill can generate its own power and complement power from the solar system. The windmill will generate electricity and the electricity will be used for lighting and for heating electric elements at night for heat generation. The heat will be used for drying. Wind power will also be used to operate a fan for forced convection through the solar tunnel dryer. This will enhance drying. Wind power and solar power, both renewable energy types shall complement each other.

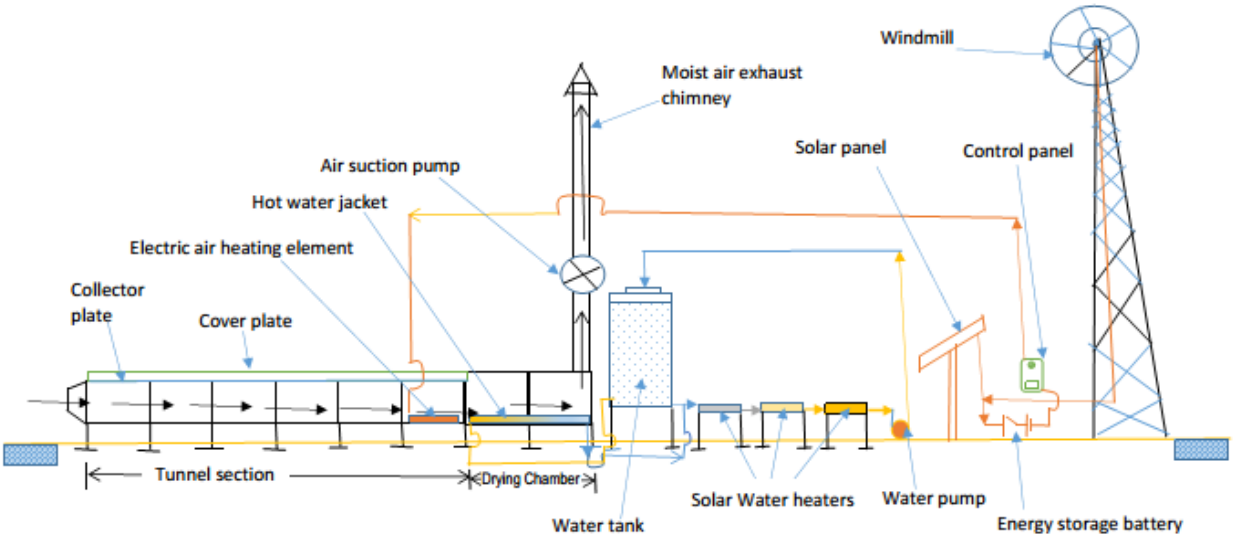


Figure 1.3: Schematic view of the Hybrid windmill solar tunnel dryer installed in Kipini, Kenya

The wind blows mostly after sunset and sunshine is available only during the day, if it not raining. When the sun shines, the solar system produces DC electricity to drive the drying system in the solar tunnel dryer and to charge batteries or to produce AC electricity after passing through an inverter. When the windmill operates, its energy is stored in the deep cycle batteries which generate electricity used in heating the DC coil in the drying chamber at night to enable drying. Enough energy is also generated to operate the fans in the solar tunnel dryer for forced air convection through the dryer to enhance drying. Wind power therefore complements solar power. Drying (recorded by weight loss) is achieved continuously. The power can also be used for other activities in the locality where fish is being processed. The introduction of a novel hybrid solar tunnel dryer-windmill system is a more efficient method of ensuring that drying is continuous and uses renewable energy, compared with the conventional solar dryers; this novel design was one of the objectives and innovations of this project. Therefore, further improvements in fish drying methods will help to enhance the quality and handling capacity of dried fish by artisanal fishermen to reduce post-harvest losses and improve on food security at the Kenyan coast with the aid of two major sources of renewable energy namely sun and wind.

In this study dying models were used to evaluate the performance of the hybrid windmill solar tunnel dryer during the drying of *Siganus sutor* (Rabbit fish or Tafi), one of the most popular fish at the Kenyan coast.

1.3 The fishery and biology of *Siganus sutor* fish in Kenya

The Kenyan coastline extends from Vanga (4^{o} 40.2' S, 39^{o} 11.5E) in the south towards Tanzania and 1^{o} 39.8' S; 41^{o}, 33.4' E towards Somalia in the North and covers 83,603km^{2} (Horril and Kamau, 2001; Newell, 1959).

The Kenyan marine fishery is divided into industrial, semi industrial and artisanal fisheries. The artisanal/small scale fishery is the most important fishery in the marine sector and Kenyan coast. There are over 14,000 fishers using diverse gears with over 3000 vessels that operate in the shallow reef lagoons and bays (Fisheries frame survey report, 2016, KMFRI State of Fisheries report 2018).

They use various methods to catch the fish including reef and beach seines, seine, gillnets, fishing traps like basket traps and fence traps among others (Wambiji et al 2008).

Vessels that are used to deploy basket traps include mashua (2%), ngalawa (1%), dugout (60%), dau (4%) foot fishers (20%) and mtori (1%). Most of these vessels are non-motorized and are propelled by sail or paddling (KMFRI State of Fisheries report 2018).

The traps account for 40% of fish landings and are made by local fishers. The traps target Siganidae (rabbit fish) and the main species of Siganidae are *Siganus sutor* (Figure 1.4), *Siganus canaliculatus* and *Siganus argenteus*.

These form the core group for the economically important fish to artisanal fishers. Siganids have the potential of being farmed in Kenya. The *Siganids* are made up of 2 genera comprising 25 species so far identified.

Figure 1.4: Fresh *Siganus sutor* in Kenyan waters.

The fish are herbivorous, diurnal, tolerate low salinities and feed on benthic algae and associated flora (Jones and Cooke, 1981, Frose and Pauly, 2004).

The *Siganids* (Rabbit fishes) reside in shallow lagoons with seagrass beds (Wambiji et al, 2008). *Siganus sutor* also known as "Tafi" grows to a maximum length of 45cm and are one of the most targeted reef fishes with a catch composition by weight of 63% in some areas (Wambiji et al, 2008) and is most abundant. They spawn in January to February in Kenyan waters, May to June in Tanzania waters (Wambiji et al, 2008) and appear again in Kipini area April to June caught by drift net fishers.

1.4 The market value of Siganid fish

The market prices of Siganids in different areas are Kshs. 60/kg (0.6 USD) in Kiunga (Lamu) in the North, and 250 /Kg in Shimoni south cost (KMFRI Status of Fisheries Report 2018).

The preservation of Siganids by drying will help in making it available all the times once stored hence reducing frequency of fishing in the reefs because of longer shelf life thus reducing pressure. Making value added dried Siganid fish products like dried packed fillet will increase the market value of the novel product resulting in higher earnings.

1.5 Quality of *Siganids* and safety

For fish to be solar dried, an assurance of the quality of the fish being processed is necessary. Currently on the coast of Kenya, only fish that has started to spoil is subjected to further drying. This results in low quality fish and a wrong perception amongst consumers that dried fish is undesirable. It is important to emphasize that the fish to be dried must to be as fresh as possible. Thus far, there are no quality indicators for fresh *Siganids* currently in existence in Kenya.

One of the objectives was to establish the Quality Index Method (QIM) for freshness of *Siganids*. The QIM was based on organoleptic attributes of the eyes, skin, gills, abdomen and texture using the standard scores of 0-3 demerit points or index points. It is the sum of these scores that are summarized and used to grade the *Siganid* fish. A quality score chart using the Quality Index Method for *Siganid* freshness in Kenya has been developed (Odoli *et al*, 2013). Coupled with quality are the quality costs during processing and the relationship between the raw material and final product quality. This innovative way of ensuring fish quality was investigated in the present novel study on dried fish.

The quality of raw material determines the success of any food processing establishment. Fish is a highly perishable commodity and loses its quality immediately after catch (Zugarramurdi et al., 2004). One of the main processing techniques in the fish processing industries is filleting. This process is influenced by many factors including rigor mortis. Freshness, type of fish (lean or fatty) and experience of personnel influence the yield and quality of the fillets (Zugarramurdi et al., 2004). As the filleting time and yield also depend on freshness, a knowledge of freshness quality of fish is important.

The solar dried *Siganids* that are dried by improved processing methods like solar drying require that they meet certain internationally recognized hygiene standards. Development of a generic HACCP system for solar dried *Siganids* is therefore important as there is no HACCP system for solar dried fish in existence. All parameters that go into an HACCP system such as biological, chemical and physical parameters must be considered including the microbiological load in the process value chain and risk analysis. Consequently, the hazard analysis and monitoring scheme was carried out for solar dried *Siganids*, including the determination of possible microbiological, physical and chemical food safety hazards, Critical control points (CCP) and Control points (CP) for each process step. The process steps from purchasing to

sales (from reception to transport to the consumer) including production and quality were investigated in this study so that all the delivered raw material and dispatched final product were at or below regulatory safe limits.

1.6 Moisture and water activity

Moisture and water activity are two parameters that affect storage of dried fish products because when the fish muscle is dry, it again becomes hygroscopic and the moisture absorbed is available for microbial growth. The safer moisture level that prevents microbial growth during storage is below 25 % and below 15% prevents mould growth (Bala, 2001). Total moisture content is also useful for weight loss during drying. The water activity is used to determine the available moisture for microbial growth and values of 0.6 to 0.85 are ideal as 0.85 prevents growth of *Staphylococus aureaus.*

Water or moisture content is the total moisture contained in food and is usually written as a percentage of the total weight:

Mw (Wet basis) = (W-D/W) x 100

Mw = Moisture content on a wet basis

W = Wet weight

D = Dry weight

Water activity indicates the availability of water that promotes biological reactions. Water activity determines microbial growth. A reduction in water activity causes a reduction in growth of microorganisms (Oduor-Odote et al, 2010).

1.8 Total Volatile Basic Nitrogen (TVB-N)

Total Volatile Basic Nitrogen (TVB-N) are one of the spoilage indicators of fresh or even dried fish. The composition of Total Volatile Bases is ammonia and amines like trimethylamine and dimethylamine. Bacterial enzymes drive the formation of these amines during storage of fish. As bacteria utilize these nitrogenous compounds, various compounds like hydrogen sulphide, dimethyl sulphide and methyl mercaptan are formed. These are typical components of spoilt fish (Zahid et al, 2011).

If drying is slow, odours and flavours associated with enzymatic and bacterial spoilage may develop leading to the formation of nitrogenous volatile bases (TVB-N) from protein and free fatty acids (Huss 1998). Determination of Total Volatile Basic Nitrogen is commonly used to evaluate the extent of spoilage in fresh fish (Dhaouadi et al, 2006; Anderson 2008; Torry advisory note no.92; Loughran and Diamond 2000, Castro et al 2000). The TVB-N concentration of 30mg/100g is the legal permitted for fresh fish (Anderson, 2008; Torry note no. 92). TVB-N limits for solar dried Siganid fish are still not known. This is because no studies have been made in that field.

1.9 pH

Microorganisms grow optimally within certain ranges of pH (Ghaly, 2010). There is normally an increase in pH of fresh fish samples during ambient or chilled storage. The increase in pH is attributed to an increase in alkaline compounds like ammonia and volatile bases formed because of microbial action during fish muscle spoilage. The changes in pH relate to the bacterial type present, free fatty acids and level of glycogen converted to lactic acid (Huss, 1995; Susanto et al, 2011). The pH decreases during the initial stages due to rigor mortis and then increases again during storage due to spoilage bacteria, causing the release of amines (Susanto et al, 2011). Type of species, fish catching methods, biology, season and methods of killing can affect pH levels**.** Studies of pH changes during drying and storage of solar dried *Siganid* fish have not been reported as yet.

1.10 Microbiological evaluation

Evaluation of microbiological changes of fish including solar dried fish during storage also gives a more complete understanding of factors that affect quality. The microbial flora after fish landing depends on the environment that the fish came from and can vary (Shewan, 1977). There is a balance in the physiological status of the body that prevents bacteria from attacking the fish when it is alive though this breaks down upon death of the fish for bacteriological spoilage to start (Eyo, 2001). The awareness of the consumer on high quality fish products is increasing therefore processes that avoid any hazards must be put in place and the public assured (Abolagba & Uwagbai, 2011). A system of fish quality inspection and control in the dried fish sector must be introduced in Kenya.

1.11 Biochemical changes

1.11.1 Lipid oxidation

Fatty fish and fish oils are nowadays considered beneficial to human health and there is an increase in their consumption. Apart from being rich in essential amino acids, lipid soluble vitamins like A and D, it is also rich in highly unsaturated fatty acids including eicosapentanoic acid (20:5 n-3) and docosahexaenoic acid (22:6 n-6) with health benefits to humans (Shahidi and Miraliakkbari, 2004). Fish oils are composed of 75 % triglycerides and 25% phospholipids. The fatty acids common in fish oils include saturated fatty acids like myristic, palmitic and stearic, monounsaturated fatty acids –palmitoleic, oleic, 11-eicosenoic and 11-docosenoic acid.

The polyunsaturated fatty acids are docosahexaenoic acid (DHA, 22:φ6) and eicosapentaenoic acid (EPA 22:5φ3). These polyunsaturated fatty acids can have 5 or 6 double bonds making them susceptible to lipid oxidation resulting in rancidity. The process of rancidity results in release of many chemicals. Some of the products include mutagens, promoters and carcinogens like fatty acid hydroxides, hydroxides, endoperoxides, cholesterol and fatty acid epoxides, enals, aldehydes and free radicals. The polyunsaturated fatty acids need stabilization to avoid deterioration. Natural antioxidants can be used as one way of stabilizing the oxidising fatty acids in fish muscle.

It is this high level of polyunsaturated fatty acids which make fish susceptible to spoilage through oxidation with the resulting products affecting texture, flavor, colour and nutritional value (Gardner, 1979). Lipid oxidation takes place in both fresh and dried fish products (Oduor-Odote & Obiero, 2010). There is normally a balance through numerous complex systems that control pro-oxidant and antioxidant factors. This balance is lost during processing and storage and lipid oxidation sets in. It depends on handling time and levels of oxidants in the fish tissue (Alghazeer et al, 2008; Pereira de Abreu et al, 2011).

Lipid oxidation involves a reaction of free fatty acids with oxygen resulting in removal of a hydrogen atom from one of the double bonds in the fatty acids. The resultant product is a radical which attacks more fatty acids forming more radicals in the form of unstable hydroperoxides and peroxidases. These are the primary products of lipid oxidation and they combine finally to produce volatile or non-volatile stable compounds like aldehydes including malondialdehyde, pentanal, hexanal some of which influence odour and flavours contributing to rancidity. The

aldehyde groups can also react with sulfydryl groups of proteins, nucleic acids and related amino acids influencing muscle toughness (Esterbauer, 1982; Hoberman & San George, 1988; Addis, 1986; Gerrard and Brown, 2002; Nair, Cooper, Vietti, and Turner, 1986).).

The classical mechanism of lipid oxidation involves three phases known as the initiation phase where the free radicals are formed then the propagation phase where more radical are formed from the free fatty acids and the termination phase as illustrated below:

Initiation: $RH + O_2 = R\bullet + .OH$

Propagation: $ROO\bullet + RH = R\bullet + ROOH$

$ROOH = RO\bullet + OH\bullet$

Termination: $R\bullet + R\bullet = RR$

$R\bullet + ROO\bullet = ROOR$

$ROO\bullet + ROO\bullet = ROOR + O_2$

RH in this case is any unsaturated fatty acid,

R**.** is a free radical produced by extracting a labile or reactive hydrogen from a carbon atom next to a double bond, ROO**.** is a peroxyl radical and OH**.** a hydroxyl radical. ROOH is a hydroperoxide (Frankel 2005).

Most studies on lipid oxidation in fish muscle have been carried out during chilled or frozen storage, microwave cooking or conventional cooking methods (Yarnpakdee et al, 2012; Cho et al., 1989; Regulska-Ilow and Ilow, 2002; Gimenez et al 2011). Balogun (1988) carried out a study on lipid characteristics during sun drying of freshwater Clupeids. There are no reported studies on lipid oxidation during the process of solar drying of fish. This is important because lipid oxidation can contribute to flavor and off-odour of fish (Yarnpakdee et al, 2012; Selli et al, 2009; Azad Shah et al, 2009).

Studies of lipid oxidation during solar drying will help to establish the fate of the polyunsaturated fatty acids that normally provide health benefits, in solar dried fish.

1.11.2 Evaluation of lipid oxidation

PV and TBARS are the main indicators commonly determined for lipid oxidation although there are others like anisidine, free fatty levels which are also used. (Giménez et al, 2011).

1.11.2.1 Peroxide Value (PV)

The hydroperoxides formed during lipid oxidation is measured as peroxide value. The maximum limit for peroxide value for fresh fish oil is 7-8mEq /Kg (Boran et al, 2006).
Once formation of secondary oxidation products starts, using peroxide value as an indicator alone may not give reliable results but is to be used with other indices (Gimenez et al 2011, Weber et al, 2006). It is not known what levels of peroxide value will be during solar drying and storage of solar dried fish. The limit of PV for fish oil is 8meq O_2/kg of oil (Boran et al, 2006).

1.11.2.2 Thiobarbituric Acid Reactive Substances (TBARS)

Over time, the hydroperoxides decompose to form secondary and more stable products like aldehydes which contribute to rancidity. One of the products is malondialdehyde (MDA) which is measured as TBARS though other secondary compounds that may not have necessarily have originated from lipid oxidation are also measured. The aldehydes like glutaraldehye and malondialdehyde with difunctional aldehydic groups can react with the nucleophiles and sulfhydryl groups of proteins and nucleophilic acids as well as related amino acids resulting in increased toughness and change in functionality. The stable secondary products influence texture, flavor and odour. In fish oil, the limit for TBARS which is measured as mg malondialdehyde/kg is 7 to 8 (Gimenez et al, 2011; Boran et al, 2006; Aghazeer et al, 2008; Esterbauer, 1982; Hoberman & San George, 1988, Shahidi, 1998).
Thus, lipid peroxidation products like peroxides and malondialdehyde were studied to monitor oxidative rancidity during solar drying and storage of solar dried fish.

1.11.3. Interaction of lipid oxidation products with proteins

During storage of dried fish and just like in fresh frozen fish storage, degradation due to lipid oxidation continues to occur (Oduor-Odote and Obiero, 2009; Badii and Howell, 2001). Lipid oxidation results in products that affect texture and in case large quantities of lipid oxidation products like peroxides are accumulated, they can be toxic when eaten (Barber & Berheim, 1967). Biochemicals in foods that are prone to lipid oxidation damage, are proteins and amino acids. Lipid hydroperoxides interact with protein/amino acids in a complex manner due to hydroperoxides and the secondary products that decompose thereafter. Proteins on exposure to peroxidized lipid form hydrophobic associations/and or hydrogen bonds. The secondary products also produced from peroxides also bind to protein (Sessa and Rackis, 1977; Erickson et al 1976). Aldehydes, one of the main secondary products bind to hydrophobic sites in the protein. The lipid-protein complex is due to electrostatic and hydrophobic interaction. Some aldehydes bind amino groups by making Schiff base adducts (Gardner, 1979).

Other modifications to protein structure due to lipid hydroperoxides and secondary products include radical reactions producing protein radicals, protein-protein and lipid-protein bonds, protein and amino acid destruction, activated oxygen and oxidation, reaction with secondary products like malondialdehyde among others (Gardner, 1979, Auborg 1999, Frankel 1998). It is this cross-linking of oxidized lipids with proteins that contributes to protein denaturation ending up by affecting nutritional and organoleptic properties (Tironi et al 2010).

1.11.4 Protein structure

After water, protein makes up the second largest component, about 20% of fish fillet. During thermal processing, protein denaturation contributes to quality changes in fish muscle. Protein macromolecules are composed of about 20 amino acids, which comprise essential and non-essential amino acids that are needed for health and growth. Two essential amino acids, methionine and lysine are generally found in high concentrations in fish amino acids (Lehninger, 2008).

Each amino acid has an amino, a carboxyl and an R group (Figure 1.5). The different properties of amino acids are derived from the R group side chain. They influence properties like solubility, reactivity of proteins with other nutrients and compounds. The amino acids, which

are the main subunits of proteins include leucine, isoleucine, valine, glycine, proline, alanine and methionine and are in the category of nonpolar aliphatic amino acids. Phenylalanine, tyrosine and tryptophan are the aromatic fatty acids. Serine, threonine, cysteine, asparagine and glutamine are the polar uncharged amino acids while the positively charge amino acids are lysine, arginine and histidine. Amino acids that are negatively charged are aspartatic and glutamatic acid (Lehninger, 2008).

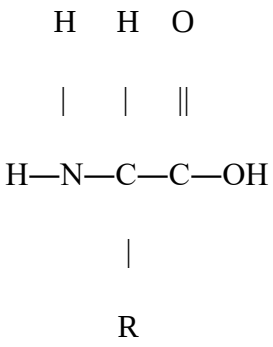


Figure 1.5: Structure of amino acid

Proteins comprise primary, secondary, tertiary and quaternary structure (Damodaran 2008). The primary structure of the protein is a linear sequence of amino acids in a polypeptide chain (Lehninger, 2008). The amino acids are covalently attached by peptide bonds. One amino group from one amino acid links with the carboxyl group from another amino acid to make a peptide bond with the removal of one molecule of water (Figure 1, 6).

There are two forms of secondary structure of proteins namely the α -helix and β-sheets (Damodaran 2008). The α-helix is a coiled structure. Each oxygen of the carbonyl group forms a hydrogen bond with amide-hydrogen of the amino acid further down, to make the polypeptide chain stable. They also make it rod-like with the R groups or the side chains pointing outwards from the chain (Lehninger 2008).

The second secondary structure is the β-sheet that resembles a corrugated ribbon like sheet structure. The hydrogen bonds in the β-sheet occur between atoms of two separate sheets, not in the same strand. In a sheet, the beta strands can be found facing the same way or the opposite way (Lehninger, 2008). The third structural arrangement is the tertiary structure. This is a three-dimensional arrangement of a polypeptide chain which contains helices, beta sheet and random coil structures. The structure is held together by non-covalent hydrogen, electrostatic and hydrophobic bonds and covalent disulphide linkages. The quaternary structure is made up of

two or more polypeptide chains arranged in a three-dimensional complex. Proteins can further exist as dimers, trimers and tetramer subunits (Nelson 2008) e.g. myoglobin.

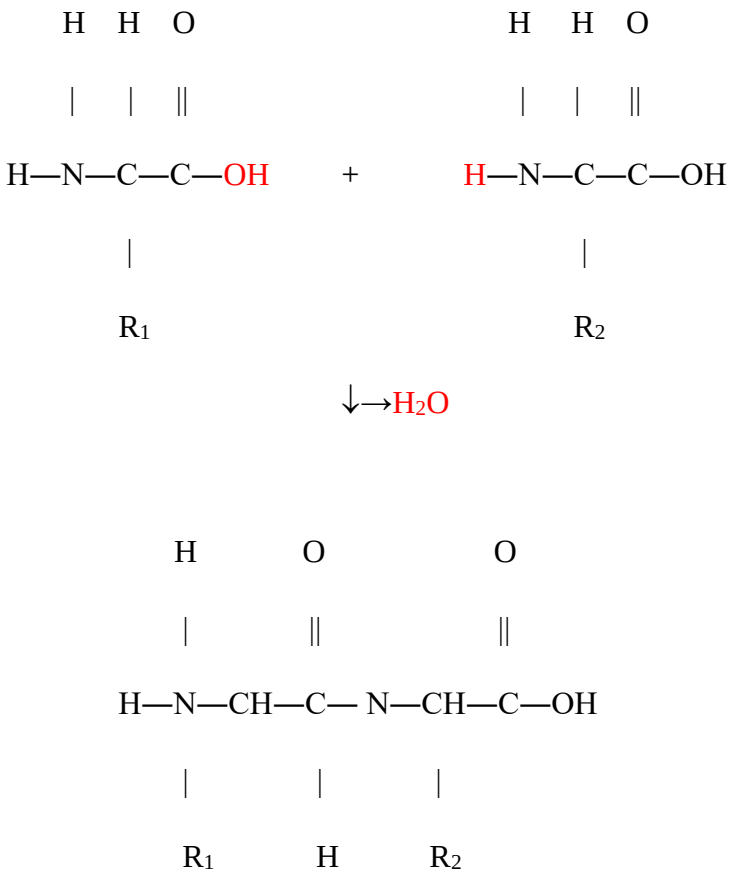


Figure 1.6: Peptide bond

1.12. Rheological properties

During the drying of fish protein structure is altered simply by loss of water. When the dried fish is stored, biochemical changes occur that are attributed to lipid and oxidation and protein denaturation. These changes are both physical and biochemical. The physical changes can be measured by studying rheological and thermal properties mainly of protein.

1.12.1 Protein denaturation during processing

Denaturation is modification or unfolding of protein and is produced by heating, freezing, chemicals and shear. For most proteins, denaturation can be considered as an irreversible endothermic transition. The heat denaturation involves the cooperative or non-cooperative transition of a protein from its folded to unfolded state due to the breakdown of secondary and tertiary protein structures. The first stage of denaturation involves non-covalent bonds and is

reversible. At higher temperatures, covalent linkages like disulphide bonds lead to aggregation and irreversible protein denaturation (Comfort 1995); Howell and Saeed, 1999). This process is also known as gelation. Gelation of proteins occurs when polypeptide chains uncoil and then interact at specific sites to form a cross-linked network

The intentional unfolding and the interaction of protein molecules with aqueous medium and induced aggregation in cooking governs the structure, flavour and texture and other qualities of the food products. It also contributes to the nutritional qualities and to the physical stability of the foods during storage. However, in preserved fish and fillets texture changes due to protein denaturation are undesirable as they lead to toughening of the fish muscle due to crosslinking of the myofibrillar proteins.

1.12.2 Rheological properties of proteins

Rheological studies can help understand what happens during processing and storage of proteins. Rheology, from the Greek word rheos is defined as the science of flow and deformation of materials governed by the force, deformation and time. Viscosity, which is resistance to flow, is used to describe the consistency of fluid materials. Elasticity is usually associated with the properties of solids and structure. Water has the lowest viscosity and a rubber ball is perfectly elastic. However, food materials are viscoelastic, neither completely liquid or elastic but with properties in between the two (Saeed and Howell, 2004).

Four main terminologies in rheology; viscocity, elasticity, viscoelasticity and elastoviscosity are used to explain the flowing or deforming behaviour of matter over a time period. In viscous material the applied energy is dissipated into the sample as heat while an elastic material stores the given energy in the form of elastic potential energy (Comfort, 1995).

G’ (the storage modulus) and G’’ (loss modulus) which are calculated from the strain/stress amplitude and phase lag as a function of time are used to determine the viscoelasticity properties of the materials. G; can be referred to as the elastic property while G’’ denotes the viscous property. An ideal liquid and perfect solid will have phase angle at 90^{o} and 0^{o} respectively. When the phase angle is between 0-90^{o} C, it is considered a viscoelastic material. However, for substances with gel forming ability, phase angle at 45^{o}C should not be used as a transition phase from viscous to elastic material. Instead, the point where the substrate changes from viscous to elastic material, the so called “gelation point or “cross over point” is obtained when log G’’=

Log G'. Not every substance with a gelling ability will have a gelation point. G' (storage modulus) has in some studies been found to be higher than G'' viscous modulus meaning higher elastic properties or already a gel /solid like (meaning the protein structure was capable of storing energy rather than letting it be dissipated as heat). The gelation or crossover point for fresh fish protein components like myosin are between 15 to 40^{o}C (Badii and Howell 2002).

Gelation properties contribute to protein functionality. The onset of G' in a rheogram rising to a particular temperature determines the degree of protein unfolding whereas a lower gelling temperature indicates less energy is required to unfold proteins prior to aggregation. Proteins which are already unfolded will enter G' at lower temperatures. For a protein to gel, it must first denature (unfold) and then cross-link to a three dimensional structure; but on high heating the denatured proteins can aggregate. Freezing and storage affect gelling properties of fish protein (Comfort, 1995; Badii and Howell, 2001). During storage of dried fish, toughening can take place and the ability of the protein to then contribute to gel strength. in the manufacture of meat products, for example is decreased. Loss of gelling ability is related to the denaturation and aggregation of myofibrillar proteins.

1.12.3 Thermodynamic properties

The heat effects of denaturation and aggregation of proteins are usually small and have opposite signs namely heat absorption (endothermic) and heat release (exothermic), respectively. Differential Scanning Calorimetry (DSC) helps to characterize the thermodynamic and conformational changes in proteins subjected to a range of temperatures. DSC helps to study thermal properties of muscle proteins during heat processing and storage and to see the effect of other nutrients on proteins (Thorarinsdottir et al, 2002).

The DSC scans produced from thermal denaturation of proteins are described in terms of peak temperatures (Tm) and enthalpy changes. The denaturation enthalpy change is expressed as ΔH. There can be either endothermic or exothermic reactions that correlate with the remaining ordered three-dimensional content of a protein. The thermally induced changes that cause protein denaturation are recorded as a differential heatflow. This is what is displayed as a peak in the thermogram (Skipnes et al, 2008).

The DSC therefore detects structural melting or unfolding of the molecule due to heating. During the process of transition of protein from its native conformation to the denatured one,

there is rupture of inter and intramolecular bonds with the process occurring in a cooperative manner.

An endothermic reaction has significant uptake of heat that is recorded in the thermogram. Once the thermogram is analysed, two parameters can be determined namely the peak transition temperature or denaturation temperature or maximum temperature (Tmax) and enthalpy change ΔH. There is a correlation between ΔH and amount of ordered secondary atructure. The ΔH value is derived from endothermic reactions like breakdown of hydrogen bonds. Exothermic reaction is indicative of protein aggregation and hydrophobic interactions (Skipness et al 2008).

Protein undergoes reversible denaturation by cooling. Changes in enthalpy can be related to denaturation. A sharp transition peak i.e. denaturation in a narrow temperature gap is denoted as highly cooperative. Myosin is made up of 6 cooperative domains with transition temperatures ranging from 43 ^{o}C and. If the transition peak is broad, then it means there is loss of cooperativity via dissociation of oligomers.

When ΔH is reduced, it signifies some loss of protein structure with temperature, in this case drying. Muscle comprises three types of protein namely actin, myosin and sarcoplasmic proteins. They however also show different endotherms depending on the part of the body. This variation could be due to differences in relative quantity of constituent protein, the basic structure of the protein and interaction of constituent proteins. In a study for cod, the thermogram showed between 3 to 8 denaturation peaks when scanning from 0 to 110 ^{o}C and these transitions match specific proteins. The first protein to be denatured is myosin at Tm 40-44 ^{o}C (enthalpy values from 44 to 820 KJ/Mol), sarcoplasmic proteins 55-60 ^{o}C, actin at 60-76 ^{o}C and collagen combined at 42-44^{o}C (Skipness et al 2008; Badii and Howell 2002). The transitions for fresh and dried fish are different. T_{max} shows the highest temperature required for denaturation. Increases in denaturation temperature with storage can denote aggregation and a structure that is stable and denatures in a cooperative way.

If protein structure is unfolded, the temperature of denaturation and Δ H is lowered (Howell and Saeed, 1999). The presence of antioxidants may stabilize the protein and can cause lowering of the denaturation temperature. Phenolic compounds may suppress thermal denaturation of protein probably by reducing the number of hydrogen bonds that are disrupted. Hydrogen bonds influence denaturation temperature and ΔH (Howell and Saeed, 1999). A decrease in ΔH also shows partial denaturation. If there is a marked increase in temperature of denaturation, it is possible that the heated protein formed a complex and stable structure.

Interaction of phenols with denatured protein before DSC analysis may be responsible for low ΔH values which can be observed in certain cases. Hydrogen bonds responsible for phenol-protein binding may break during the heating, contributing to an endothermic peak and the overall heat enthalpy value. High Δ H means that the protein is resuming its normal stable conformation with an increase in denaturation temperature. It is however possible that protein-phenol complexing may be by hydrophobic interactions. When hydrophobic interactions decrease, the ΔH would also decrease. The exact nature of phenolic-protein interactions is not known.

1.13 Antioxidants

Oxidation damages nutritional and organoleptic properties of oily foods. Lipid oxidation occurs via the initiation stage in the presence of oxygen or metal ion or enzymes and hydrogen is abstracted near the methylene bond (Frankel, 2005). This is followed by the free radical propagation stage and finally the interacting of radicals to terminate the autooxidation. The primary products are lipid hydroperoxides, which are very unstable and degrade to secondary oxidation products (aldehydes, ketones, alcohols, hydrocarbons) that affect food quality. As a means of preservation, antioxidants can scavenge free radicals; quench singlet oxygen; have chain-breaking reducing action; chelate metals or inhibit specific oxidative enzymes. Antioxidants act by mixed and cooperative mechanisms (Craft et al, 2012).

Depending on their mechanism of action antioxidants are classified as primary or secondary, (Reische et al., 1998). Primary antioxidants are chain breaking antioxidants that scavenge free radical that delay or inhibit the initiation step or interrupt the propagation of autoxidation. Primary antioxidants in foods include tocopherols, butylated hydroxyanisole (BHA), butylated hydroxytoulene (BHT), propyl gallate (PG) and tertiary-butyl hydroquinone (TBHQ).

Secondary antioxidants like citric acid, lecithin, ascorbic acid and tartaric acid (Zuta et al, 2007) act as metal chelators, provide hydrogen to primary oxidants and break down hydroperoxides formed during propagation step. They act on singlet oxygen and absorb ultraviolet radiation. Secondary antioxidants work synergistically with primary antioxidants to improve antioxidant activity (Reische et al 1998).

There are restrictions in utilization of synthetic antioxidants such as BHT, BHA, and TBQH as they are not considered safe for food use and customers currently prefer "natural products" (e.g. ascorbic acid, α-tocopherol or phenolic compounds) from both terrestrial and lately aquatic plants (Zuta et al, 2007).

Brown algae is a good source of compounds with antioxidative action, and is found in different regions of the world like Indonesia, Japan, Canary Islands, Norway, the Brittany coast, the Aegean Sea, Korea, Iceland, Saudi Arabia, Thailand, Mexico, Ireland, Brazil, Hawaii and New Zealand. Most of the compounds are phenolic with different antioxidant activities (Balboa et al, 2013). The fucoidan fraction content and structure in macroalgae depends on the extraction and the depolymerization procedure that alters the antioxidant activity.

A simple and reliable *in vitro* antioxidant activity tests normally via quenching free radicals by hydrogen donation, the ability to transfer one electron and other assays (Balboa et al, 2013). *In vitro* antioxidant capacity has been used to compare different compounds, but not their performance in food systems. The complex behaviour is influenced by the substrate, solubility and phase distribution of the antioxidant and its interaction with the nutrients and physical and atmospheric conditions (Decker, 1998).

The onset and rate of ipid oxidation in fish can be delayed by the addition of antioxidants (Pereira de Abreu et al, 2011). Due to possible toxicity and side effects (Safer and Al-Nughamish, 1999) of commercial synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) there are strict regulations for their use in certain countries. Therefore, interest in natural products has increased rapidly, particularly those from aquatic plants.

1.14 Natural antioxidants

A diverse range of products like tea, wines, beer, rosemary, oregano, spices, herbs, clove, blue berries, mustard can neutralize free radicals and subsequent reactions that minimize loss of quality (Pereira de Abreu et al, 2011). Turmeric, macroalgae and plant macrophytes like water hyacinth can be used as a source of natural antioxidants (Stankovich, 2004; Shanab and Shalaby, 2012; Balboa et al, 2013; Chan et al, 2009) and were adopted in the present study.

1.14.1 Curcuma longa

Curcuma longa L. is of Indian origin and belongs to the Zingibareceae family. This plant is tropical and is grown globally including South East China. The ground rhizomes of Curcuma longa L called turmeric, and its active constituent, curcumin, are used as a spice and colouring in curry powders and other condiments. Turmeric is extracted from the root of the turmeric plant by boiling, drying and finally grinding to a powder. A more concentrated form can be produced by solvent extraction of the active ingredients curcuminoids. The concentrate, also known as curcumin is an orange yellow crystalline powder that is soluble in oil (Stankovich, 2004). The health properties of turmeric may be due to anti-oxidant, anti-cancer and anti-inflammatory properties (Stankovich, 2004; Chan et al, 2009). A study of the different parts of the plant indicated that rhizomes contained a higher amount of phenols and could scavenge radicals more efficiently than the leaves of *C. longa*; however, the leaves had superior metal chelating ability.

1.14. 2 Euchema denticulatum

There are more flora and fauna in the marine aquatic environment acting as a new source of a number of new compounds with unique structures (Balboa et al, 2013; Blunt et al, 2012). These compounds have biological activities useful in the cosmetics, pharmacy, food and biotechnology industries. Some of the flora, like certain macroalgae, live in stressful aquatic conditions that require them to deal with free radicals and other oxidants by producing secondary metabolites that protect against oxidative stress (Balboa et al, 2013). Based on pigmentation alone, seaweeds (Figure 1.7) are classified into 3 groups – brown (Phaeophceae), red (Rhodophceae) and green (Chlorophyceae) and all contain active antioxidants at different levels (Balboa et al, 2013).

Figure 1.7: Seaweed farming and harvesting in the South coast of Kenya

Red and brown algae are known to be rich in various bioactive compounds also with antioxidant activity. Some of the bioactive compounds have been identified from methanol extract fractions including catechin, flavonols, flavonol glycosides and phlorotannins. These compounds have unique structures believed to contribute towards their strong antioxidant activity, for example the polyphenol phenol rings that acts as an electron trap for free radicals (Zakaria et al, 2011).

Despite the diversity of seaweeds along the Kenyan coast, no studies have been reported on their antioxidant activity. *Euchema denticulatum* is now farmed in Kenya and is a reliable source of raw material. Thus far, only ecological and culture studies have been documented on this species in Kenya. Thus, this study can provide further useful bioactivity data and possibly increase shelf life of fish and other foods when used as a source of antioxidants. Further, it would also help to promote the consumption of algae as a food products rich in polyphenolic antioxidants.

1.14.3 Water hyacinth

One of the alien aquatic plants in Kenya is the water hyacinth *Eichhornia crasipes* (Mart) Solms. Its origin can be traced back to the Amazon, Brazil and it is spread all over South America, the tropics and sub tropics. In 2010, its presence was registered in 62 countries (Shalaby, 2012; Gao and Bo, 2004; Shanab). The water hyacinth forms dense monocultures

over water and disrupts the food chain and ecosystem structure (Dandelot et al, 2008). In Lake Victoria, Kenya (Figure 1.8), it has influenced fishing and fishermen lifestyles even blocking routes for fishing vessels. The water hyacinth inhibits growth of certain microalgae in the same environment (Dandelot et al, 2008) due to allelopathy (Awad, 2008; Jin et al, 2003). Just like the macro algae, the water hyacinth produces certain metabolites and green algae and germination of seeds and growth of seedlings can be inhibited by crude allelochemical extracts from *E. carassipes* (Awad, 2008; Jin et al, 2003).

Figure 1.8: Water hyacinth in Lake Victoria blocking maritime transport and fishing areas

As mentioned earlier, the human body and food is protected from adverse effects of ROS by free radical scavengers or antioxidants (Matsukawa et al, 1997). There natural antioxidants are preferred as there are reservations about using synthetic ones (www.intechpen.com; Grillo and Dulout, 1995). Phenolics, flavonoids, glutathione have been identified in the water hyacinth fractions. Water hyacinth therefore just like other marine organisms has shown presence of bioactive compounds with antioxidant activity. The flavonoids identified by phytochemical studies in water hyacinth may be responsible for antioxidant activity and they also have high bioavailability (Abd El-Baky et al, 2008).

1.14.4 Mechanism of antioxidant activity

To limit lipid oxidation, both synthetic and natural antioxidants can be used. The antioxidants control lipid oxidation by binding competitively with oxygen and retarding the initiation step. The antioxidants also minimise propagation of free radicals by binding or distruction and form

more stable forms of non-reactive antioxidant radicals. They also inhibit catalysts and stabilize hydroperoxides. When the antioxidants break the oxidative chain reaction by reacting with the fatty acid peroxy radicals to form stable antioxidants, the end products include non-reactive antioxidant radicals (Gardner, 1979, Howell and Saeed 1999, Jayanthi and Lalitha, 2012). Phenolic substances used as antioxidants can react with proteins in a manner that reduces ΔH values. In complex food systems ΔH is influenced by the environment and presence of other proteins and nutrients. The hydroxyl groups of the phenolic compounds hydrogen bond with protein groups exposed by heat denaturation. This reaction may accompany the prevailing protein-protein aggregation as the proteins denature and increase the exothermic contribution thereby reducing the enthalpy change DSC values.

1.15 Packaging and Storage

An area which is ignored in the dry chain Kenyan fishery is packaging; it is part of the dry fish value chain that extends shelf life of a product. Packaging facilitates distribution and display of the product information and provides consumer appeal (Bykowski and Dutkiewicz 1996). Packaging in the fishery industry encompasses a wide range of products from moulded polystyrene containers, paper, laminated cartons with polyethylene or aluminum foil, sheets of metal, metal foil and plastics including nylon polythene in retail. The packaging for fish reduces odour and makes it convenient to carry with other purchases (Bykowski and Dutkiewicz 1996).

From a food safety point of view, packaging prevents contamination of food and enhances food preservation by maintaining the atmosphere in a controlled or modified atmosphere package or a vacuum package or by preventing rehydration of a dried food. Packaging acts as a barrier for microbial agents, moisture and oxygen that would further cause deterioration of fish during storage therefore helps extend shelf life (FAO 2014). Packaging provides an opportunity in the fish value chain for the product information to be displayed. Fish is highly nutritious with the polyunsaturated fatty acids and proteins playing a key role in health. Nutritional information, may boost consumer confidence, improve sales to benefit fish traders.

Modified atmosphere packaging, controlled atmosphere packaging, reduced oxygen packaging and vacuum packaging have gained attention in the recent past (Hun, 2005; Robertson, 2013 Taheri and Motalebi, 2012). The most effective and widespread packaging method in use

currently is vacuum packaging that has been tried in the dry fish chain industry (Erkan, 2016; Ogongo et al, 2015;Ganguly and Kumar, 2014) where there is an increasing demand for high quality fish by consumers and targeted export markets. The fisheries sector in Kenya needs to be part of the improved fish value chain and implement packaging of dried fish products for improved quality and income for better food security and reduction of post-harvest losses.

One problem encountered after solar drying of fish is appropriate storage conditions. In many cases in Kenya, the dried fish is stored in jute sacks or baskets or just kept in the open. Storage in these conditions is unsatisfactory because biochemical and microbiological changes occur and affect quality (Verma et al, 1995; Sarkardei and Howell, 2007; Oduor-Odote and Obiero, 2009).

Little work has been done on storage of solar dried fish as most focus has always been on frozen fish with packaging and lately in the presence of certain antioxidants (Rodríguez et al (2009); Manju et al, 2007). Packaging under vacuum or modified atmosphere conditions is one of the major developments in food and fish packaging. Vacuum packaging delays spoilage while maintaining high quality, assures safety and reduces economic loss of fishery products (Goppal et al, 1999; Manju et al, 2007).) To date there are no studies reported on biochemical changes with emphasis on lipid oxidation during storage of solar dried *Siganids* in Kenya. When fish muscle is dried and stored, it absorbs water because of its hygroscopic nature and leads to biochemical deterioration. Thus, improved storage of dried fish products using normal polythene (thin layer, plane films) and vacuum packaging compared with non-packaged fish were investigated.

1.16 Conclusion

Countries have adopted drying as one measure of preservation of food and with the abundance of sunlight in Kenya, a solar tunnel dryer to produce a quality assured solar dried fish product and other products like vegetables can be adopted. Information on fish solar dryers and drying is to be developed in this study.

This study therefore investigated the design and fabrication of a solar tunnel hybrid dryer to dry *Siganus* fish in Kenya continuously in all weather conditions; its effectiveness in reducing the

microbiological load and biochemical quality changes, effect of natural antioxidants during storage, carbon footprint and the collection of data for use in HACCP development of solar dried *Siganid* fish.

1.17 Aims and objectives

1.17.1 Aim

The aim of this study was to design and fabricate a hybrid windmill solar-tunnel dryer and to evaluate the nutritional quality, quality costs, shelf life and safety including carbon footprint of the solar dried *Siganus sutor*.

1.17.2 The objectives

(i) Design and fabrication of a hybrid wind mill solar tunnel dryer in Kipini, Kenya
(ii) Evaluation of drying characteristics of Siganid fish product
(iii) Investigation of the microbiological changes and biochemical (protein and lipid oxidation) of solar dried *Siganids* during storage with or without natural antioxidants
(iv) Evaluation of rheological and thermodynamic properties of solar dried Siganids during storage
(v) Development of a quality management guide for HACCP and carbon footprint for solar dried *Siganids*

CHAPTER 2

CHAPTER 2

2.0 Construction and modelling the drying of fish in the hybrid windmill solar-tunnel dryer

2.1. Introduction

The aim of this study was to design a dryer for artisanal fisherfolk to use in villages on the Kenyan coast. Fishing is a major form of livelihood and traditionally fish is dried on rocks and sand which is unhygienic as it is prone to infestation and is dirty. As refrigeration is very expensive and many villages do not have adequate electricity, the aim was to build a dryer that would use solar heating during the day. At night and during the rainy season, to facilitate continuous drying, wind energy can be converted to electricity using a windmill on the coast. These sustainable forms of energy supplies are cheap and more accessible in remote villages and can make drying of fish and other produce possible in a safe hygienic way. The performance of the dryer will however be compared with existing drying models

Modelling is a tool for establishing the ability/availability of use of scientific tools and methods to predict what happens in real life scenarios. It is not possible to measure the temperature of the sun, because no one has ever reached the sun. Models have been developed to predict what is happening in the sun. In the same breadth, it may not always be possible to open a drying system and measure how the material is behaving. This opening interferes with the environment in the drying system. It is necessary to be able to predict all the parameters of the drying material and its environment, if we are to carry out commercial production as opposed to experimental procedures. This is useful in design of systems, where it is possible to use a certain model to predict what will happen, as a system is developed. It is also useful in monitoring the performance of systems, because any deviation from the expected behaviour as predicted by the model could be monitored and if a part of a system is failing, corrective action can be taken. The model for use in simulation can be developed from scratch or one can use an existing model. In this layer drying, several researchers have developed different models to predict the loss of moisture by a drying material under thin layer conditions. Thus, in most cases, some researchers in drying have opted to establish which of the models would best describe the drying of a certain material under certain drying conditions.

The process of establishing which model best describes or suits the drying of a certain material under certain drying conditions requires that a model is used to simulate data, and the data is compared with experimental or actual data from a certain drying environment. Thus in this case, 18 models are used to generate data. The data generated by each model is compared with the actual data using drying coefficient (R^2), Root Mean Square Error (RMSE) and χ^2 as tools for evaluating the comparative performance of the model against actual data. The requirement is that the model with the highest value of R^2, and the least values of RMSE and χ^2 is the best model. This is the criteria used in determining the best performing model for the dryer.

2.2. Materials and methods

2.2.1 Dryer construction at Kenya Marine and Fisheries Research Institute (KMFRI)

The dryer was designed and constructed as described below. The components were the solar collector, the drying chamber, the hot air chamber, the roof of the dryer, a photovoltaic system, a windmill with a wind generator, storage batteries, charge controllers, solar panels, a DC coil, water recirculation pump, solar panels, solar heating, panels for water, insulated water reservoir tank, copper tubing and wiring and plumbing accessories.

2.2.1.1 Solar Collector

The solar collector was constructed as the main direct heat generation section of the dryer. The collector had 3 parts namely drying chamber, hot air chamber and the dome shaped roof.

The body of the collector was 9.75 m long, 0.9 m high and 1.2 m wide. The upper part was covered by the dome shaped roof and the lower part was made of insulation block made of metal tube frames and wooden blocks(board).

2.2.1.2 The body of the collector

2.2.1.3 The frame

The main frame was constructed by welding using 5.08 cm (2 inch) 2.54 cm by (1 inch) hollow black metal tubes and 5.08 cm (2 inch) angle bars (Figure 2.1). The angle bars provided support

for the floor boards and side boards. They were joined to give a total length of 9.75m (32 feet) long divided into 4 partitions of 2.4 m (8 feet) by 1.2 m (4 feet each excluding the inlet and outlet chutes. A 2.54 cm (1inch) by 0.96 cm (3/8th inch) angle bar was welded at the base of the main frame of the dryer to support the side boards to be introduced.

Figure 2.1: Construction of the main frame of collector for the hybrid windmill solar tunnel dryer with the boards and aluminium sheets

2.2.1.4 The boards

The insulation boards were made of two 3 mm triply sandwiching 50 mm styropol. The top triply was laminated with a 0.3 mm alluminium sheet and a 3mm triply at the bottom laminated with a 32 gauge galvanized sheet (Figure 2.1). The sides were bound by a 2.5 mm by 5 mm timber. The walls of the dryer were covered with insulated blocks made of two 3 mm plywood sandwitching a 20 mm styropol and the inside triply was laminated with a 0.3 mm aluminium sheet on the inside and the outside triply was laminated with a 0.3 mm galvanized sheet on the outside. The blocks were were bound using a 25 mm angle bar and 2.54 cm (1 inch) flat

2.2.1.5 The drying and hot air chamber

The wall of the drying chamber had a height of 0.8 m, 2.4 m length by 1.2 m width with provisions for the drying trays (Figure 2.2). The hot air chamber is basically for heating the ambient air mainly by the solar collector. The chamber was 2.13 m long 1.2 m width and 0.9 m high (including the stands). The upper part was covered by the dome shaped roof and the lower part was covered by an insulation board at the base and walls made of two 3 mm triply sandwiching 50 mm styropol. The inner triply was laminated with a 0.3 mm alluminium sheet and a 3 mm triply at the outer laminated with a 32 gauge galvanized sheet. The sides were bound by an angle bar of 3.50 cm (1⅜ inch) by 0.32 cm (⅛ inch).

Figure 2.2: a) The collector and hot air chamber b) Drying chamber

The drying chamber was fabricated in the same way as the hot air chamber and was at the extreme end of the dryer and had a height of 0.8 m with provisions for the drying trays. At one end of the drying chamber was attached the solar collector and at the other end was the chimney allowing for wet air exhaust. The chamber allowed for two drying trays. To enhance the heat distribution in the drying chamber, provision was made to allow for air exhaust at the bottom and upper part of the dryer.

2.2.1.6 The roof of the dryer

Materials: Flat bar of 3.8 cm by 0.42 cm 1/6th inch (1 ½ inch by 1/6 inch), galvanized wire gauge 20, bolts and nuts M10 7.62 cm long (3 inches), drill bit M10, timber 5.08 cm by 2.54 cm (2 inches by 1 inch) 19.5 m (64 feet) each 2.44 m long(8 feet); timber of 2.54 cm by 2.54 cm (1inch by 1inch) 21.9 m each 2.43 m long (72 feet each 8 ft long), tough bond or Contac

glue (HENKEL) 4 litres, wire nails 5.08 cm (2 inch), quantity 1 kg, U V stabilized polythene 200 µ gauge (AMIRAN), silicon tube (black), 5 pieces, roofing nails.

Roof design

The main frame of the roof was made of flat bars reinforced with galvanized wire. The roof was made into a dome shape to enhance drainage during the rainy season. As a first step, the width of solar dryer collector area was measured to help determine the mid- point or the ridge. The ridge or the apex of the dome was 10.0 cm (4 inches) high.

The width of the dome shaped roof was measured by taking the total length across from one side of the dome roof to the other side and considering the 10 cm (4 inch) height of the apex. An extra 10 cm (4 inches) and 5 cm (2 inches) on each side) was added to the total width of the dome shaped roof. This was equivalent to the total length of the flat bar used to make the roof of the dryer (Figure 2.3). The width of the entire dryer roof therefore consisted of full length from one side of the dryer to the other including the apex; a total of 2 ½ bars of 5.5 m (18 feet) length each were used

Figure 2.3: The collector roof of the dryer: Before (a) and after (b) roof installation

The flat bars were cut into desired length using a hacksaw (Raider Ultra). A drill machine (Makita from Germany) with drill bit M10 was used to make holes that fit M10 bolts and nuts 7.6 cm (3 inches) long on both ends of each flat bar. The flat bars were fastened on to the wall of the dryer to form the dome shaped roof and painted with red oxide metal paint as an undercoat to avoid rust, followed by black board paint. Some pieces of 5 cm (2 inches) by 2.5 cm (1 inch) timber measuring 10 cm (4 inches) in length were cut and firmly fixed on the existing stands of the dryer to provide support to the 5 cm by 2.5 cm (2 inches by 1inch) timber rail which ran lengthwise and all-round the upper layer of the wall of the dryer and just at the base of the dome

dryer roof. The timber measuring 5.08 cm by 2.54 cm (2 inch by 1 inch) timber was fixed to the wall of the dryer using 5.08 cm (2 inch) wire nails. The timber was painted black using black board paint. The galvanized wire (20 gauge) was then looped over the flat bars at intervals of 0.3 m (1 foot) each throughout the entire length of the collector.

The stabilized UV polythene (200 µm gauge, AMIRAN Kenya) was then laid on top of the frames made of the pieces of flat bar and galvanized wire to prevent the UV polythene from sagging. The overlapping UV polythene was then rolled on to 2.54 cm by 2.54 cm (1 inch by 1 inch) timber and Contac glue was used to further firmly fix the polythene on to the 2.54 cm by 2.54 cm timber (1 inch by 1 inch). The 1 by 1 timber with the polythene rolled was then then fixed onto the 5.08 cm by 2.54 cm (2 inch by 1inch) timber rail that was running lengthwise and all-round the dryer using iron sheet roofing nails to help provide base support for the UV polythene. Any open spaces below the roof or in the dryer were sealed with silicon (Pattex, Smart Universal in 280 ml tubes) using a standard silicon gun to make the chamber air tight and to prevent any heat loss. The entire wall of the dryer was then painted black using black board paint to maximize heat absorption.

2.2.1.7 The lower chute

The lower end chute measured 60 cm by 124.5 cm at the dryer connector and narrows to a 50 cm vertical square end after a length of 1 m at the chute connector. It was made of 0.2 cm mild steel sheets painted silvery on the inside and black on the outside.

2.2.3 Installation of photo voltaic part

2.2.3.1 Power Source

The electrical power required by the dryer is for running the two 12V DC fans at day time. The power was obtained from a 100 W solar panel and was stored in 100 Ah deep cycle battery. Figure 2.4 shows the circuit layout for the power system. The power circuit ensured that the fans could be operated independently and simultaneously. Provision was also made for other power needs like lighting. The charging of the battery was controlled by a 30 A charge

controller. Figure 2.5 shows the circuit layout for the power system. The following are the considerations that are followed in operating the control panel in the power source:

- The power switches should always be maintained off whenever the dryer is not in use.
- However, the connection between the battery and the solar module should always be on.
- The electrolyte level in the battery should be checked before operation to ensure that it has.

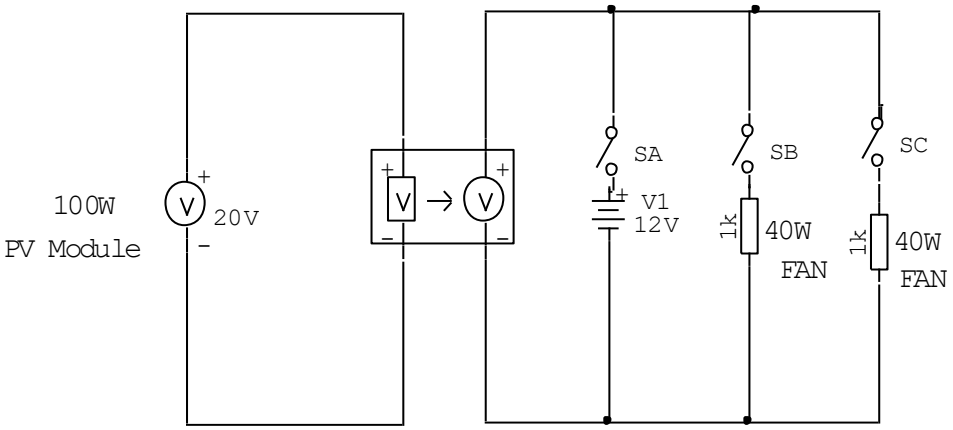


Figure 2.4: Circuit layout of the power supply system

- The LED indicator on the charge controller (Figure 2.5) has the following colorations, which should be noted, to indicate the level of charge (voltage) in the power system: Red for low charge, amber for medium charge and green for fully charged capacity. If the LED indicator is red, this indicates low charge capacity in the battery. It is thus prudent to use the solar panel in directly running the fans. Hence switch A is maintained open. If the LED indicator is amber in colour, it means that the level of charge in the battery is at a medial level. So, it is the prerogative of the operator depending on the availability of sunshine to either use the solar panel or the battery to run the power to the fans. In case of low sunshine, it is recommended that the battery be used while in cases of maximum sunshine it is advisable to save the battery. If the LED indicator is green in colour, it means that the operator can use either the battery or the solar module to run both fans since there is adequate power supply.
- When no drying is in progress, switches B and C should be maintained open while switch A is closed to facilitate the charging of the battery.
- At times of a low drying load inlet fan is the one to be operational, thus switch A and B should be open. This facilitates the charging of the battery in conjunction with the inducement of air into the collector tunnel to dry the fish.

- The operator should ensure that the panel is free from dust accumulation before the system is started. This ensures that there is efficient conversion of solar rays into electrical power.
- The outlet plug from the control panel to the dryer should be fitted into the outlet socket (See Figure 2.5) to facilitate transfer of power from the control panel to the dryer unit. The control panel can then be switched on.

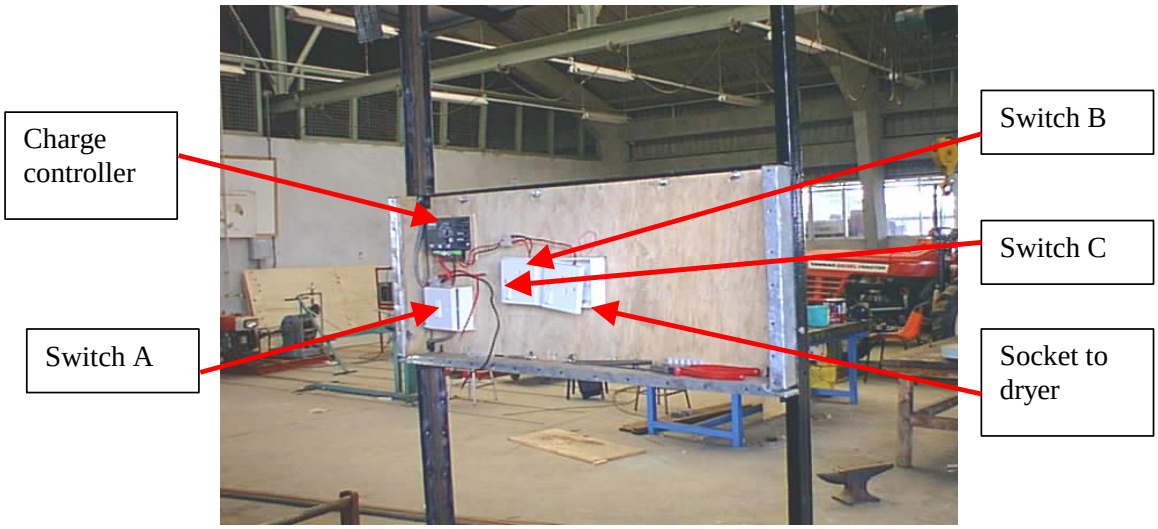


Figure 2.5: Locations of switches A, B and C and the charge controller .

2.2.3.2 DC heating coil

The DC heater (ELAC, South Africa) was placed in the area before the drying chamber. It comprises of a DC breaker for protection, a thermostat for temperature regulation, a 400 W DC element and a blow fan (Figure 2.6). The heater operates on a 12 Volts DC supply. The thermostat is used to set and regulate the amount of heat dissipated by the heater. Once the set temperature has been attained, the thermal switch turns off the heater until the temperature drops below the set value. The cycle is repeated indefinitely.

Figure 2.6: DC coil glowing with heat in drying chamber of the hybrid windmill solar tunnel dryer in Kipini, Kenya

2.2.4 The windmill system

This consists of wind generator, wind controller, battery bank, inverter, associated controls and mounting.

2.2.4.1 The mast

This is a heavily fortified galvanized steel tower of approximately 10 m height on which the wind generator is mounted (Figure 2.7).

2.2.4.2 The wind generator

The wind generator makes a rotary motion when it is windy to generate a 3-phase power supply which is then passed on to the wind controller. The wind controller in turn converts the AC power from wind into DC power which is suitable for battery charging. It also assists in regulating battery charging to protect the battery from overcharging.

Figure 2.7: The wind generator about to be hoisted and the windmill in operation for the hybrid windmill solar tunnel dryer in Kipin, Kenya

2.2.4.3 The battery bank

The battery bank (Figure 2.8) acts as the power storage and supply current to the inverters, fans and the D.C coil. The inverter provides alternating current (A.C) to serve the circulation pump and the community

Figure 2.8: The battery bank and inverter for the hybrid windmill solar tunnel dryer in Kipini, Kenya

The solar panels substitute the battery charging during day time. A charge controller protects the batteries from overcharging. The associated controls include isolating switches for different sub circuits and a changeover switch for selecting either wind power or solar power.

To facilitate DC current transmission a 10.0 mm² armoured cable was laid from the wind generator to the power control unit built near the solar tunnel dryer. Electrical wiring to the fans and the D.C coil in the drier were shortened thus reducing voltage drop. The whole dryer system is shown in Figure 2.9.

Figure 2.9: The drying system of the hybrid windmill solar tunnel dryer in Kipini showing the tunnel dryer, drying chamber, windmill and hot water system

AC power was also supplied to some houses of the local community members to provide lighting (Figure 2.10).

Figure 2.10: Community house provided with electricity from the hybrid windmill solar tunnel dryer in Kipini

The community members mainly female were trained on how to use the dryer and on how to utilize the dryer to dry other products like mangoes during seasons when fish catches are low (Figure 2.11).

Figure 2.11: Members of Kipini BMU drying fish and other farm produce like mangoes during low fishing season in the hybrid windmill solar tunnel dryer in Kipini, Kenya

2.2.5 Drying of *Siganids* and drying characteristics

2.2.5.1 The preparation of salted and unsalted the fish samples

Siganid fish (Tafi) was purchased from the Shimoni fish landing site in the south coast of Kenya, which is 100 km from KMFRI offices in Mombasa. The fish were eviscerated, thoroughly washed and stored in ice boxes with ice then taken to KMFRI offices where they were kept overnight, before being further transported to Kipini drying site, 240 km on the North

Coast of Mombasa. At Kipini, the fish were filleted and washed thoroughly, before being cut into samples, approximately 5 mm thick. Three pieces of fish were selected for the determination of initial moisture content of the fish before drying. A minimum of 270 pieces were prepared and divided into two sets of samples, each containing at least 135 pieces. One sample batch was soaked in 5% brine (sodium chloride) and the other in 0% concentration (water), for 12 hours. In addition, two fish samples were prepared by eviscerating and filleting of fish. One of the sample was brined while the other was soaked in water, for 12 hours. These two samples were used to measure the weight loss of the fish as drying progressed (Oduor-Odote et al 2015)

2.2.5.2 The fish drying process

After sampling for initial moisture content, the rest of the fish were spread in the same tray such that no sample was placed on top of another, which is typical of thin layer drying. The arrangement of the samples was such that air that has been used to dry a sample, would not be used to dry the next sample. The trays were then placed in the dryer for the fish samples to dry. The drying process was monitored by weighing the two samples every 2 hours using a cell powered electronic balance (Decker, USA) at two-hour intervals from the start of the experiment. This was continued until there was no noticeable change in weight of these samples, when the experiment was stopped.

To analyse the drying process, samples were selected randomly from each treatment at the start of drying, and subsequently at two-hour intervals. The samples were picked in triplicate at any sampling time. Since there was no equipment for detailed analysis of the samples at the experimental site, the samples were weighed to determine their wet weight ($\mathbf{W}_w$). They were then placed and wrapped in properly labelled pieces of aluminium foil, and placed in airtight seal-lock plastic bags, which were subsequently placed in ice boxes to reduce biological activity in the samples. The sampling was repeated at 2-hour intervals, until the end of the experiment. The samples were then taken to the Kenya Marine Fisheries Research Institute (KMFRI) laboratories (300km) in Mombasa, where they were placed in an air-oven at a temperature of 105^{o}C for 24 hours and moisture determined according to AOAC methods (1990). Upon removal of the samples from the oven, they were cooled in a desiccator, dry weights, W_d corresponding to the recorded wet weights W_w, were recorded. The dry basis moisture content

M for each sample was determined using equation 1. Based on the Handerson and Pabis thin layer drying model and according to observations by Kingsly *et al.* (2007) and Uluko *et al*. (2006) for material drying under varying range of relative humidity, which is typical of solar drying, the moisture ratio (MR) was determined using equation 2. In this equation M_o, k and t are the initial moisture content on dry basis (kg/kg, d.b), the drying rate constant recorded per second and the drying time in seconds, respectively. Based on the analysis, moisture ratio drying curves were developed.

$$M = \frac{W_w - W_d}{W_d} \tag{1}$$

$$MR = \frac{M}{M_o} = Ae^{-kt} \tag{2}$$

The partial differential equation for moisture diffusion can be written as in equation 3, in which D_f is the effective diffusivity. In addition, the solution to equation 3, for thin layer drying of fish in a tray is as given in equation 4. In which d is half thickness of the drying fish (Vasić *et al*., 2012).

$$\frac{\partial M}{\partial t} = D_f \nabla M \tag{3}$$

$$MR = \frac{8}{\pi^2}\sum_{n=0}^{\infty}\left(\frac{1}{2n-1}\right)^2 \exp\left(-(2n-1)^2\left(\frac{\pi}{d}\right)^2 D_f t\right) \tag{4}$$

In typical drying processes, the value of t is large (Sacilik and Unal, 2005), the first term of the series shown in equation 4 obtained by considering only first term of the series n = 1 and neglecting the higher term is then used to evaluate moisture ratio as shown in equation 5 (Mudgal and Pande, 2007; Hassini, 2006). Then based on equations 2 and 5, the effective diffusivity of the *Siganid* fish can be evaluated as shown in equation 6.

$$MR = \frac{8}{\pi^2}\exp\left(-\left(\frac{\pi}{d}\right)^2 D_f t\right) \tag{5}$$

$$D_f = k\left(\frac{d}{\pi}\right)^2 \tag{6}$$

2.2.5.3 Modelling thin layer drying of fish

The actual moisture ratio data obtained from the experiment was fitted to thin layer drying models to get the best model fitting the experimental drying data. In total, 18 models (Table 1) were considered as cited by Barbosa *et al.* (2007), Gunhan *et al*. (2005) and Alibas (2012). In order to obtain the best model based on the two sets of data, the *solver* function in excel spreadsheet (MS Excel 2008^{TM}) was used to optimise the sum of the difference of the squares between the simulated and actual data, for a minimum value. The values obtained were taken through the various statistical measures of goodness of fit. In Table 2.1, the parameters a, a_o, b, c, g and h are coefficients, n is drying exponent, k, k_o, k_1, k_2 are drying rate coefficients specific to each equation, t is drying time and L is the thickness of the drying sample

Table 2.1: Mathematical thin layer drying models used to model the drying of fish in the hybrid windmill solar tunnel dryer in Kipini, Kenya

S/No	Model Name	Equation
1	Lewis	$MR = \exp(-kt)$
2	Page	$MR = \exp(-kt^n)$
3	Modified page	$MR = \exp(-kt)^n$
4	Handerson and Pabis	$MR = a\exp(-kt)$
5	Yagcioglu et al (Logarithmic)	$MR = a\exp(-kt)$
6	Two-term	$MR = a\exp(-k_1t) + b\exp(-k_2t)$
7	Two term exponential	$MR = a\exp(-kt)+(1-a)\exp(-kat)$
8	Diffusional approach	$MR = a\exp(-kt)+(1-a)\exp(-kbt)$
9	Verma et al	$MR = a\exp(-kt)+(1-a)\exp(-gt)$
10	Modified Handerson and Pabis	$MR = a\exp(-kt)+b\exp(-gt)+c\exp(-ht)$
	Simplified Fick's diffusion Equation	$MR = a\exp\{-ct/L^2\}$
12	Modified page equation-II	$MR = a\exp\{-c(t/L)^2\}$
13	Midilli and Kucuk	$MR = a\exp(-kt^n) + bt$
14	Weibul Distribution	$MR = a - b\exp(-kt^n)$
15	Logistic	$MR = a_0 / 1+(\exp(-kt))$
16	Jena and Das	$MR = a\exp(-kt + b\sqrt{t})+c$
17	Demir et al.	$MR = a\exp(-kt)^n+c$
18	Alibas	$MR = a\exp(-kt^n+bc)+c$

Source: Barbosa *et al.* (2007), Gunhan *et al*. (2005) and Alibas (2012)

2.2.5.4 Measures of performance of the models

If y_i is an observed value, and has an associated modelled value f_i then, the mean of the observed data, $\bar{y}$, for a total of N number of observations, is given by equation 7.

$$\bar{y} = \frac{1}{N}\sum_{i=1}^{N} y_i \tag{7}$$

In addition, the coefficient of determination, R^2, can be used to test the linear relationship between measured and modelled values, as computed in equation 8.

$$R^2 = \frac{\sum_{i=1}^{N}(y_i - \bar{y})^2 - \sum_{i=1}^{N}(y_i - f_i)^2}{\sum_{i=1}^{N}(y_i - \bar{y})^2} \tag{8}$$

The root mean square error, RMSE always positive represented as "zero" in an ideal case, provides information on the short-term performance and may be computed from equation 9.

$$RMSE = \sqrt{\frac{1}{N}\left(\sum_{i=1}^{N}(f_i - y_i)^2\right)} \tag{9}$$

The reduced chi-square, χ^2, may be calculated as in equation 10, where n is the number of constants in the equation. The lower the values of the reduced χ^2, the better is the goodness of fit.

$$\chi^2 = \frac{\sum_{i=1}^{N}(y_i - f_i)^2}{N - n} \tag{10}$$

These parameters were used in the evaluation of the goodness of fit of the thin layer drying models to the drying data.

2.3 Results and discussion

2.3.1 Performance of the fabricated dryer in the drying of fish

The relationship between the moisture content of salted and unsalted *Siganid* fish and drying time as well as dryer and ambient temperature is presented in Figure 2.12. The figure shows that the moisture content for the drying *Siganid* fish under the two treatments reduced as the drying time increased. The figure further shows that the moisture loss was exponential.

This trend has also been reported by Guhnan *et al*. (2005), Alibas (2012) and Kituu *et al*. (2014). As illustrated by Figure 2.12, the reduction in moisture content for the fish under the two treatments shows similar trends. However, the figure shows that the moisture ratios for the unsalted fish reduced relatively fast compared to the salted fish, which agrees with the observations made by Graivier et al. (2006) and Jittinandana (2002).

The reduced drying associated with the salting of fish could be attributed to the binding of moisture by salt in the fish flesh. On the other hand, a two way statistical *Student's t- test* did not show the existence of any significant difference between the moisture contents of the salted and unsalted fish during drying (t_{stat}=*-1.4832*, $t_{crit,\ 5\%}$=*2.0686*).

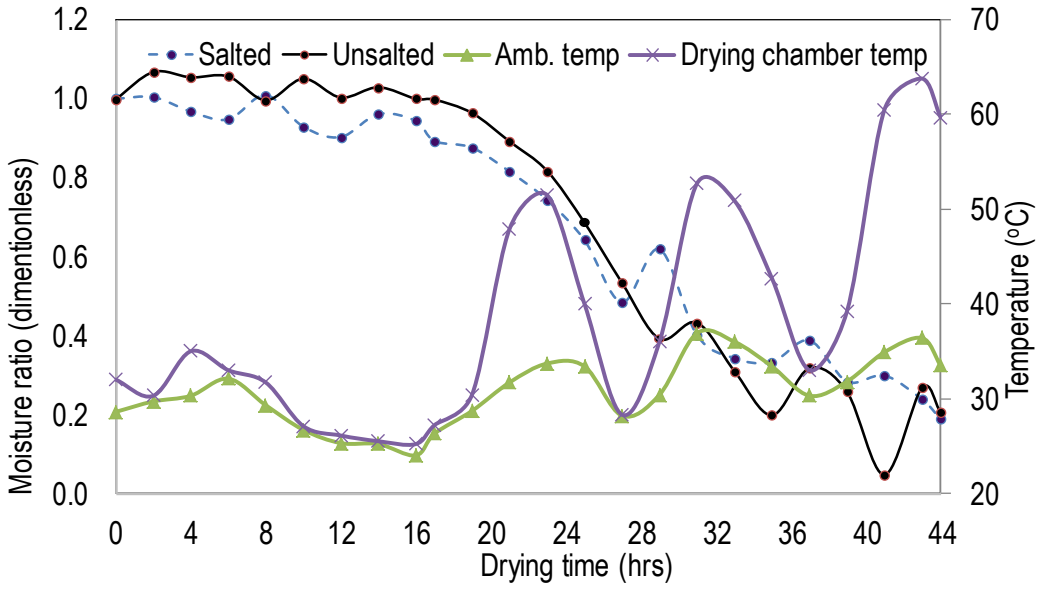


Figure 2.12: Variation of moisture ratio, solar dryer and ambient temperatures during drying of Siganid fillet in the hybrid windmill solar tunnel dryer in Kipini, Kenya.(Amb = Ambient temperature, Drying chamber temp= Drying chamber temperature)

As observed from Figure 2.12, the harnessing of energy by solar tunnel dryer can be demonstrated by the high temperatures observed in the drying chamber, compared to the ambient temperatures. The figure further shows both the ambient and drying chamber temperatures increased, particularly the latter. However, despite the higher temperatures developed in the drying chamber, a two-way statistical analysis did not show the existence of significant difference between the ambient and drying chamber temperatures over the entire drying period which was a cloudy day ($t_{stat}=9.6689\times10^{-16}$, $t_{crit,\ 5\%}=2.0687$). In addition, the mean temperatures in the drying chamber were not high enough to cause protein denaturation of the fish proteins.

Figure 2.13 presents the variation of ambient and plenum chamber relative humidity during the drying period. The Figure shows a high ambient relative humidity, above 25%, throughout the drying period. This is characteristic of relative humidity along the Kenyan coast, due to the high water vapour in the atmosphere. The high levels of ambient relative humidity must have reduced the drying rate of fish and this can lead to prolonged drying periods, and consequently to spoilage of fish. In addition, for the first 18 drying hours, the ambient and drying chamber relative humidity values were almost indistinct.

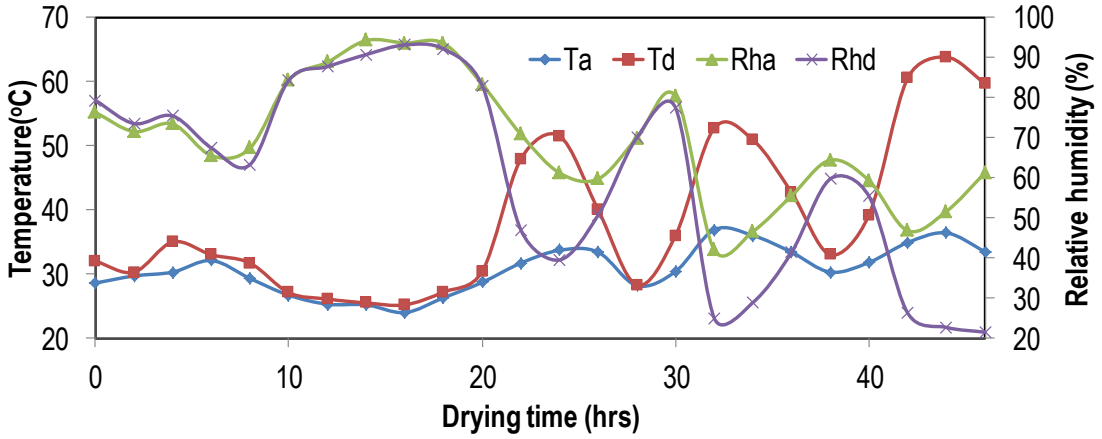


Figure 2.13: Variation of ambient and drying chamber temperatures and relative humidity with drying time in drying of Siganid fillet in the hybrid windmill solar tunnel dryer in Kipini, Kenya (Ta=Ambient temperature, Td= Dryer temperature, Rha= Ambient relative humidity and Rhd=Relative humidity inside the dryer

Since humidity is related to air temperature, this behaviour is attributed to the ambient and drying chamber temperatures. The temperatures were low, demonstrated similar trends, and were almost indistinct within the stated drying period. However, beyond the 18th drying hour, the ambient temperatures increased, and subsequently, the drying chamber temperature increased. However, the increase in drying chamber temperature was much more than the increase in ambient temperature, which demonstrated energy harnessing by dryer. The increase

in the temperatures must have resulted in reduction of the ambient and plenum chamber relative humidity values, and hence improving the ability of the system to dry the fish to a better quality. This is because lower air humidity results in increased moisture absorption potential (Mujaffar and Sankat, 2005). A two-way statistical Student's *t*-test showed a significant difference between ambient and drying chamber relative humidity ($t_{stat}=7.2543$, $t_{crit,\,5\%}=2.0687$).

This further augments the view that the solar dryer was able to harness solar energy for drying. The high solar dryer temperatures must have resulted in significant reduction in the relative humidity in the dryer compared to the ambient humidity resulting in increased drying potential for the solar tunnel dryer. This agrees with the observation by Sahoo (2012) who noted that relative humidity reduces exponentially as air temperature increases. The safe moisture content for fish brined in 0 to 15% salt are 0.15 and 0.35 kg/kg, on a dry basis (db) respectively (Brugay *et al*, 2003). The final moisture content of 0.2kg/kg, db was shelf stable for dried fish. The drying of the fish to stable values of moisture content was due to higher chamber temperatures in the dryer, which must have resulted in better drying conditions for the *Siganid* fish.

Figure 2.12 shows the relationship between moisture ratio and drying time for the brined and non-brined *Siganid* fish. The drying equations with respective R^2 values are as shown in equations 11-12. In these equations, MR_s and MR_{us} are the moisture content values for salted and unsalted fish samples, respectively. It is seen from the results that there was an exponential decrease in moisture ratio with drying time and a strong correlation existed between moisture ratio and drying time with high R^2 (0.7192-0.8733). The drying rate constant (k) values ranged from 0.037-0.05 per hour for the salted and unsalted *Siganid* fish. However, the values decreased with increase in brine concentration. This implies that an increase in salt concentration binds water to the fish flesh, and this makes it unavailable for drying.

$$MR_{us} = 1.676\exp(-0.05t), \quad R^2 = 0.7192 \tag{11}$$

$$MR_{s} = 1.3697\exp(-0.037t), \quad R^2 = 0.8733 \tag{12}$$

Based on the equation 6, the drying rate constants and the thickness of the materials, values of the effective diffusivity (D_f) were 6.53 x 10^{-12} and 6.63x 10^{-12} m^2/s for the salted and unsalted *Siganid* fish, respectively.

The Diffusivity is calculated in reference to equations 2 and 6 which are considered to be similar. Therefore A in equation 2 is equated to 8/π2 in equation 5. Note that equation 2 can be rewritten as $MR = Aexp(-kt)$

Therefore the terms in the last brackets can be written as

$$MR = Aexp(-kt) = \frac{8}{\pi^2} exp\left(-\left(\frac{\pi}{d}\right)^2 D_f t\right)$$

From this equation it clear that

$$k = \left(\frac{\pi}{d}\right)^2 D_f$$

Or

$$D_f = \frac{kd^2}{\pi^2}$$

The value of k is determined by developing equations from the graphs. These equations are given as equations 11 and 12. From these equations, the values of **A** and **k** for both salted and unsalted conditions were determined as the values before the t in the exponential (exp) terms. The parameter d in equation 4, 5 and 6 is given as half of the thickness of the fish. In section 2.2.5.1. it is indicated that the fish samples were cut into pieces of approximately 5mm thickness. Thus ***d*** would take the value of 2.5mm or 2.5×10^{-3}m. Thus with the values of ***k*** and ***d*** known, then $\boldsymbol{D_f}$ could be determined.

These values further augment the observation that the unsalted samples dried much faster than the salted samples. The low values of R^2 of 0.7192 and 0.8733 for unsalted and salted fish, respectively, indicated that the Lewis model may not be the best model that describes the drying of *Siganid* fish in the hybrid windmill -solar tunnel dryer. It was therefore necessary to evaluate the thin layer drying model that best describes the drying of *Siganid* fish in this dryer.

2.3.2 Modelling of the drying of fish in the dryer

The performance parameters of the various thin layer drying models in Table 2.1 were as shown in Table 2.2 with actual values. The various magnitudes of the performance parameters used to test the performance of the models are shown in the table. Based on R^2, *RMSE* and reduced χ^2, the table further shows that different models performed differently, in modelling the drying

of fish in this dryer. Based on the performance criteria, where the best performing model would have the highest value of R^2, and least values of RMSE and reduced χ^2, for salted fish, models 2, 9, 12 and 16 had the highest R^2 values at 0.9665, 0.9432, 0.9665 and 0.9154, respectively. Similarly, using RMSE, the same models had values the least values at 0.0539, 0.0691, 0.0539 and 0.0843, respectively. Thus, based on R^2 and RMSE, models 2 and 12 were the best performing models. In addition, based on χ^2, the models had the best performance at 0.0032, 0.0055, 0.0033 and 0.0081, respectively.

The page model is of the form

$$MR = \exp(-kt)^n \quad (5)$$

This equation can be rewritten as

$$MR = \frac{1}{\exp(kt)^n} \quad (6)$$

This implies that for the same value of t, a material with high value of k would have lower values of MR. This implies that the higher the value of **k**, the lower the rate of loss of moisture. This accounts for the reason as to why the unsalted fish has a lower value of $k = 4.78\times10^{-6} hr^{-1}$, and losses moisture faster than the salted material with a higher value of $1.0573\times10^{-4}\ hr^{-1}$.

Thus, the best model for the drying of the salted fish in the hybrid windmill solar tunnel dryer was model 2, with the highest R^2, and the least RMSE and reduced χ^2. The trend was similarly observed for unsalted fish, in which the models 2, 9, 12 and 16 had the highest values of R^2 (0.9434, 0.9228, 0.9434 and 0.8940, respectively), lowest values of RMSE (0.0840, 0.0980, 0.0840 and 0.1148, respectively) and χ^2 (0.0077, 0.0110, 0.0081 and 0.0151, respectively).

Since model 2, the Page model, had the highest of R^2 and least RMSE and reduced χ^2 in the drying of unsalted fish in the hybrid windmill solar tunnel dryer, it was considered the model that describes the drying of unsalted *Siganid* fish in this dryer. Thus, it can be inferred that model 2, the Page model, best describes the drying of salted (5%) and unsalted *Siganid* fish drying in the hybrid windmill-solar tunnel dryer. A two-way statistical analysis did not show a significant difference between modelled and actual moisture ratio for salted ($t_{stat}=8.0267\times10^{-2}$, $t_{crit,\ 5\%}=2.0687$) and for unsalted ($t_{stat}=2.8740\times10^{-5}$, $t_{crit,\ 5\%}=2.0687$) at 5% level of significance.

Table 2.2: Model performance parameters of various thin layer drying models in Table 2.1

S/No	Model parameter Values		R^2		RMSE		χ^2	
	Salted	Unsalted	Salted	Unsalted	Salted	Unsalted	Salted	Unsalted
1	k=0.0213	k=0.0215	0.7616	0.6549	0.1417	0.2079	0.0210	0.0451
2	k=1.05725×10^{-4}, n=2.5664	k=4.7870×10^{-6}, n=3.4946	0.9655	0.9434	0.0539	0.0840	0.0032	0.0077
3	k=0.1459, n=0.1459	k=0.1466, n=0.1466	0.7616	0.6540	0.1417	0.2079	0.0219	0.0471
4	a=1.1819, k=0.0277, n=1	a=1.2882, k=0.0310, n=1	0.8327	0.7736	0.1186	0.1678	0.0153	0.0307
5	a=1.1819, k=0.0277, n=0	a=1.2882, k=0.0310, n=0	0.8327	0.7736	0.1186	0.1678	0.0161	0.0322
6	a=0.5910, k_1=0.0277, b=0.5910, k_2=0.0277	a=0.6441, k_1=0.0310, b=0.6441, k_2=0.0310	0.8327	0.8557	0.1186	0.1678	0.0169	0.0338
7	a=1, k=0.0213	a=1, k=0.0215	0.7616	0.6540	0.1417	0.2079	0.0219	0.0471
8	a=1, k=2.1277×10^{-2}, b=1.0037	a=1, k=2.1485×10^{-2}, b=1.0037	0.7616	0.6540	0.1417	0.2079	0.0201	0.0432
9	a=5.0488, k=0.0594, g=0.0816	a=8.0298, k=0.0714,g=0.0907	0.9432	0.9228	0.0691	0.0980	0.0055	0.0110
10	a=0.3940, k=0.0277, b=0.3940, g=0.0277, c=0.3940, h=0.0277	a=0.4294, k=0.0310,b=0.4294, g=0.0310, c=0.4294, h=0.0310	0.8327	0.7736	0.1186	0.1678	0.0188	0.0376
11	a=1.1820, c=0.1824, L=2.5668	a=1.2882, c=0.2054, L=2.5725	0.8327	0.7736	0.1186	0.1678	0.0161	0.0322
12	c=0.01734, L=2.7009, n=2.5664	c=0.0065, L=2.8063, n=3.4962	0.9655	0.9434	0.0539	0.0840	0.0033	0.0081
13	a=1.3840, k=0.1401, n=0.5372, b=0	a=1.4307, b=0.0617, n=0.8233, b=0	0.6389	0.7085	0.1748	0.1906	0.0367	0.0436
14	a=0.6754, b=0, k=1.0958, n=1.0791	a=0.6904, b=0, k=1.1109, n=1.0935	0.00	0.00	0.2899	0.3527	0.1009	0.1493
15	a=0.1289, k=0308	a=0.1802, k=0.0349	0.8034	0.7181	0.1286	0.1872	0.0180	0.0382
16	a=0.8604, k=0.0612, b=0.2368	a=0.8130, k=0.0841, b=0.3450	0.9154	0.8940	0.0843	0.1148	0.0081	0.0151
17	a=1.1819, k=0.1664, n=0.1664, c=0	a=1.2882, k=0.1762, n=1, c=0	0.8327	0.7736	0.1186	0.1678	0.0169	0.0338
18	a=0.3387, k=1.9999, b=0, c=0.6612, n=4.3963	a=0.3218, b=1.9999, c=0, n=4.4121	0.0545	0.0332	0.2819	0.3468	0.1004	0.1519

2.3.3 Drying temperatures in the hybrid windmill solar tunnel dryer at night

The drying temperatures recorded were as shown in table 2.3 and figure 2.14; they indicated that a higher temperature inside the dryer were recorded.

Table 2.3: Humidity, temperature and wind speed at ambient conditions and inside the the hybrid windmill solar tunnel dryer system at night

Conditions		Dryer condition			Ambient air temperatures, humidity and wind speed		
	Time	Temp	Humidity	W/Speed	Temp	Humidity	W/Speed
All systems on, cloudy weather	9.00 am	30.3	81.6	0	28.8	75.2	2.5
Conditions	10.00am	35.9	54.1	0.4	30.4	67.6	0.8
	11.00am	39.2	47.3	0.3	32.1	67.1	1.1
	12.00 am	39.2	51.2	0.4	31	68.9	2
	1.00pm	38.3	64.4	0.6	29.3	64.4	0.5
	2.00pm	33.6	66.2	0.6	31	71	2.2
	3.00pm	30.9	66.9	0.3	28.9	76	2.6
	4.00pm	29.6	75.9	0.4	27.8	75.9	0.9
	5.00pm	27	78.3	0.2	27.1	81.1	0.5
	6.00pm	26	82.6	0.4	26.4	79.7	0.9
Only the heater is on	7.00pm	27.3	76.9	0.6	26.6	77.7	1.4
	8.00pm	30.8	72.5	0.8	26.4	78.6	2.4
	8.15pm	34.2	66.6	0.8	26	81.2	2
	8.30pm	34.8	60.5	1.1	25.6	84.3	1.9
	8.45pm	35.2	51.3	1.2	25.2	80	2.5
	9.00pm	29.9	73.3	1.5	25	86	2.3
Heater switched off	9.15pm	28.6	73.9	0.9	24	85.1	2.8
	9.30pm	26.6	76.8	1.6	23.3	86.6	3

It is noticed that when the heating coil was in use at night, the temperatures in the drying chamber from 7 pm to 9 pm ranged between 27.3 ^{o}C and 35.2 ^{o}C which can allow drying as opposed to the range of 24 ^{o}C to 25 ^{o}C in ambient air.

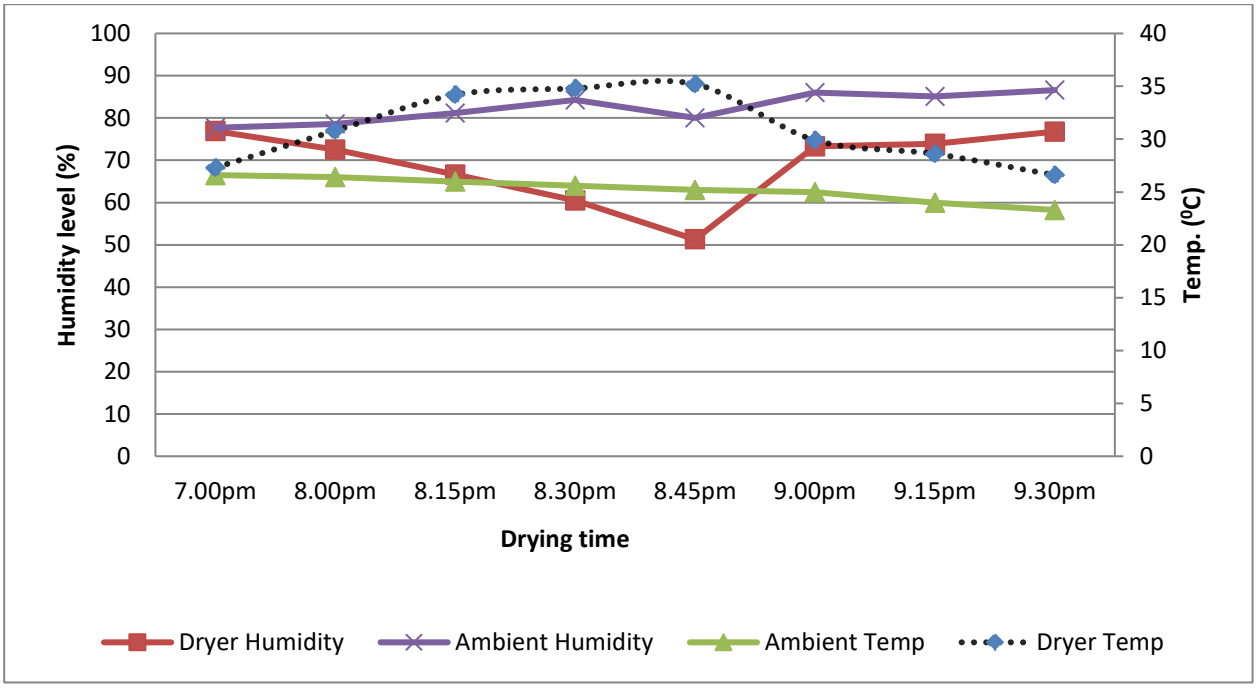


Figure 2.14: Changes in temperature and humidity with time during the drying of Siganus sutor using integrated windmill solar tunnel dryer.

2.4 Conclusions

The results of this study indicate that the hybrid windmill solar tunnel dryer is capable of harnessing solar energy compared with open sun conditions, as the ambient temperatures were always lower than the plenum chamber temperatures, despite the temperatures not showing any statistical difference ($t_{stat}=9.6689\times10^{-16}$, $t_{crit,\ 5\%}=2.0687$). In addition, it showed that the moisture content of fish reduced with drying time, and the reduction was exponential (Figure 2.12), both under salted and unsalted conditions. Based on the Handerson and Pabis model, the drying rate constant for the salted and unsalted fish was 0.0371 and 0.0377 hr^{-1}, respectively, while the values of the effective diffusivity (D_f) were found to be 6.53 x 10^{-12} and 6.63x 10^{-12} m^2/s for the salted and unsalted fish, respectively.

The Page model was established as the best model that describes thin layer drying of *Siganus sutor* fish in the integrated wind-solar tunnel dryer.

Drying temperatures of 35 ^oC achieved at night can facilitate drying and prevent spoilage and the final moisture content attained was 19.98% suitable for shelf-life storage.

CHAPTER 3

CHAPTER 3

3.0 Biochemical, Microbiological and Quality of Siganus Sutor fillets

3.1. Introduction

The quality of raw fish can substantially influence the dried product quality. The fish quality changes further during processing by solar tunnel drying and subsequent storage. The objective of this chapter was to assess the effect of delayed processing on biochemical microbiological and quality changes of *Siganus sutor* fillets starting from catch, during handling and processing and storage using the fabricated solar tunnel dryer (Chapter 2).

The main biochemical and nutritional changes monitored were water activity and moisture, which affect biochemical and microbiological properties; pH; lipid oxidation parameters peroxide value and thiobarbituric acid related substances (TBARS) that cause rancidity and production of toxic substances and TVB-N which alters the product quality. The influence of raw material quality (fresh or stored) on the solar tunnel dried product yield and productivity costs was also assessed as it affects eating quality, creates wastage and increases cost to the fish processor. This was done over a 4 hr delayed processing period. Finally, the influence of delayed icing over 10 hrs on the drying and biochemical properties was investigated to understand the changes and improve the quality of the final dried product. The microbiological quality and safety of the products were also assessed in freshly landed fish and solar dried fish together with the assessment of the bacterial quality of the ice and water used for chilling fish.

3.2. Materials and Methods

3.2.1 Influence of processing time on fillet yield and quality

3.2.1.1 Fish handling

The study was conducted between 27-30th of November 2013 in Kipini, North coast of Kenya. Arrangements were made with selected fishermen going to sea to sex and gut the *Siganus sutor* fish (Rabbit fish or Tafi) immediately after capture and group them based on time of capture

and size. Upon landing and prior to the study, fish were kept temporarily iced. The fish were then inspected further to confirm gutting. They were immediately washed and put on the processing table and separated into three batches.

Each batch was assessed for freshness to determine the quality of freshness by observing the gills, the skin and the eyes. The % yield was determined while filleting the *Siganids* before being transferred to the clean cooler box. One batch was filleted immediately by experienced personnel and was used as fillets at time 0. The second batch was filleted after 2 hours and the third batch after 4 hours and were recorded as time 2 and time 4 respectively. All the fillets were chilled immediately after filleting to reduce autolysis and bacterial activities. Samples were randomly picked for yield (cost) estimation, microbial and biochemical analysis following designed sampling protocols described below. Fresh samples for microbiological analysis were taken and transported to the laboratory immediately for analysis 240 km away to Kenya Marine Fisheries Research Institute (KMFRI), Mombasa.

3.2.1.2 Influence of Raw Material Quality on Process Yield

Organoleptic assessment of fresh Rabbit fish was performed to group fish in different quality grades using the Quality Index Method (QIM) scheme developed for *Siganids* as shown in Table 3.1. From a previous study, a score of beyond 15 out of a maximum of 21, had been used as unfit for human consumption (Odoli *et al* 2013). Based on this, 4 quality groups can be categorized as score ranging from 0-3, 4-7, 8-11 and 12-15 as shown in Table 3.1(Odoli et al 2013)

Table 3.1: Quality Index Scores for Siganids

Quality parameter		**Description**	**Score**	**Sample codes**		
Skin	Colour/ appearance	Fresh, bright metallic	0			
		Dull metallic	1			
		Dull yellowish near the abdomen	2			
	Mucus	Clear, no clotting	0			
		Milky, clotted	1			
		Yellow and clotted	2			
	Odour	Fresh sea weed	0			
		Neutral, musty	1			
		Hey, sour	2			
		Rotten, ammonic	3			
	Texture	In rigor	0			
		Finger mark disappears	1			
		Soft and Leaves mark	2			
Eyes	Cornea	Clear	0			
		Milky	1			
	Pupils	Clear & back	0			
		Opaque	1			
		Grey	2			
	Form	Convex	0			
		Flat	1			
		Sunken	2			
Gills	Colour/ appearance	Bright light red	0			
		Becoming discoloured/light brown	1			
		Grey-brown, brown, grey, green	2			
	Mucus	Transparent/clear	0			
		Milky, clotted	1			
		Brown, clotted	2			
	Odour	Fresh seaweed	0			
		Neutral/musty	1			
		Sour, mouldy	2			
		Rotten	3			
Quality index (0-21)						

A quality method for dried fish, based on organoleptic assessments, is yet to be developed. The organoleptic quality of *Siganids* freshness were assessed by observing the skin, gills and eyes at time 0 (initial time) time 2 (after 2 hrs) and time 4 (after 4 hrs) of delayed time to drying.

Three quality categories were identified based on storage time at ambient temperature; for purposes of this study, the ones that were sampled at 0 hr were referred to as grade 1, those sampled after 2 hr storage as grade 2 and grade 3 for those sampled after 4 hrs. The fish were held in ice at sea so these grades are valid for very fish Siganids. Each quality level had five pieces of fish later referred to as replicates. Upon grading, the respective costs were determined based on the time taken for filleting the replicates and yield based on the fillets weight as a percentage of the whole fish weight.

3.2.2. Biochemical analysis during delayed processing

Samples were kept iced in a cooler box for 3 days prior to transportation to the laboratory. The parameters tested included Water activity, Moisture, pH, peroxide value (PV), total volatile base nitrogen (TVB-N), thiobarbituric acid number (TBARs).

3.2.2.1 Water Activity (a_w)

Water activity in the three categories of fish in delayed icing for 0, 2 and 4 hrs which for purposes of this study were called grade 1, 2 and 3 were measured using water activity meter (DECAGON-USA) and the results recorded accordingly.

3.2.2.2 Moisture content

Moisture content was analyzed using AOAC 1990 method of analysis.
Approximately 5.0 grams of sample was weighed and recorded accordingly in a pre-weighed aluminum foil and then placed in an air oven to dry at 105^{o}C for 24 hrs. The sample was removed and re- weighed again, and the weights recorded accordingly. Moisture content was calculated as follows:

$$\text{Moisture content X (\%wb)} = \frac{initial\ weight - final\ weight}{initial\ weight}\ x\ 100$$

$$\text{Moisture content (\%db)} = \frac{mass\ of\ water}{mass\ of\ dry\ solid}\ \text{X100}$$

3.2.2.3 pH

Changes in pH of the *Siganid* fish samples were measured for each batch using a pH meter Make: HANNA; Model No. Hi 9025

3.2.2.4 Peroxide Value

The peroxide value is the amount in ml of 0.002 M sodium thiosulphate solution needed to titrate the iodine released from potassium iodide as a result of the activity of the peroxides contained in 1 g of fat. The principle of the method involves taking a sample for analysis in an acetic acid and chloroform solution, titrating it with a potassium iodide solution, and titrating the iodine released using a sodium thiosulphate solution (Regulska-Ilow, 2002).

PV was determined according to Alghazeer et al, (2008). Air was expelled from a conical flask by using nitrogen gas prior to start of the experiment. Fish oil (0.5 g) was weighed into the flask then 10 ml chloroform and 15 ml acetic acid was added. 1 ml of fresh saturated potassium iodide was then added. The flask was sealed and swirled gently to mix the solution for 1 min then placed in a dark cupboard for 1 min. Subsequently about 1 ml of starch solution was added. The coloured solution was titrated with 0.002 N (M) sodium thiosulphate solution. A control sample without fish oil was also analyzed. Peroxide value was calculated as below and results expressed as MilliEquivalents O_2/kg follows:

$$PV = \frac{(PV\ titre - PV\ blank) \times N \times 1000}{Weight\ of\ fat\ used.}$$

3.2.2.5 TBARS

Thiobarbituric acid value (TBARS) was determined as according to Pearsons (1981) as follows;

The sample (10 g) was macerated with 50 ml distilled water for 2 minutes and then washed into a distillation flask with 47.5 ml distilled water. 2.5 ml of 4 M HCl was then added to the solution. Antifoaming glass beads were put in the heating flask to reduce overflow of solution. The solution was heated using an electric mantle and a distillation unit to allow the collection of distillate. The distillate (50 ml) was then collected and 5 ml of the distillate was transferred into a glass stoppered tube using a pipette in triplicate. To each 5 ml solution, 5 ml of TBA reagent was added shaken and boiled for 35 minutes, cooled for 10 minutes. The optical density was

then measured using spectrophotometer at 538 nm against a blank. Calculation was then done using the below formula

7.8 *D Where D = Sample Absorbance-Blank

3.2.2.6 TVB-N

TVB-N was estimated using Conway's diffusion unit. Ten grams of the sample was weighed and 10 ml of 20% TCA added. The samples were then blended using a pestle and a mortar for 3 minutes and then kept in a refrigerator for 5 minutes. The samples were then precipitated using 10 mls of 20% TCA and allowed to stand in refrigerator again for 30 minutes. The samples were then removed from the refrigerator, filtered and centrifuged at 2000 rpm for 5 minutes. The filtrate was then made to volume using distilled water to 100mls.

Boric acid (2 ml) was quantitatively transferred in to the Conway diffusion unit and covered with a greased cover glass. Potassium carbonate (1ml) was then quickly added to the Conway outer chamber with immediate closure of the lid. The solutions of the outer chamber were then mixed gently by rotation and incubated at 37^{o}C for 90 minutes. After removing the cover 0.02N sulfuric acid was used in the titration of the solution of the inner chamber until a pale pink color appeared and the volume of the titrant recorded.

The TVB-N values were the computed using the formula below

TVB-N (mg%) = 14* N * (X-Y) * 100/W (mg N per 100g)

N= normality of the H_2SO_4

X = ml of H_2S0_4 used for titration of the sample

Y = ml of H_2SO_4 used for titration of blank

W = Weight of sample taken

3.2.3 Biochemical changes during solar drying

3.2.3.1 Fish handling

Fresh *Siganids* (Tafi) were obtained and treated as in section 3.1.1 above. The icing was delayed for 0, 2, 4, 6, 8 and 10 hrs and quality index used according to Table 3.1 to establish quality. Fresh samples for microbiological analysis were transported to the laboratory immediately for analysis.

3.2.3.2 Drying

The hybrid windmill solar tunnel dryer fabricated in Kipini was used to dry the fillets (Chapter 2). The samples were removed from the cooler box and put on the trays at an inclined angle to drain excess water and were transferred into the dryers after 30 minutes and placed in the solar dryer trays in 6 different batches according to the time of delay before start of drying. The delay in time was 0, 2, 4, 6, 8 and 10 hrs delayed processing. Each batch was monitored for weight loss by taking measurements of triplicate samples every 2 hrs.

Physical parameters like temperature and humidity inside the dryer were also taken every 2 hrs as the drying continued using temperature-humidity DECKER (USA) data logger.

The drying was ended when there was no further change in weight with time hence no further weight loss. The samples for moisture loss analysis were then separately wrapped in aluminum foil in batches, put in the transportation containers and transferred to the laboratory for analysis. The fish dried here was only for biochemical and microbiological parameters during drying and storage.

3.2.3.3. Biochemical parameters during drying

Samples for the determination of water activity, moisture content, pH, PV, TBARS, TVB-N were taken and carefully wrapped in aluminum foil and then put in seal-lock polythene bags and kept in an icebox for analysis in the laboratory.

3.2.4 Microbiological analysis

3.2.4.1 Sample collection and preparation for analyses

The *Siganus* fish species samples for analysis were taken from the same batch for quality analysis in section 3.2.3 above. The fresh fish samples were aseptically wrapped in sterile aluminium foil, labeled, stored in ice in a cooler box, and immediately transported to KMFRI laboratory within 5 hours of purchase for microbiological analyses. At the end of 3 days of solar drying, dried fish samples were also aseptically collected and taken to the laboratory for storage and analysis. Processing water and storage ice was also analyzed for bacteriological loads using the most probable number (MPN) method.

3.2.4.2 Isolation and enumeration of bacteria

Spoilage Specific Organisms (SSO's) which cause sulphide food spoilage were cultured by inoculation of 1ml of the final dilution of the same homogenate on selective iron sulphite agar (ISA) plates and subsequent incubation at 25°C for 5 days. The media used for the enumeration of food spoilage microorganisms contain sulphite reductase enzyme in their cytoplasm. The enzyme catalyzes the intracellular metabolism of sulphites into hydrogen sulphide (H_2S) which contributes prominently to the off-oduor associated wish sulphide fish spoilage. Consequently, the bacteria are identified as black colonies on the ISA plates. The black coloration is a result of the formation of a precipitate yielded by the reaction between an iron (III) salt (a constituent of the ISA) and the produced sulphide.The Siganid fish samples were homogenized in the laboratory and 25 g of fish tissue used for Total Viable Bacteriological Counts (TPC) using the spread plate technique as described by Lakshmanan *et al*., (2002). The total viable counts for the Specific Spoilage Organisms (SSO) were determined by spread plating of the sample aliquots on iron sulphite agar (ISA) then by aerobic incubation at 25 °C for 5 days. H_2S producing bacteria were determined by counting black colonies on ISA and expressed as CFU g^{-1}.

3.2.4.3 Storage of fish for microbiological analysis

The samples were stored in a similar manner as for biochemical analysis in section 3.1.1 in three different ways. One set of samples was kept in an ordinary polythene bag and sealed using a sealing machine, one kept in open dishes and the third one vacuum packed using vacuum

packer. Sampling was done after every 14 days for one month to assess the shelf life of each, by analyzing the biochemical and microbiological as well physical parameters used as indicators of spoilage. Temperature and humidity were also monitored daily in the laboratory to determine the changes in the condition of ambient air surrounding the *Siganid* fish during the storage.

3.3 Results and discussion

3.3.1 Quality scores during delayed icing for 0-4 hrs category

There are various ways of evaluating fish quality but sensory techniques still remain the most reliable and easy to use (Ólafsdottir et al, 1997). The sensory methods used commonly involve presenting raw and cooked fish to trained panelists to quantitatively evaluate, using preset objective terms and numerical scales. This Quantitative Descriptive Method (QDA) has found wide application in seafood evaluation (Johnsen and Kelly, 1990; Gines et al 2004). The QDA, however, is limited because the panelists must be properly trained to give a thorough description of quality (Nelsen and Green, 2007).

Currently, Quality Index is used that has advantages because it addresses the unique characteristics of spoilage patterns and indicators of each species (Nelson and Green, 2007). Therefore there is no general quality index scheme and has to be specific for each fish species. The parameters evaluated like eyes, gills, appearance, odour, and texture are specific. In Quality Index Method (QIM) system, there is a linear relationship between Quality Index and storage time; this make it possible to estimate the remaining shelf life (Nelson and Green, 2007). When using QIM there is little need for sample preparation because the whole raw fish is evaluated. Moreover, the training needs for the panelists are not as elaborate as in the QDA system (Nelson and Green 2007). QIM can be used in farms, making it easy to carry out quality evaluations (Martinsdóttir et al 2001). In this study, the QIM method for Siganus sutor (Odoli et al, 2013) fish was used to evaluate quality.

Most fish that is dried in Kenya have either started to spoil or incurred delays in processing; this leads to low yield if the fish are to be filleted and higher costs because the filleting takes a longer time. The QIM system is used to assure product quality before processing starts. In this study the QIM was used to evaluate quality of Siganid fillets during delayed processing.

The quality scores were based on the QI scheme. The results were then computed as shown in Figure 3.1. The scoring scale was designed in QIM format such that score 0 meant the freshest, score 1 was slightly fresh while score 3 meant the least fresh. The results show that the freshness quality of the fish assessed decreased with time and those assessed after 4 hours had the poorest quality. From the score, the fish were still of good quality after 4 hr delayed processing with a QI maximum of 1.2 for the gills.

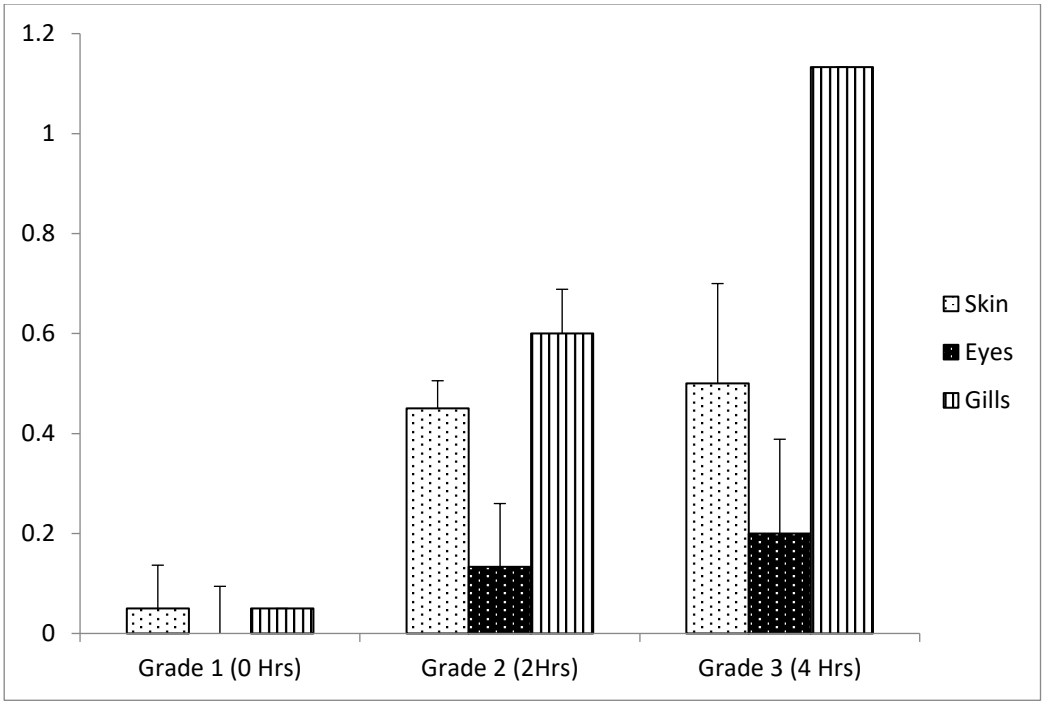


Figure 3.1: Quality assessment of fresh Siganids during ambient storage at 0hr, 2 hr and 4 hr delayed processing

3.3.2. Influence of Raw Material Quality on Process Yield and Labour Productivity

3.3.2.1 Quality index during delayed icing for 0- 4 hr category

The quality index (QI) was reported based on average of 5 fish per trial (grade) and showed a linear relationship with time of storage by delayed icing. For purposes of this study only, the freshest fish sampled at 0hrs after landing was named grade 1, the one sampled after 2 hrs grade 2 and the one sampled after 4 hrs grade 3. According to Figure 3.2 there was a strong correlation of $R^2 = 0.9735$ observed with the groups that were significantly different ($p < 0.05$). The noted difference showed premium quality grade 1 scoring lower than good quality grade 2 and marginal quality grade 3 accordingly.

The results indicated that attributes gradually deteriorated with time and obeyed the QIM scheme; fresh fish just after catch was given lower scores which increased with time, coming close to a maximum score at the end of shelf life (Martinsdottir *et al*., 2001). It is therefore worth noting that the QIM was successfully used to categorize samples into quality grades. Five pieces of fish used in the current study was in accordance with the guidelines for evaluation of freshness of whole fish (Martinsdottir *et al.* 2001). A minimum of three (large fish) to 10 (small fish) random samples is recommended in the guidelines as this should be taken to cover the biological differences in spoilage rate of fish. All the quality grades were within the consumption limits as the QI for the analyzed fish had a maximum score of 21 whereas, grade three that was scored higher had QI of 6.

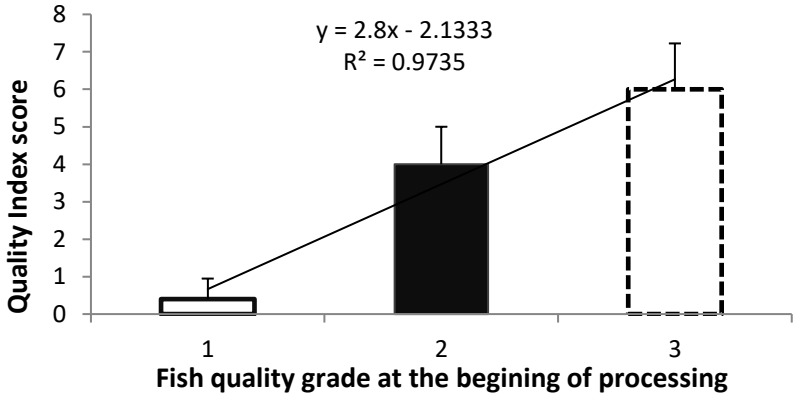


Figure 3.2: Quality index (Max QI=21) of Siganus sutor freshness grades. Averages (±SD) over each quality grade analyzed (average score, N=5). 1= stored for 0 hours; 2= stored for 2 hours; 3= stored for 4 hours.

3.3.2.2 Individual quality parameters in QIM scheme

The average scores for the individual quality descriptors varied considerably within the groups (Figure 3.3). However, subsequent quality grouping recorded consistently higher scores except form of the eyes. Figure 3.3 further depicts that skin mucus and skin odour should be excluded from the scheme as they were either difficult to evaluate or did not reflect on quality for Siganid fish and recorded minor changes in the groups (Sveinsdottir*et al*, 2003).

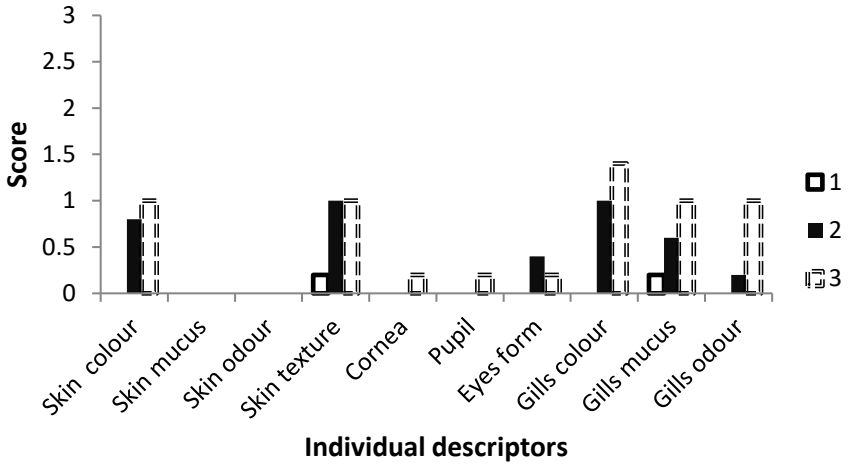


Figure 3.3: Average score for individual quality descriptors (attributes) of *Siganus sutor* evaluated with QIM scheme Rabbit fish (N=5). 1= stored for 0 hours; 2= stored for 2 hours; 3= stored for 4 hours.

3.3.2.3 Influence of raw material quality on yield and labour

The most basic value addition to fish is filleting for convenience eating and culinary preparations. In Kenya, filleted fish costs 6 to 8 times more than the price of whole fish because of convenience and cultural resistance to fish eating as some communities simply do not eat fish because of appearance, hard labour of scaling. In contrast, fillets are easy to cook and store. Species, sex, type, and structural anatomy of the fish determine yield, type and quality of fillets obtained (Rora et al, 2001).

Maintaining freshness of fish fillets by applying controls like HACCP at landing, reception and during processing is necessary for optimum quality costs (Zagaramundi et al, 2004; 2007). Quality of fish fillets is influenced by physical, biochemical and biological condition of the fish (Zugarramurdi et al, 2004). The quality is expressed in texture changes. The factors responsible for the quality of the texture includes age of the fish, size, fat content, amount and properties of proteins, connective tissue and stress handling (Coppes-Petricorena 2010) The post-harvest factors that are also important include rate and extent of decline in pH, status of rigor mortis, extent and rate of breakdown of myofibrils and connective tissue due to proteolysis, nucleotide degradation, temperature and time of storage of the fish (Kiessling et al, 2004). Fresh fish muscle firmness decreases in the initial storage period because of protein hydrolysis and deterioration of the tissue (Kiessling et al, 2004).

Traditionally filleting is carried out at after the onset of rigor mortis thougt it is important to consider state of freshness and storage costs. It is not easy to control the onset of rigor in an

industrial set up where catches are from the wild as opposed to farmed fish where harvesting can be controlled (Borderías and Sánchez-Alonso, 2011).

Figure 3.4 shows the yield diminished with a reduction in fish quality; grade 1 recorded higher fillet yield of about 54% compared to grade 2 and 3 that recorded 50% and 45% in that order. However, the yield is observed to be inversely correlated with production time reported during filleting, as longer time (minutes) were observed with subsequent grades. Earlier studies in cod filleting showed the relationship between raw material quality and productivity and lack of icing on board (Zugarramurdi et al., 1995; Zugarramurdi et al., (2004). Longer storage time produced lower yields and required slightly longer time for filleting. The difference looks insignificant for few samples but in an industrial set up it would translate to big economic losses in terms of time and yield. Because freshly caught fish are still in rigor, the muscles are easily cut than when the fish loses rigor. Fillets tend to stick to the bones and therefore give low yields and increases time to fillet. Although this is a preliminary study involving short storage time periods, it is nevertheless indicative of the fact that quality and production costs have a relationship, and further studies can be undertaken to provide proper advice to industry

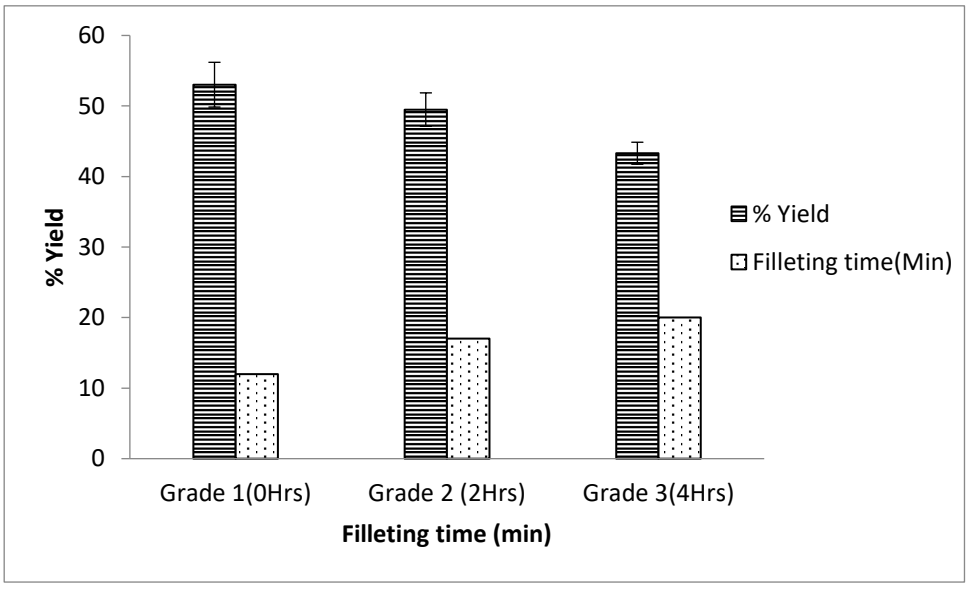


Figure 3.4: Influence of raw material quality of Siganus sutor on filleting time and yield because of delayed icing for 0, 2 and 4 hrs. (N=5). 1= stored for 0 hours; 2= stored for 2 hours; 3= stored for 4 hours.

3.3.3. Biochemical parameters during delayed processing for 0-4 hr category

Table 3.2 shows the results for the biochemical parameters during delayed processing for 0, 2 and 4 hrs.

Table 3.2: Biochemical changes during delayed processing at 0, 2 and 4 hrs

Grade 3	% MOISTURE CONTENT		WATER ACTIVITY		pH		TVB-N		TMAN	PV		TBARS	
	Value	Standard Deviation	Value	Standard Deviation	Value	Standard Deviation	Value	Standard Deviation		Value	Standard Deviation	Value	Standard Deviation
0 hrs	73.5	0.0522	0.88	0.0050	6.65	0.0141	13.8	0.1939	nd	21.65	0.7127	0.367	0.0055
2 hrs	73.8	0.4023	0.88	0.0000	6.54	0.0141	27.8	0.0025	nd	89.11	0.5530	2.098	0.0055
4 hrs	74.8	0.2879	0.88	0.0100	6.5	0.0071	42.0	0.1995	nd	106.12	0.8250	3.011	0.0552

Nd (not detected)

During the 4-hour delayed icing period there was increase in TVB-N, PV and TBARS. pH decreased and TMA was not detected.

3.3.3.1 Water activity

Water activity is related to microorganisms that cause spoilage of fish (Abbas et al, 2009). Upon death of fish, bacteria gain entry into the fish body through the gills into the blood vessels and also through the belly cavity lining and eventually through the skin. When bacteria get access to the flesh, they grow rapidly producing off-odour and flavours (Johnson et al 1994). The main factors that contribute to fresh fish spoilage in the whole complex process of inter-related systems are protein degradation leading to formation of products like hypoxanthine, trimethylamine, hydrogen sulphide; development of oxidative rancidity with the formation of ketones, aldehydes and esters and the action of microorganisms (Gram et al, 2002). Spoilage of fish eventually influences flavours, odours, colours or texture of the fish, leading to fish loss which is an economic burden (Abbas et al, 2009).

It is now established that water activity is a key factor that influences the spoilage process in fish (Abbas et al, 2009) and is used in many cases as a Critical Control Point for HACCP. The

water activity correlates with bacterial growth (Scott, 1957) and the growth of bacteria is inhibited at specific water activity values. Water activity of 0.85 is considered a safe level for pathogen growth (Abbas et al, 2009). In fresh products the water activity is high. In the standard definition, water activity is a measure of the water available in a food that microorganisms can use for growth. The microorganisms include bacteria, yeast and mould.

The optimum water activity limits or slows down fat oxidation, enzymatic reactions and protein denaturation therefore maintains product quality and extends shelf-life (Abbas et al, 2009; Wolf et al, 1984). Water is a solvent for chemicals and for microbial and enzymatic reactions. Therefore, water activity is a measure of the availability of water to participate in such reactions.

There was no change in water activity levels as seen in Table 3.2 in the initial storage periods for fish stored from 0 to 4 hrs. The water activity value of 0. 88 was however high and adequate to support microbial growth. This is what is seen from the increase in TVB-N levels in the same period because microbial growth is affected when water activity is below 0.85 (Abbas et al, 2009). Dehydration of foods helps to extend shelf life by reduction in water activity (aw). Low water activity inhibits microbial growth.

3.3.3.2 pH

Microorganism have a minimum and optimum pH for growth. pH change is because of various deterioration processes in the fish after harvesting. These processes include biochemical, autolytic and microbiological breakdown mechanisms. Spoilage mechanisms or processes can occur independently or concurrently, and the relative importance will vary with the fish species (size, lipid content, maturation stage); environmental conditions (feeding activity and availability, microbial load), slaughter method, catch method, post-mortem handling, storage and conditions of processing (Viji et al, 2009; Isabel et al, 2009).

pH is one of the physico-chemical parameters used as an indicator to assess degree of freshness or spoilage in fish (Huss 1988). In freshly caught fish, pH values of 6.0 to 6.5 are reported and pH values 6.8 to 7.0 are acceptable, although there are some fish that can have alkaline meat just after being caught. The pH value therefore, cannot be used as the only indicator for fish freshness (Huss, 1998; Viji et al, 2015; Huss 1995; Kose and Erdem (2001). In the post-mortem period, breakdown of nitrogenous compounds may increase pH, while oxidation of lipids, and metabolism of glycogen is responsible for reduction in pH (Huss, 1995). The pH can decrease

or increase in fresh fish (Viji, 2015). A decrease is attributed to accumulation of lactic acid by anaerobic glycolysis and release of inorganic phosphates by degradation of ATP. An increase in pH is due to the breakdown of nitrogenous compound containing products leading to an increase in amines and other volatile bases (Binsi et al, 2007). The physical properties of a fish muscle is affected by a post mortem reduction in pH. This is because with a drop in pH, there is a reduction in the net surface charge on the muscle proteins. This causes partial denaturation and loss of some water holding capacity (Huss, 1995).

In this part of the study, changes were in the range of 6.65 for fish sampled at 0 hr and 6.5 after 4 hrs (Table 3.2); pH ranges were within the norm. The low pH levels are as a result of stress at the time of death which causes depletion in energy reserves especially glycogen being converted to lactate. It could also be due to increased production of free fatty acids during hydrolysis of fats (Özogul et al 2005). The *Siganids* are sometimes caught using basket traps which are left overnight. The fish in these traps struggle for a long time trying to escape from the trap, hence metabolizing glycogen to lactic acid, leading to a lower pH.

3.3.3.3 Peroxide Value

Peroxide value measures the amount of hydroperoxides. In this study, during the 0 to 4 hr delay in icing period, PV increased from 21.65 $mEqO_2$/kg at 0 hr to 106 $mEqO_2$/kg after 4 hr. A value of 20 $mEqO_2$/kg is proposed as the maximum limit for fish oil but there is no maximum standard value agreed for PV as higher PV values have been obtained (Alghazeer et al, 2008), even in this study without detection of spoilage. The PV levels confirm that one indicator alone may not be used to draw conclusions on deterioration, therefore more parameters are usually analyzed.

3.3.3.4 TBARS

In this study, the TBARS increased from 0.367 mg malondialdehyde /kg at 0 hr to 3.011 mg malondialdehyde/kg after 4hr of delayed processing storage (Table 3.2). The delayed processing showed a linear increase with time from 0 to 4 hrs. TBARS are formed from unstable hydroperoxides during lipid oxidation and contribute to rancidity. The increase in TBARS corresponded to the formation of hydroperoxides which was slower in breakdown than formation of TBARS. The value of TBARS in this short storage period was less than the upper limit set for most fish oils of 7 to 8 mg malonaldehyde /kg (Gimenez et al, 2011; Boran et al, 2006) and 27 mg malondialdehyde for fish mackerel (Özogul et al 2005).

3.3.3.5 TVB-N

Microbial growth responsible for spoilage of fish, produces several compounds including biogenic amines like histamine, cadeverine, and putrescine. The other products include organic acids, aldehydes, sulphides and ketones. They have unpleasant odours and off-flavours (Dalgaard et al, 2006). Total volatile basic nitrogen (TVB-N) include ammonia and amines that are formed during degradation of protein and non -protein nitogeneous compounds. The hydrolytic enzymes from micro-organisms catalyse this process (Manat et al, 2005).

Trimethlylamine (TMA) is found in marine organisms in varying concentrations and their levels also depend on the species and the environment. The precursor is Trimethylamine Oxide (TMAO). TMA is formed as a result of bacterial breakdown of TMAO (Huss 1995). Other products formed are dimethyl amine (DMA) and formaldehyde. The analysis of TMA does not give information on early autolytic changes in spoilage but will give changes due to spoilage later (Huss 1988) The upper limit for human consumption for TMA is 10-15 mg N TMA/100g (Huss, 1988). Some workers have not found it useful as an indicator of spoilage (Magnusson and Martinsdottir, 1995; Smith et al. (1980b). In this study, more focus is on TVB-N.

The TVB-N values in the fresh fish are 4 to 20 mg N/100g (Huss, 1998). In this study, the initial TVB-N values at the beginning in the 0 to 4 hr storage period was 13.8 mg N/100g. In a separate set of *Siganid* fish, processed in the 10 hr delay category, the TVB-N values were 4.15mg N/100g at the beginning before the start of the drying process; this is within the limits for fresh fish (Huss 1995).

TheTVB-N levels rose from 13.8 to 42 mg N per 100 g of fish muscle from 0 hr to 4 hr of delayed processing (Table 3.2). TVB-N release depends on bacteria decomposition of fish flesh. Freshly caught fish have TVB-N values of 5 mg and 20 mg N per 100 g of muscle and 30-35 mg N per 100g of muscle is the level of acceptability for iced stored fish in cold regions (Ozogul et al 2005 and Huss 1998) although in warm water fish, this could be higher. Parameters for

fish quality are influenced by many other factors so one indicator cannot be used (Huss, 1995; Viji et al, 2009; Isabel et al, 2009).

3.3.3.6 Conclusions on delayed icing on 0-4 hr category

It was concluded that the raw material quality had a direct impact on yield and production costs of *Siganus sutor*. There was an increase in filleting time and lower filleting yield during delayed processing. The levels of PV, TBARS, TVB-N, TMA increased during delayed processing as a sign of quality deterioration. The pH values reduced during delayed processing. Further studies could be done to document all production costs. There is a need to further link the developed QIM scheme for *Siganus sutor* to precisely characterized quality with storage time.

3.3.4 Drying Siganid fish during delayed processing (0, 2, 4, 6, 8 and 10 hr delayed processing)

3.3.4.1 Moisture content in fish samples

Moisture content is important for storage of fish and the levels of moisture also help to determine the end of drying. The targeted moisture content at the end of drying is 15% to prevent mould growth. Bacterial growth is prevented at moisture content less than 25% (Bala and Mondol, 2001). In the present study, moisture content was primarily used to determine the end of the drying process although it was also used as an indicator in monitoring storage of the dried Siganid fish. The fish that were dried after delayed processing for 0, 2, 4, 6, 8 and 10 hr all dried to 15 % moisture which is the shelf stable target for dried fish. Whereas water activity is the preferred method for monitoring fish storage, moisture content measurements can complement water activity.

The drying trends for *Siganus sutor* fish are shown in Figure 3.5. At the beginning of drying, the % moisture dropped from 72.77±1.08 to 14.6±0.07 (for fish processed after delayed icing of 0hr. The moisture dropped from 72.93±5.02 for the fish processed after 2 hr of delayed icing to 14.75±0.95. After 4 hr of delayed icing, the moisture content in the *Siganid* fish dropped from 72.35±1.15 to 16.88±0.22. In the fish that was processed after 6 hr of delayed icing, the moisture content dropped from 71.66±1.39 to 12.00±1.30. The moisture content in the fish that

was dried after delayed icing dropped from 73.43±1.76 to 15.53±1.84. In the fish that was processed after 10 hr delayed icing, the moisture dropped from 73.69±0.40 to 13.64±0.47. The fish therefore were all dry to about 15 % which is the shelf stable moisture content that does not favour bacterial or mould growth.

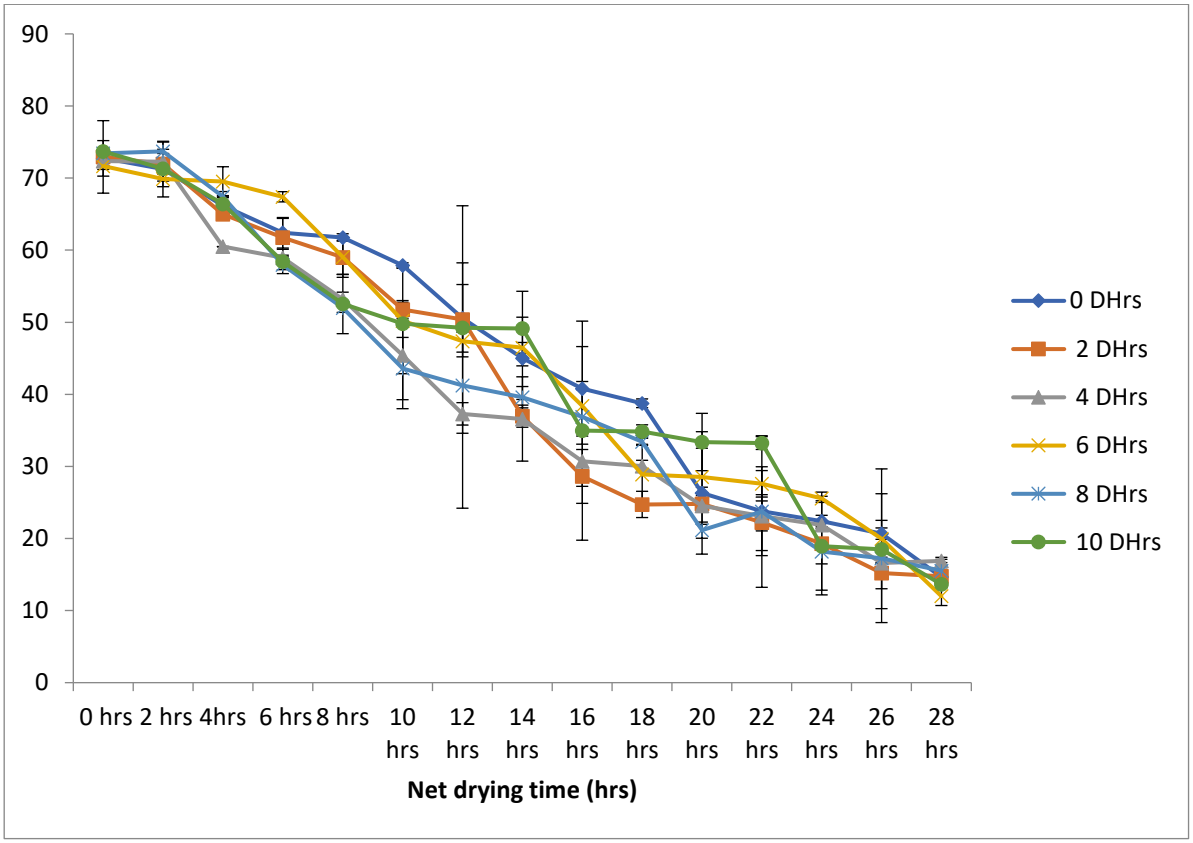


Figure 3.5: Changes in moisture content during drying of Siganids after delayed icing of 0, 2, 4, 6, 8 and 10 hr

3.3.5 Biochemical changes during drying (0, 2, 4, 6, 8 and 10 hr delayed processing)

3.3.5.1 Water activity in fish samples undergoing delayed filleting

Fish samples subjected to delayed filleting (0, 2, 4, 6, 8 and 10 hr) were just named as 0hr, 2hr, 4hr, 6hr, 8hr and 10hr. The water activity during drying was reduced irrespective of delayed icing before drying at 0, 2, 4, 6, 8 or 10 hr (Figure 3.6). The fish that was filleted at 0 hr in this lot, had a faster loss in water activity than the one that was filleted last after 10 hr delayed processing. The drying was for 3 days. Water activity is the available water for microbial growth and is unbound water. In fish stored for 0 hr before drying, the water activity dropped to a much lower level (0.6) which is good for storage (Abbas et al, 2009). That means that the fish, after

achieving a lower water activity level, could have a longer shelf-life. This still supports the importance of processing fish when still fresh. There could be biochemical and microbiological reactions retaining the water or not making it available in stale fish and not releasing it during drying, as fast as in the fresh fish; this viewpoint can be further investigated.

3.3.5.2 pH

During and after the process of drying, the pH values for the fish processed after 0 and 2 hr were lower than those processed after 6, 8 and 10 hrs (Figure 3.7). The pH was up to 6.8 for the fish processed after 0 and 2 hr of delayed processing while those processed after delayed filleting for 4, 6, 8 and 10 hr was up to 8.9. The pH values for fish processed after 4, 6 8 and 10 hr were higher because as the delay continued, bacteria also metabolized various substrates in the fish to produce volatile basic compounds that are alkaline, hence resulting in the increased pH (Huss, 1995; and Susanto et al, 2011, Makawa et al, 2014).

3.3.5.3 Peroxide value

During the process of drying, the peroxide value was highest in the fish processed after 10 h and lowest in the fish processed after 0 hr (Figure 3.8). The PV was as low as 31.74 $mEqO_2$/kg at 0hr and reduced to 10.16 $mEqO_2$/Kg when drying in the solar tunnel dryer ended after 3 days. In the fish that was processed after 10hr of delayed processing the PV was initially at 65.57 and dropped to 20.37 $mEqO_2$/Kg. The lower PV was because the hydroperoxides are unstable at higher temperatures and break down further (Frankel 2005) to volatile and none volatile compounds and secondary products with limits being 20mEq O_2/kg (Weber et al 2008, Haque et al 2013).

3.3.5.4 TBARS

TBARS decreased during drying (Figure 3.9). The level of TBARS at the beginning of drying for fish stored for 0 hr before processing was 2.13 mg malondialdehyde per Kg and this reduced to 0.29 mg malondialdehyde per Kg at the end of drying on the 3rd day. For fish processed after 2 hr, the TBARS reduced from 2.40 mg malondialdehyde per Kg to 0.49 mg malondialdehyde per Kg and for the fish dried after 4 hr of delayed processing the TBARS reduced from 3.95 mg malondialdehyde per Kg at the start to 1.72 mg malondialdehyde per Kg at the end of drying on the 3rd day. The TBARS in the fish processed after 6, 8 and 10 hr the TBARS were 30.04, 43.87 and 68.64 mg malondialdehyde per kg respectively. This reduced to 7.8 mg

malondialdehyde per Kg for delayed processing after 6 hr, 15.6 mg malondialdehyde per Kg for delayed processing after 8 hr and 32.86 mg malondialdehyde per Kg after delayed processing of 10 hr.

The increase in TBARS at 0, 2, 4, 6, 8 and 10 hr at the beginning is due to the initial formation of hydroperoxides which break down to aldehydes and related compounds. However, during the process of drying, the TBARS decreased either due to breakdown to volatile tertiary compounds like hexanal and also due to formation of adducts with proteins (Weber et al 2008; Özogul et al 2005; Frankel 2005), which can cause toughening of fish. The limits for TBARS in fish oil is 7 to 8 mg MDA/kg but this may not apply to solar dried fish because the fish at the end of drying and at the beginning of storage is still suitable for human consumption despite the higher levels of TBARS.

3.3.5.5 TVB-N and TMA

At the end of the drying period, for the fish in the 0 hr delayed processing category, the TVB-N dropped to 0.8mg N/100 g. The TVB-N values also dropped after drying in the fish that was subjected to delayed icing after 2, 4, 6, 8 and 10 hr delayed processing period. The TVB-N values were 5.5 mg N/100g, 4.98mg/100 g. 8.3 mg N/100 g, 6.09 mg N/100 g, 11.1 mg N/100 g and 14.5 mg N/100 g. Low levels of TVB-N at the beginning of drying have been reported by Erkan (2016) prior to storage. This result means that solar drying reduced the levels of TVB-N during drying. During the first few hrs of drying however, there was an increase in TVB-N before the drop (Figure 3.10). This increase followed by a drop in TVB-N after solar drying in Siganid fish is reported for the first time. The volatile compounds could be lost during drying due to a drop in bacteria levels and high temperatures in the dryer. There is a need to further understand the levels for TVB-N in dried Siganids before storage. There was a delay in the formation of TMA levels which continued to drop during drying in the 10 hr delayed processing category (Figure 3.11). In the delayed processing period for 0 to 4 hr, TMA was undetected. The formation of TVB-N and TMA were formed by different pathways.

Bacterial growth continued until the available nutrients were depleted. Increased TVB due to increase in microbial loads has been reported (Anderson, 2008; Orban et al 2011). The TVB-N values finally reached lower values at the end of drying. The trend was slightly different for TMA-N in that there was no peak increase before a decrease, although there was still a net reduction in TMA-N at the end of the drying period. TMA was not detected in the fish fillets

that were filleted after 0, 2 and 4 hours for delayed processing and did not show any TMA throughout the drying period. The TMA levels started to be noticed in the fish that was processed after 6, 8 and 10 hr delayed processing. In the fish that was processed after 6, 8 or 10 hr delayed icing, the TMA was 0.16, 0.83 and 2.23 respectively at 0 hr; this decreased to 0.02, 0.05 and 0.02 respectively after 28 hr net drying period.

It is important to note the levels of TMA in the fish at the start of drying, irrespective of the delay were still within the allowable limits of spoilage of 5mg TMA/100g (Orban et al 2011). The pathways and rates for formation of TMA and TVB-N could be different involving different types of bacteria with different capacities to form volatile bases (Orban et al 2011) though at the end both were reduced after the drying process.

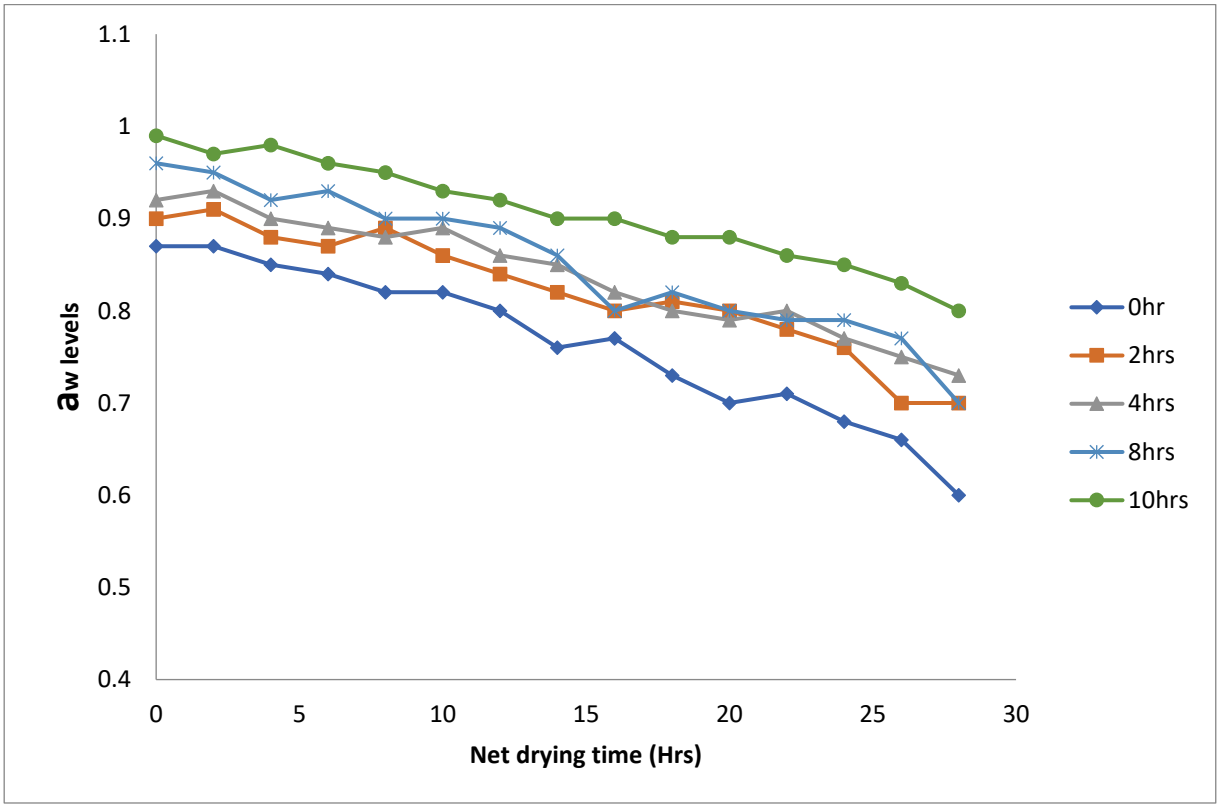


Figure 3.6: Changes in water activity during the actual solar drying of Siganid fish fillet after delayed icing for 0, 2, 4, 6, 8 and 10 hrs in the hybrid windmill solar tunnel dryer in Kipini, Kenya.

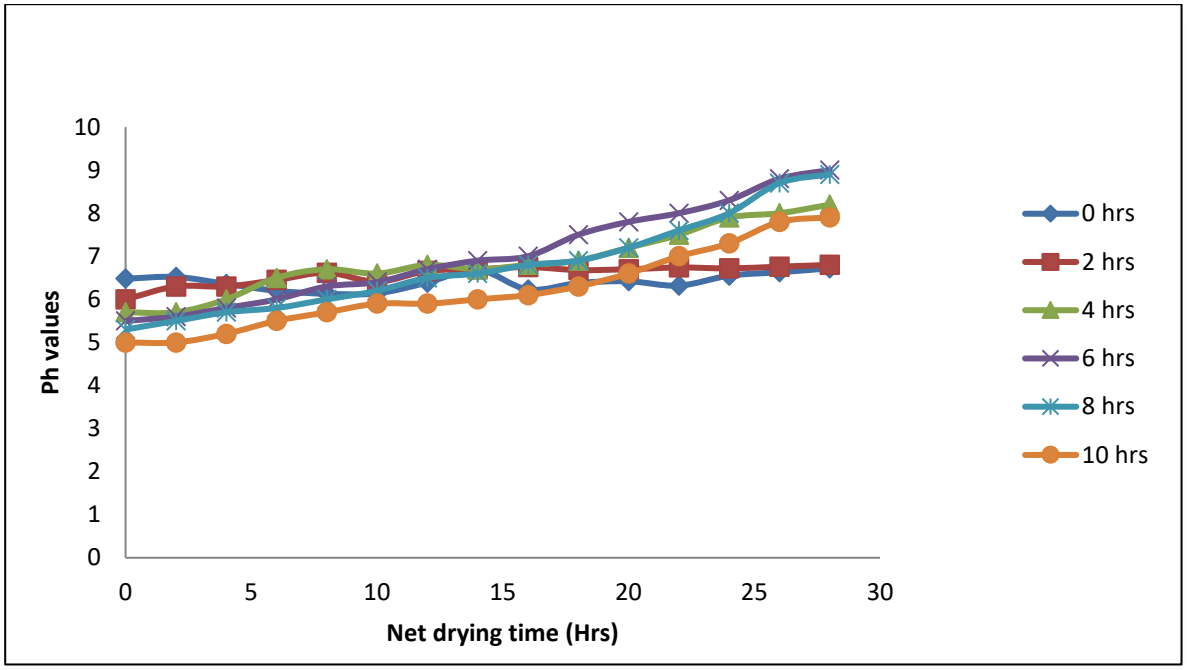


Figure 3.7: Changes in pH during the actual solar drying of Siganid fish fillets after delayed icing for 0, 2, 4, 6, 8 and 10hrs in the hybrid windmill solar tunnel dryer in Kipini, Kenya.

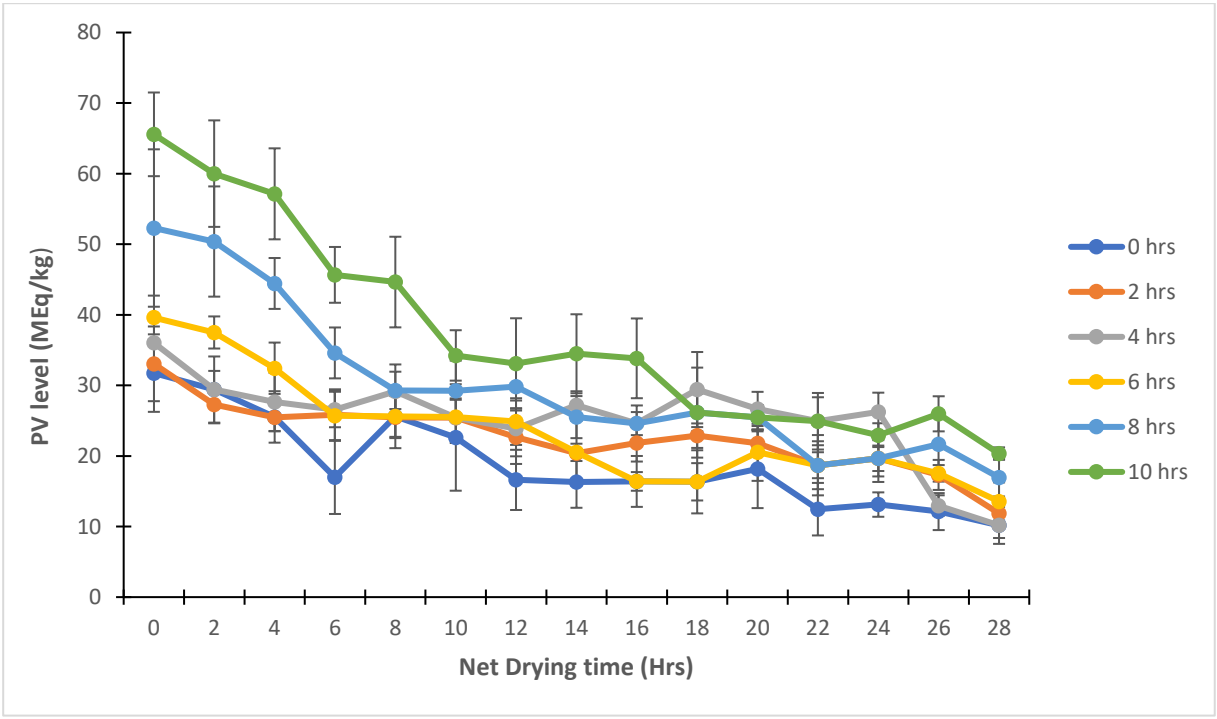


Figure 3.8: Changes in PV (peroxide value) during the actual solar drying of Siganid fish fillets after delayed icing for 0, 2, 4, 6, 8 and 10hrs in the hybrid windmill solar tunnel dryer in Kipini, Kenya.

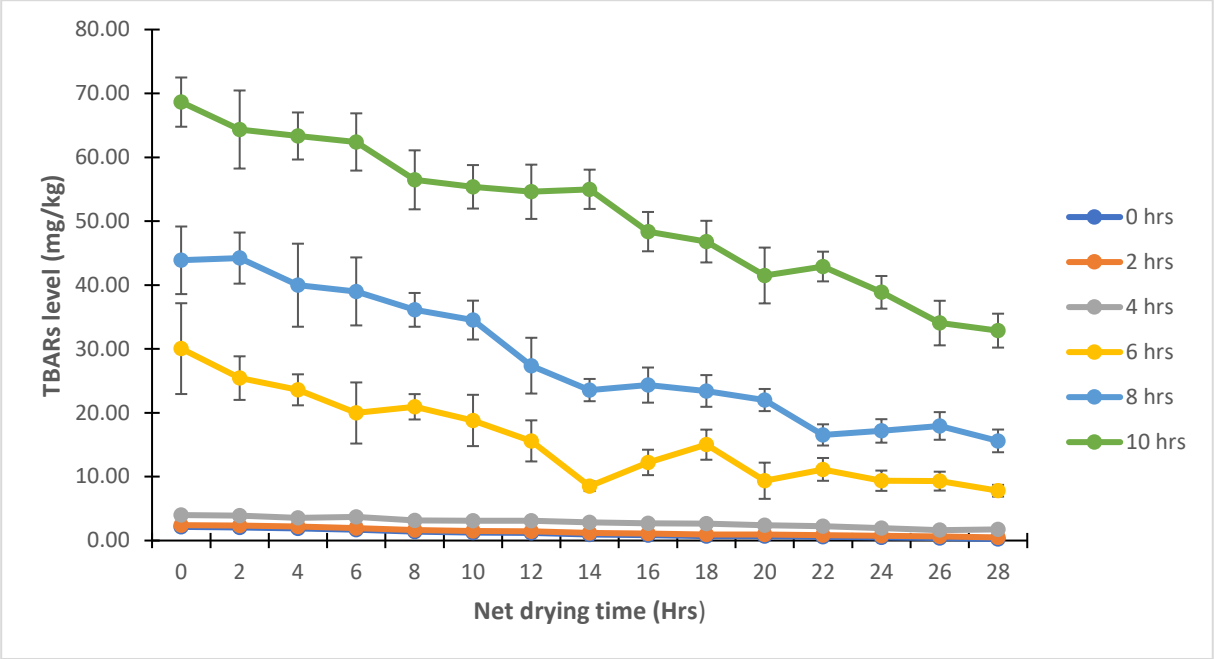


Figure 3.9: Changes in TBARS during the actual solar drying of Siganid fish fillets after delayed icing for 0, 2, 4, 6, 8 and 10hrs in the hybrid windmill solar tunnel dryer in Kipini, Kenya.

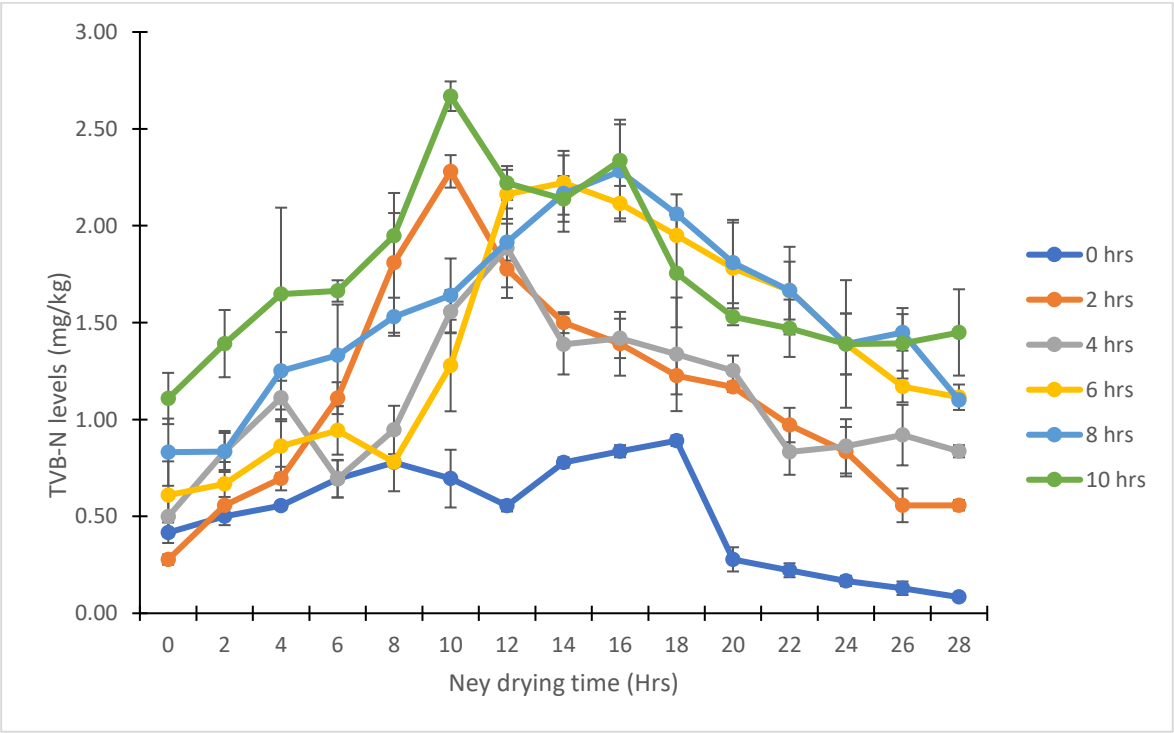


Figure 3.10: Changes in TVB-N during the actual solar drying of Siganid fish fillets after delayed icing for 0, 2, 4, 6, 8 and 10hrs in the hybrid windmill solar tunnel dryer in Kipini, Kenya.

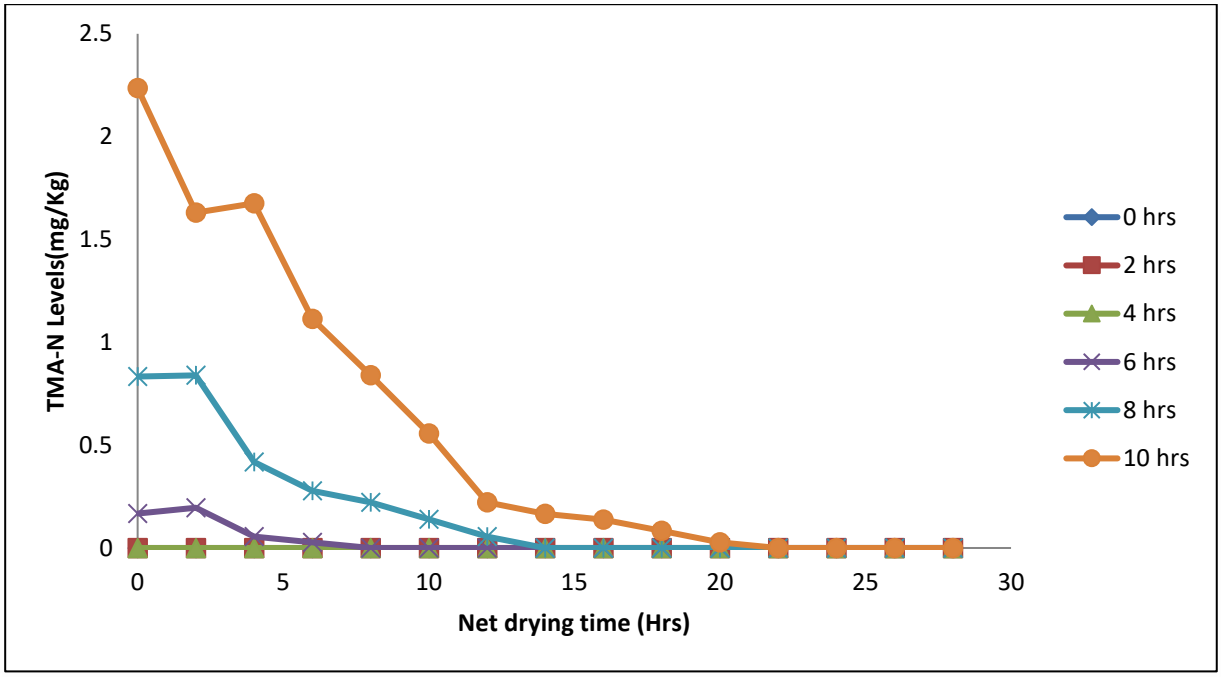


Figure 3.11: Changes in TMA during the actual solar drying of Siganid fish fillets after delayed icing for 0, 2, 4, 6, 8 and 10hrs in the hybrid windmill solar tunnel dryer in Kipini, Kenya

3.3.5.6 Conclusions

PV and TBARS decreased during drying because of unstable nature of hydroperoxides and aldehydes breaking down to secondary and tertiary products and further, interacting with proteins. TVB-N and TMA decreased owing to reduced bacterial action. The pH increased during drying. Drying reduced/altered the biochemical spoilage indicators to a minimum before storage commenced.

3.3.6 Biochemical changes during storage of solar dried fish only at 0 h of delayed icing

The fish were stored on open benches in the lab to simulate the storage conditions in a traditional set-up, the second batch was stored in normal polythene bags (wraps) used daily in the markets as an improvement which is cheap and hygienic, and the third batch of *Siganid* fillets was stored after vacuum packaging using a vacuum packer with pouches in the laboratory (Figure 3.12). Only the fish that were processed at 0 hr were used for storage trials.

i  ii  iii 

Figure 3.12: Solar dried Siganid fillet stored in the open (i) in normal polythene packaging (ii) and vacuum packaging (iii)

3.3.6.1 Moisture during storage

In the fish stored in the open, the moisture content increased from 15.15% at the start of storage to 21.46% after 75 days of storage (Figure 3.13).

For fish that was stored in normal polythene packaging bags, the increase in moisture was from 15.15 to 19. 26%. In fish stored in vacuum packaging, there was a minimal increase in moisture from 15.15% to 16.5%.

Moisture content above 25% can support bacterial growth and mould growth can take place at moisture content above 16%. The fish was still within safe limits for microbial attack despite an increase in moisture content. The absorption of moisture is due to the hygroscopic nature of the fish muscle. The role of vacuum packing is key in ensuring moisture does not increase during storage.

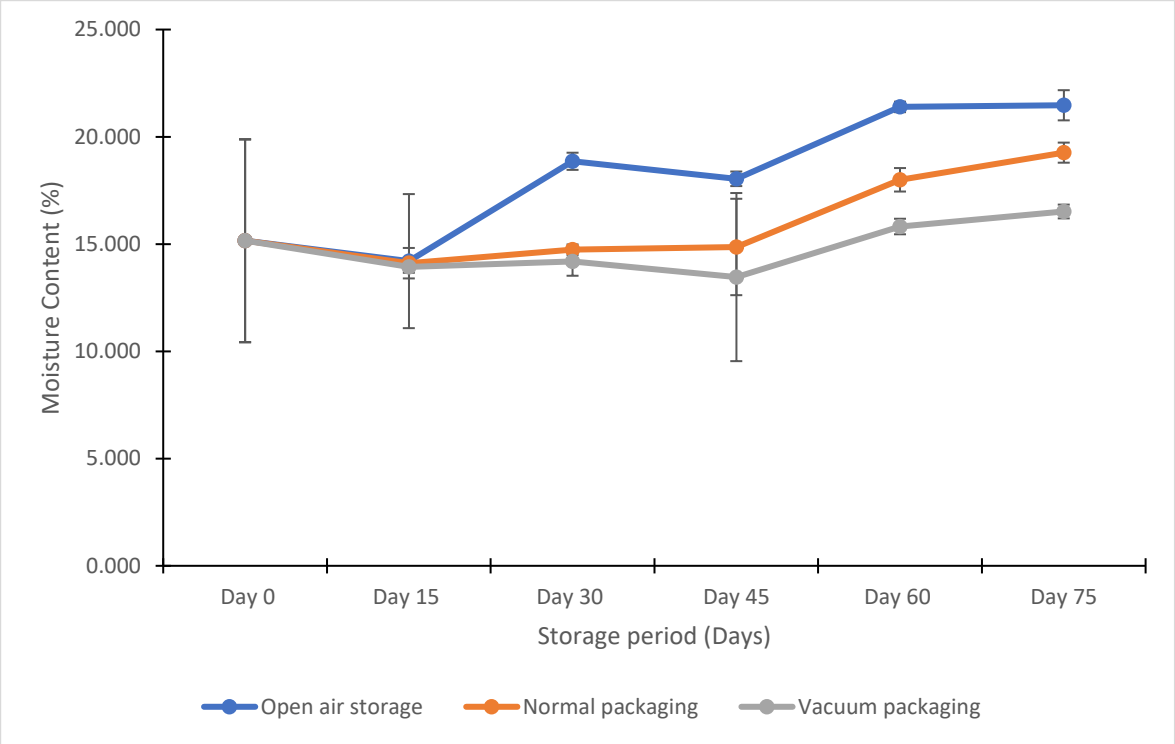


Figure 3.13: Changes in %moisture content (wet basis) of solar dried Siganid fish fillets during storage in the open air, in normal polythene packaging and vacuum packaging

3.3.6.2 Water activity

The water activity in the fish fillet that was dried without delayed processing was monitored during storage, either in the open air (no packaging), polythene bags or in vacuum packaging (Figure 3.14). The water activity at the beginning of storage for the *Siganid* fish stored in the open was 0.74 and it increased to 0.81 after 75 days of storage (Figure 3.14). The water activity in the *Siganid* fish stored in normal packaging increased from 0.74 to 0.75 and was still 0.74 at the end of storage. There was no increase in water activity for the fish stored in vacuum packaging, which showed a slight decrease to 0.72 after 75 days of storage.

Vacuum packing was responsible for keeping the water activity low (Kumar, P and Ganguly S, 2011). Dried fish can be stored safely for up to 180 days (Farid et al, 2014). Water activities below 0.8 for beef products are common and are equivalent to about 23% moisture. The water activity achieved in this study during storage is still below the threshold of 0.8 hence the fish is still safe for consumption. There is however a complex relationship between moisture content and water activity. Moisture content is useful when looking at the time to end the drying process.

Water activity provides information for evaluating spoilage on storage. It is not always correct to assume that a higher moisture content will mean a higher water activity (Karel, 1975; Potter 1968; Oduor-Odote et al, 2010).

3.3.6.3 pH

The pH during storage of the Siganid fillets was 6.83 at day 0 for the fish stored in the open. This decreased to 5.6 at the end of storage (Figure 3.15). The drop was by 1.32 pH units equivalent to 18%. In the fish stored in normal packaging the pH dropped from 6.83 to 6.05 representing an overall drop of 11.42 % while for the fish stored in vacuum, the drop was from 6.83 to 6.15 equivalent to 9.9%.

Changes in pH occur during storage but is normally faster in ambient storage equivalent to open storage. Although pH has been used to measure fish deterioration, the variation in pH is due to many factors like chemical composition, storage conditions in relation to air among others (Susanto et al, 2011).

Under normal circumstances, the pH is expected to drop, then increase again during storage. (Farid 2014). In this case the pH decreased. This means that there was some activity during storage that could be due to a factor other than glycogen and its effect was higher in the fish stored in the open than in packaged fish. The hydrolysis of lipids to fatty acids could be another reason for the decrease in pH. During storage of fish muscle in ambient conditions, El-Deen and El-Shemery (2010) noticed a decrease in pH. Also, dissolution of CO_2 into the packaging bags may lead to a drop in pH (McMekeen et al, 1982).

3.3.6.4 PV

The peroxide value was monitored during a75 day storage period in the fish that was processed at 0 hr, without delayed icing. The fish fillets were stored in the open, normal polythene bags and vacuum packaging. The peroxide value at the start of storage was 3.44 mEqO_2/kg for fish stored in the open and increased to 130 mEqO_2/kg after 75 days of storage (Figure 3.16). In the fish stored in polythene packaging, the PV increased from 3.44 at the start to 78.94 mEq O_2/kg and for fish stored in vacuum packaging the PV increased from 3.44 to 35.0 mEq O_2/kg after

75 days storage. The PV value continued to rise during storage of *Siganids* suggesting that the propagation of free radicals and primary products of lipid oxidation continued up to 75 of storage. The oxidation was increased by 3722% from the starting value for fish stored in the open, 2194% for fish stored in polythene and 917% for fish stored in vacuum packaging. Lipid oxidation is influenced by light and oxygen. The higher value of PV in the fish stored in the open and those stored in normal polythene could be because of both light and oxygen while the increase in PV in the vacuum-packed fish could be due to light mainly because the packaging was permeable to light. PV normally rise, peak and then drop due the formation of secondary and finally tertiary products, as many studies have shown (Ghlaly et al 2010). Other studies have also shown a continuous rise in PV of fish oil without peaking, and then dropping during storage (Boran et al, 2006). PV limits for fresh fish oil is 20mEqO_2/kg (Jinadassa 2014). Due to the rise and fall of values as the primary products break down to secondary products, more than one indicator should be used to monitor spoilage.

3.3.6.5 TBARS

TBARS decreased throughout the storage period (Figure 3.17). The TBARS decreased from 5.65 mg malondialdehyde per kg at day 0 to 1.98 mg malondialdehyde per kg of fish stored in the open, 2.42 mg malondialdehyde per kg for fish stored in polythene packaging and 3.08 mg malondialdehyde per kg for fish stored in vacuum packs. There was a 64% drop in TBARS in open storage, 60% drop in polythene storage and 45% drop for fish stored in vacuum packs.

TBARS are formed because of hydroperoxide breakdown from lipid oxidation with the formation of secondary products like aldehydes. The limit for TBARS is 5 mg malonaldehyde/kg for good quality fresh fish although fish can be consumed even when TBARS are 8.0 mg MDA/kg (Shallam et al 2007).

The fish for storage was therefore within consumption range for humans. The decrease in the level of TBARS is due to breakdown to tertiary products and interaction and cross-linking between aldehydes like malonaldehyde with proteins particularly tyrosine, lysine and tryptophan amino acids (Saeed and Howell, 2002, Saeed et al, 1999, Shallam et al, 2007). The rate of formation of PV can also be higher than TBARS as the latter break down and interact with other compounds.

3.3.6.6 TVB-N

TVB-N was monitored during storage of the Siganid fillet. The fish used was the fresh one that was stored for 0 hr in the 10hr delayed processing category. All were mixed randomly and fresh TVB-N values taken. The fish fillets were packed in 3 different packaging categories. The first batch was one left in the open, the second batch was stored in normal polythene bags and the third one was vacuum packed. The storage was for 75 days.

The level of TVB-N was 2.78 mg N per 100 g at the beginning of storage in the fish kept in the open and increased to 27.75 mg N per 100 g after 75 days of storage equivalent to 899% increase (Figure 3.18). In the fish packed using normal packaging, the TVB-N value increased from the baseline of 2.78 to 16.6 mg N per 100 g after 75 days of storage equivalent to an increase of 497%. The fish stored in vacuum packs and stored for 75 days the TVB-N increased from 2.78 to 8.83 mg N per 100g equivalent to 217.6%. The TVB-N levels were all within the limits allowed for fresh fish though the limits for dried fish has not been established. The allowable limits set by the EC 95/149 is 35 mg TVB-N per 100g. Similar studies have given TVB-N values of 17 to 33 mg N /100g for dried fish (Pavakar et al, 2013; Jinadasa 2014).

The lowest increase in TVB-N was in the fish stored in vacuum and the highest increase was for those stored in the open. TVB-N is considered a fish spoilage indicator rather than a fish freshness indicator. These levels apply to fresh fish (Orban et al, 2011). The TVB-N value is categorized in quality classes with TVB-N up to 25 mg N per 100g considered “high quality” up to 30 mg N per 100 g considered “good quality” and between 30-35 mg N per 100g falling in “level of acceptability” and above 35 mg N per 100g considered “spoilt” (Jinadassa 2014).

Random checks for TVB-N levels for dried fish in different markets reveal a wide variation with values as high as 53.4 mg N per 100 g going up to 98 mg N per 100 g being recorded (Prakash et al, 2001; Yamanaka et al 1986.; Jamila and Ranjitha 2009). There are no reported studies on dried fish indicator parameters for Siganids. This study is however very useful to dry chain fish processors to obtain data for spoilage of dried fish deterioration and to adopt proper storage conditions. The vacuum packaging acts as a barrier preventing spoilage agents like moisture, and bacteria from penetrating and reaching the fish

3.3.3.7 Temperature

Temperature is important during storage of dried fish because less than optimum temperatures can influence microbial growth and favour lipid oxidation. In this study, the temperature during storage of fish ranged from 30.4ºC at the beginning of storage to 23ºC at the end of storage (Figure 3.19). This was due to weather changes over time. Temperature during storage of dried fish is as critical as temperature considerations in preservation of fresh fish. The temperatures of storage influence bacterial growth (Jinadassa 2014). Temperatures of between 5ºC and 60ºC can easily favour microbial growth so storage under such conditions can lead to deterioration of fish. In the case of dried fish, the ones that are stored at temperatures indicated above (23-30ºC) can result in microbial propagation if other conditions are right. Vacuum packaging will help under such circumstances.

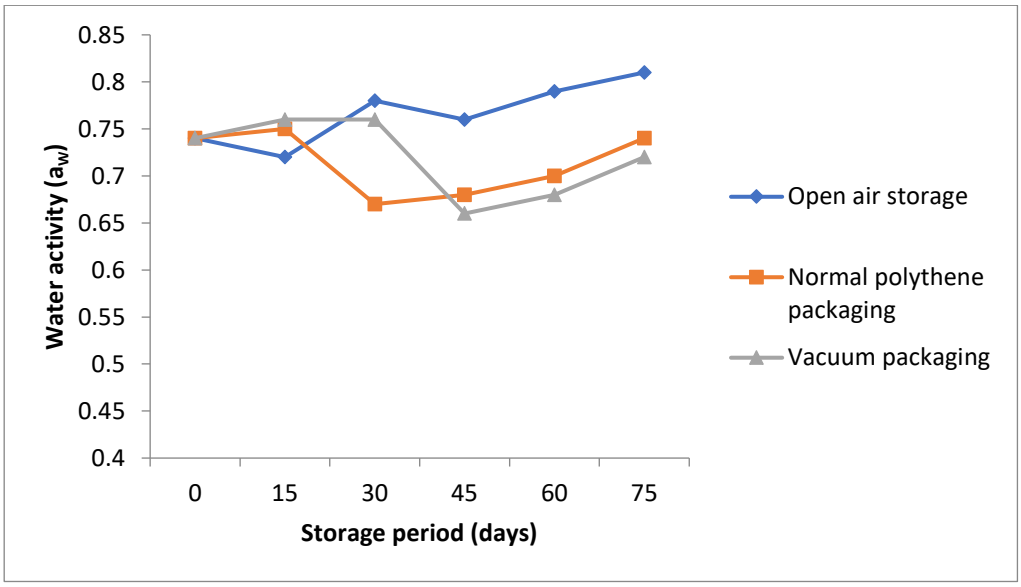


Figure 3.14: Changes in water activity of solar dried Siganid fish fillets during storage in the open air, in normal polythene packaging and vacuum packaging.

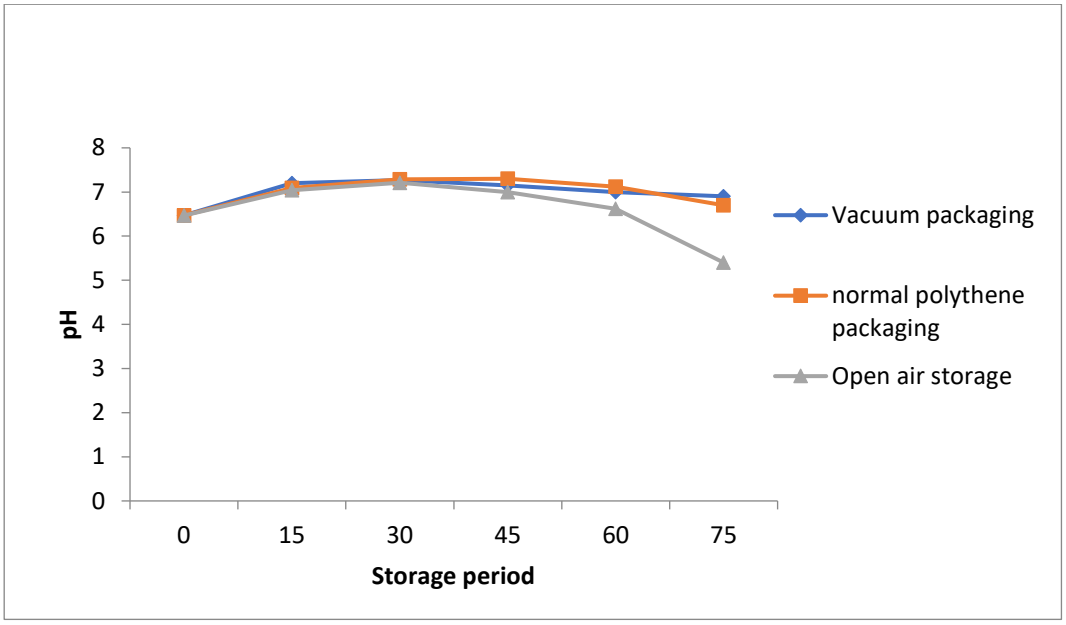


Figure 3.15: Changes in pH of solar dried Siganid fish fillets during storage in the open air, in normal polythene packaging and vacuum packaging.

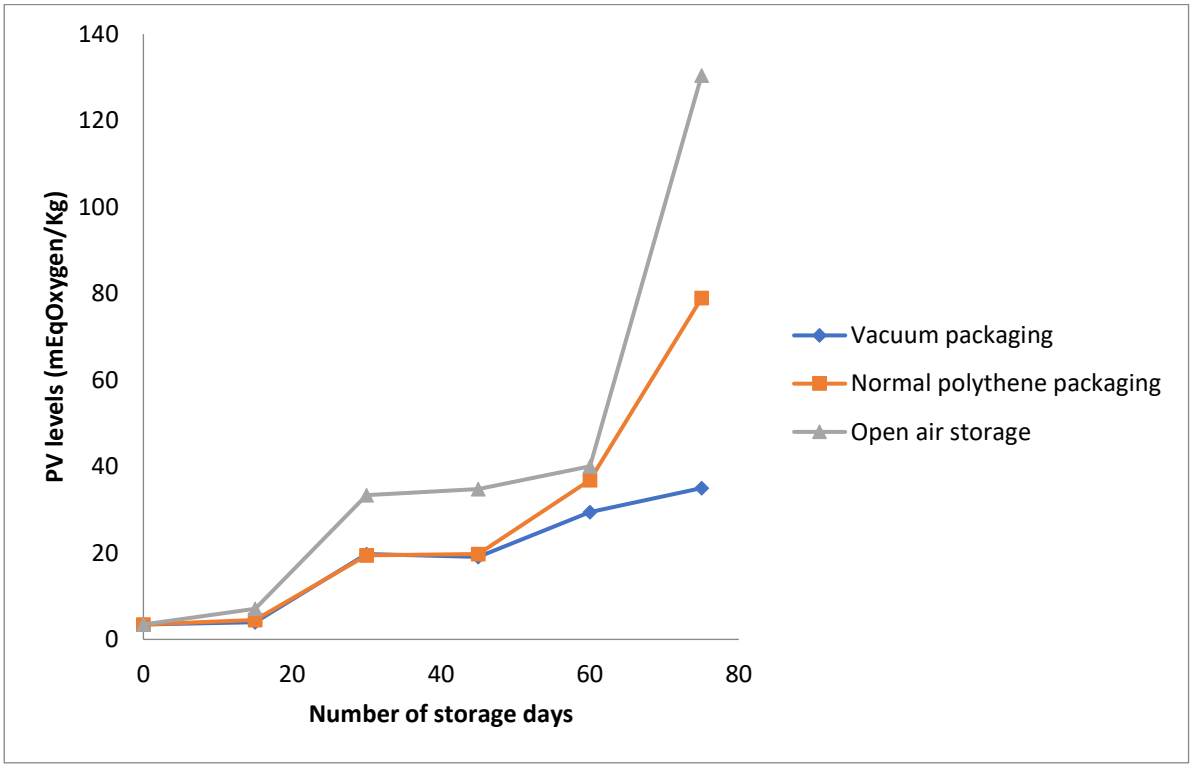


Figure 3.16: Changes in PV (peroxide value) of solar dried Siganid fish fillets during storage in the open air, in normal polythene packaging and vacuum packaging.

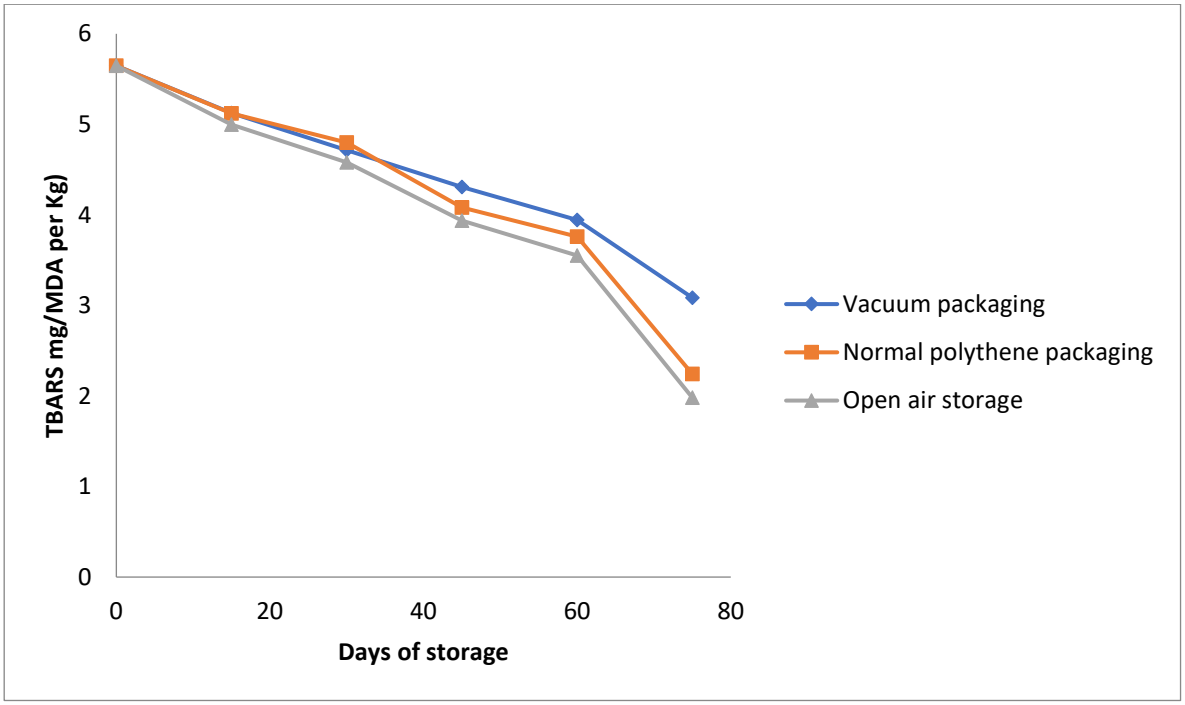


Figure 3.17: Changes in TBARS of solar dried Siganid fish fillets during storage in the open air, in normal polythene packaging and vacuum packaging.

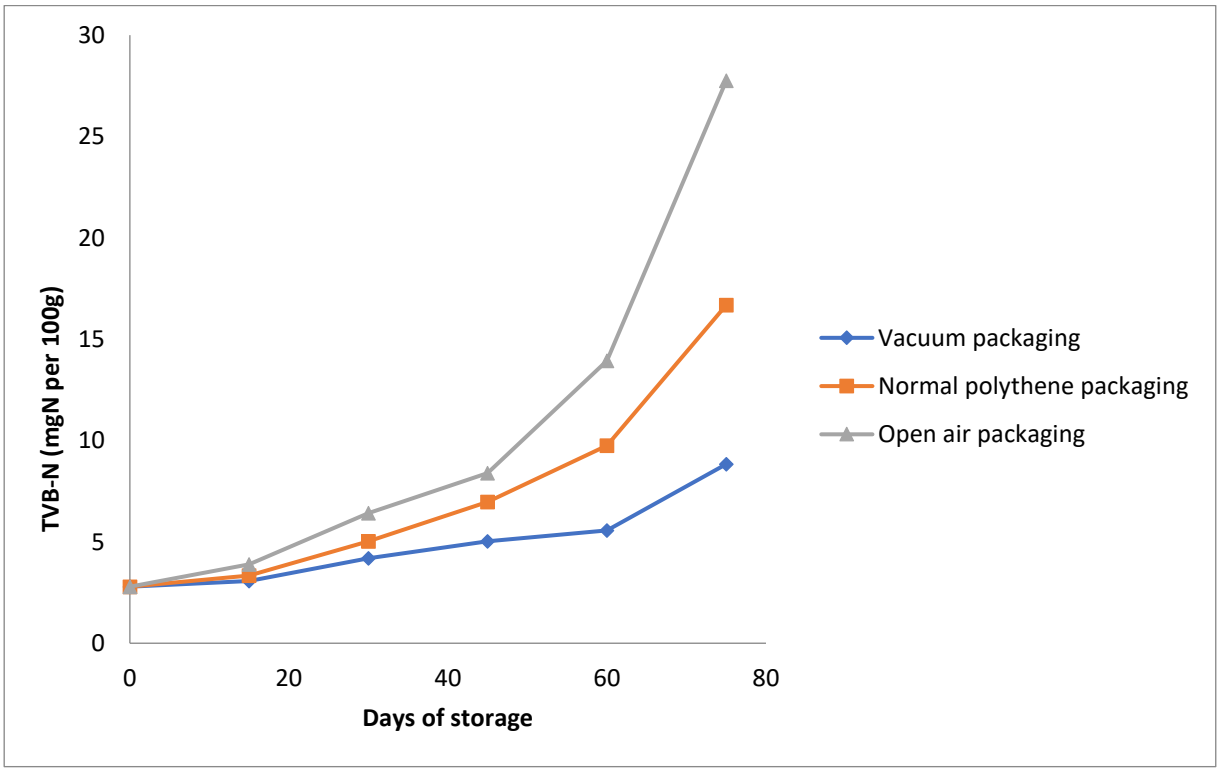


Figure 3.18: Changes in TVB-N of solar dried Siganid fish fillets during storage in the open air, in normal polythene packaging and vacuum packaging.

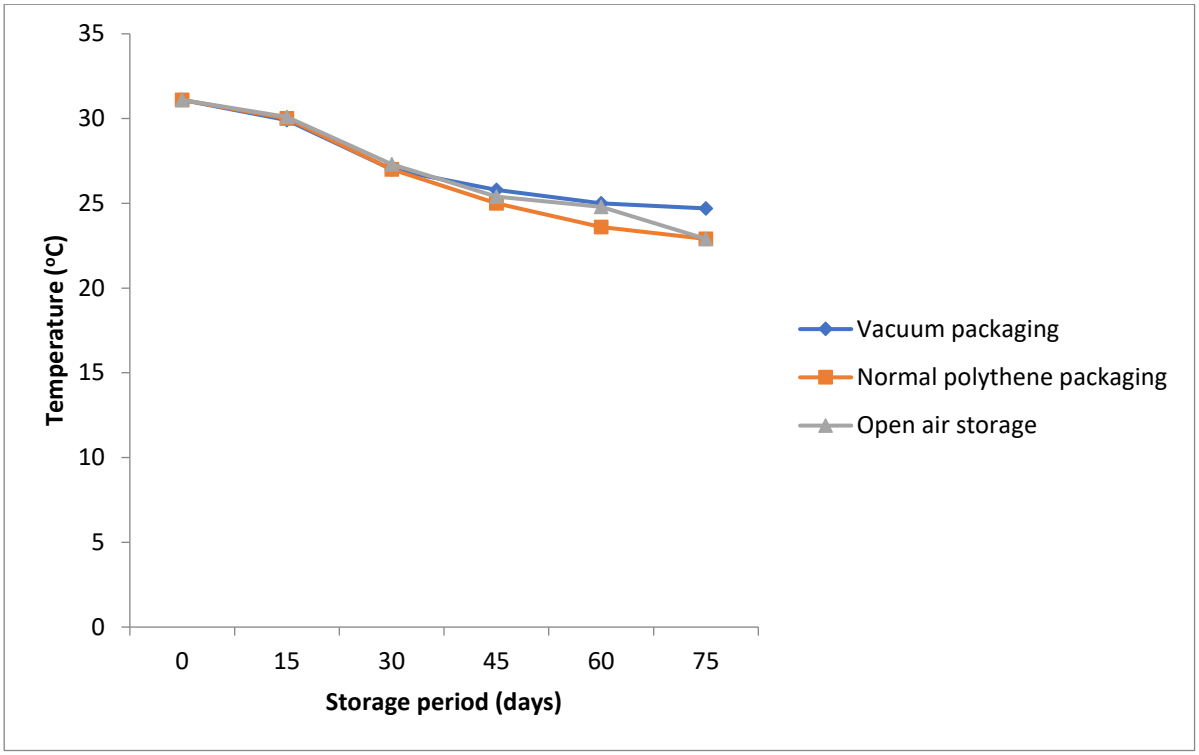


Figure 3.19: Changes in temperature surroundings of solar dried Siganid fish fillets during storage in the open air, in normal polythene packaging and vacuum packaging.

3.3.6.8 Conclusions

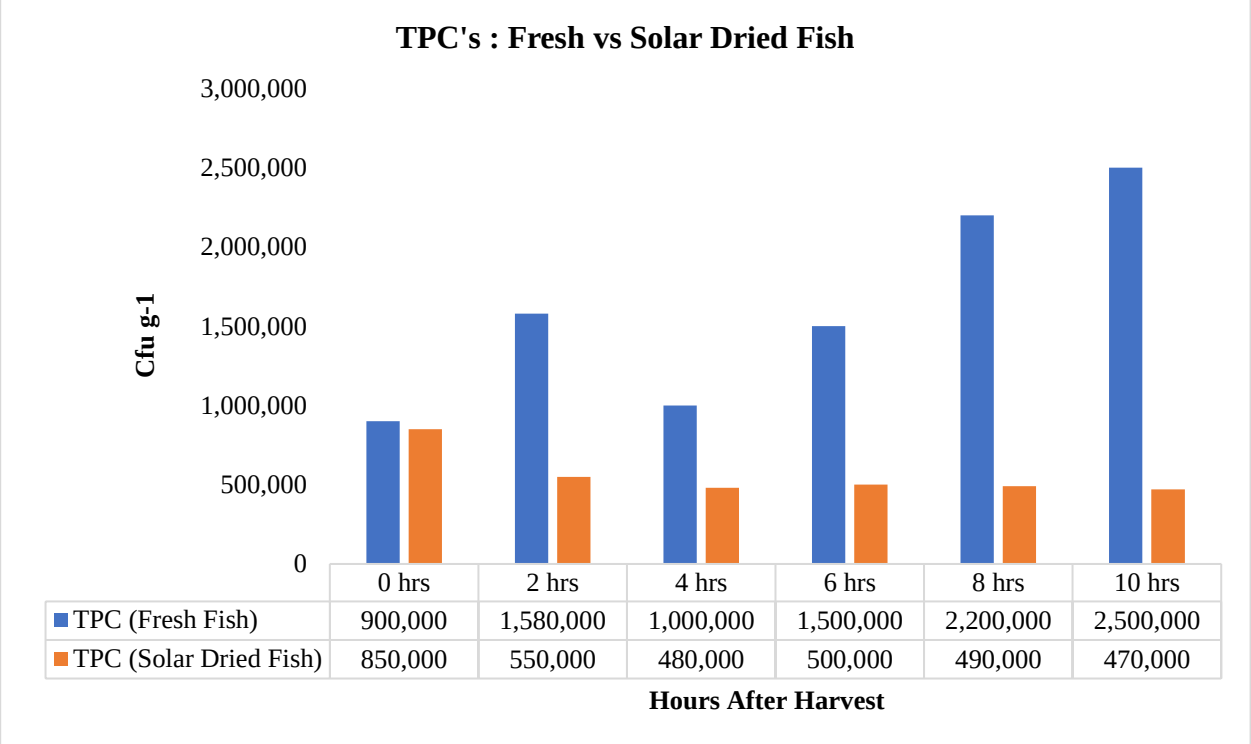
The moisture content increase was lowest in the vacuum packed stored fish. Peroxide value increased the least in the vacuum-packed fish. TBARS decreased during storage and the decrease was lowest in the vacuum-packed fish. TVB-N was also the lowest in the vacuum packed stored fish. The pH decreased during storage. Water activity increased minimally in the vacuum packed stored fish during the storage period. It is important to note that spoilage indicators for dried fish cannot be limited to one parameter, but many should be assessed. Packaging particularly vacuum packaging of dried fish products should be introduced in Kenyan laws in the Fisheries Act. However, further studies on the growth of anaerobic bacteria is needed.

3.3.7 Microbiological analysis and evaluation

The spoilage rate of post-harvest fish (*Siganus* spp) samples was tested. Temporal variation of microbial loads (total viable plate counts and spoilage specific microorganism counts) was used as an indicator of the spoilage rates with varied periods of delayed icing. These results

justify the need to dry harvested fish to minimize the rate of microbial division, thus preserving the quality of the fish and minimizing losses attributable to microbial fish spoilage.

3.3.7.1 Bacterial load of freshly harvested versus solar dried fish



	0 hrs	2 hrs	4 hrs	6 hrs	8 hrs	10 hrs
TPC (Fresh Fish)	900,000	1,580,000	1,000,000	1,500,000	2,200,000	2,500,000
TPC (Solar Dried Fish)	850,000	550,000	480,000	500,000	490,000	470,000

Figure 3.20: Total Plate Counts: Fresh vs Solar Dried Fish

The total plate counts for fresh fish ranged from 9.0 x 10^5 (immediately after harvest) to 2.50 x 10^6 (10 hours after harvest) while those for solar dried fish ranged from 8.50 x 10^5 to 4.70 x 10^5 colony forming units per gram of sample (Fig 3.20).

Drying reduced the spoilage specific organism loads from 6.0 x 10^5 – 2.90 x 10^6 cfu g^{-1} for freshly harvested fish to 8.0 x 10^4 – 9.90 x 10^5 cfu g^{-1} after drying (Fig 3.21). The maximum plate and spoilage specific counts prior to drying were slightly higher than the permitted food-safe limits (10^5). This was attributed to the conditions of the fishing areas and the time taken before preservation.

The significant reduction of microbial counts after drying illustrated its role in retarding spoilage thus emphasizing its relevance to minimizing post-harvest losses.

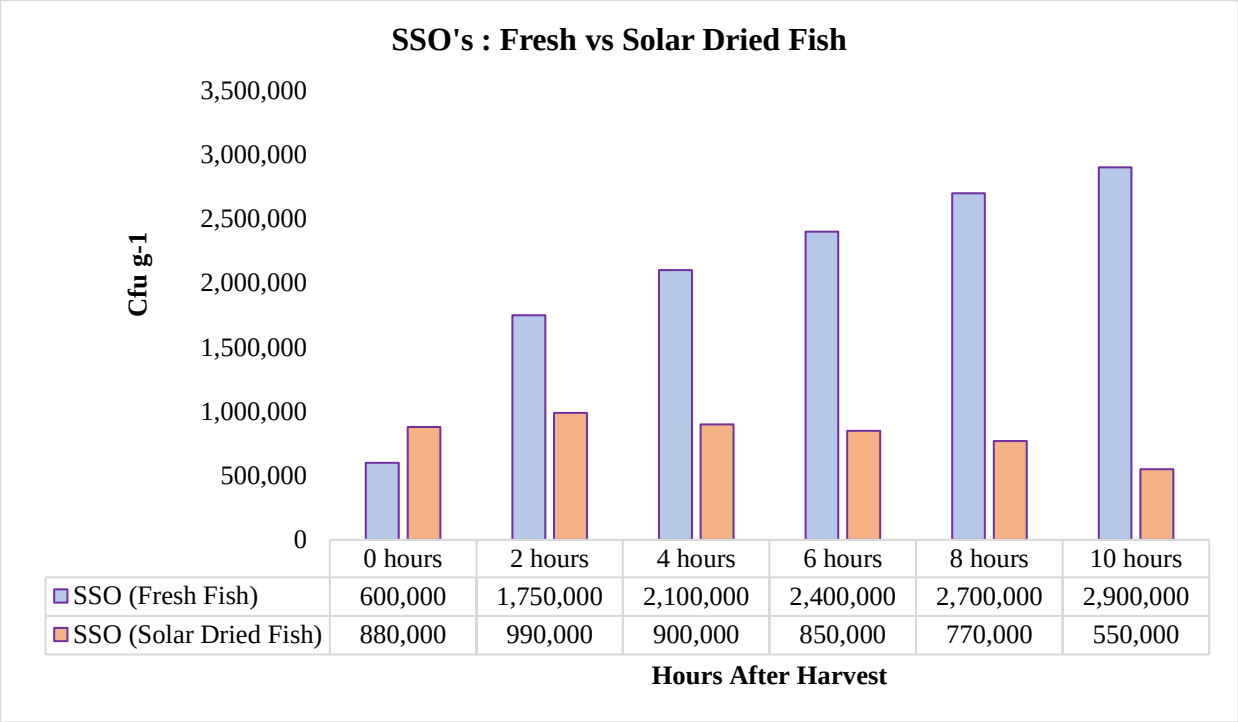


Figure 3.21: Spoilage Specific Organisms: Fresh vs Solar Dried Fish

3.3.7.2 Storage-specific, temporal variation in bacterial loads of solar dried fish

Solar dried samples were analyzed for the temporal variation (0, 14 and 28 days) in bacterial loads with exposure to different storage conditions (ambient, polythene bags and vacuum packaging). Table 3.3 illustrates the loads of total plate counts and spoilage specific organism counts obtained from samples of solar dried fish at Day 0. This was the baseline for further tests on day 14 and 28 under different storage conditions.

Table 3.3: Bacterial Counts for Solar Dried Fish (Day 0)

Sampling Time	TPC (Mean Total Viable Colony Counts) [Cfu g^{-1}]	SSO (Mean Total Viable Colony Counts) [Cfu g^{-1}]
0 hours	550,000 (5.50 x 10^5)	480,000 (4.80 x 10^5)
2 hours	530,000 (5.30 x 10^5)	480,000 (4.80 x 10^5)
4 hours	480,000 (4.80 x 10^5)	470,000 (4.70 x 10^5)
6 hours	490,000 (4.90 x 10^5)	350,000 (3.50 x 10^5)
8 hours	470,000 (4.70 x 10^5)	270,000 (2.70 x 10^5)
10 hours	193,000 (1.93 x 10^5)	93,000 (0.93 x 10^5)
RANGE	1.93 x 10^5 – 5.50 x 10^5	0.93 x 10^5 – 4.80 x 10^5

Cfu g^{-1} = Colony forming units per gram of fish flesh

Figure 3.22: Temporal variation in bacterial loads of solar dried fish stored differently

3.3.7.3 The bacteriological quality assessment of ice and processing water

The most probable number (MPN) method was used for the estimation of the bacterial load in both the processing (i.e., washing) water as well as the ice used in preservation during delayed processing.

The estimated Most Probable Number of the bacterial load in the processing water was >2,400 (Table 3.4). The MPN of the bacterial load in the washing water was rather high suggesting there was cross contamination of the fish samples during processing. This is the water available in the field.

Table 3.4: The MPN of the bacterial load in the processing (i.e. washing) water during delayed icing

Month	**Jan, 2013**
MPN Values	>2,400

MPN = Most Probable Number

The estimated Most Probable Number of the bacterial load in the preservation ice during the project period was found to be 49 (Table 3.5). This number was lower and suggested that there was no cross contamination resulting from the preservation ice.

Table 3.5: The MPN of the bacterial load in the preservation ice during the project Period

Month	Jan, 2013
MPN Values	49

MPN = Most Probable Number

3.4 Discussion-Microbiology evaluation and conclusions

Bacterial growth is a cause of fish spoilage, apart from causing disease. Bacterial numbers can be used as an indicator of fish quality. In this study the total number of bacterial count for the freshly landed *Siganus* ranged from 0.90 x 10^6–2.50 x 10^6 cfu g^{-1} of fish flesh for the TPC analysis whereas SSO ranged between 0.60 x 10^6 – 2.90 x 10^6. These levels were however higher than the maximum limits recommended by both the International Standards Organization (ISO-4833) and the Food and Agricultural Organization (FAO) of 5 x 10^5(ICMSF, 1980; FAO/WHO, 1976, 1978; WHO, 1974; and Codex, 1977). This variance in accepted limit

numbers has in fact made the developed countries such as Denmark and United States of America (USA) to set their own accepted maximum limits in a gram of flesh at 1 x 10^5 (100,000) and 5 x 10^5 (500,000) respectively. However, the observed count numbers were within the accepted limits mentioned by the Sudanese Standards and Metrology Organization (SSMO) of $5\times10^5 – 10^6 cfug^{-1}$ for fresh fish products (SDS 357). The numbers were also in the normal range of between $10^2 – 10^7 cfug^{-1}$ of fish flesh (Liston, 1980).

The existing wide range of bacterial flora counts on freshly caught fish, according to Shewan (1977), depends on the environment rather than the fish species. Thus, the considerable high microbial loads obtained from the freshly landed fish samples could possibly be due to poor handling by the fishermen at sea or the processors with different hygienic profile having regular skin contacts with the fishes. This aspect of cross contamination was effectively corroborated by the observed higher MPN values observed in the washing water during the study. Another possible explanation could be the unhygienic sanitary environment of the fish source (ocean) and the fishing vessel.

In cases of solar drying, the bacterial load counts were on average, within the normal ranges stipulated by ISO-4833 standards (Codex, 1977). However, the mean counts were highest (8.50 x $10^5 cfug^{-1}$) for TPC and (9.90 x $10^5 cfu\ g^{-1}$) for SSO. Thus, the low microbial counts obtained from the dried fish samples could be attributed to the low moisture content due to drying that made it difficult for microorganisms to grow.

This scenario changed, however, during storage and the counted numbers slightly increased with the majority still being in the range of 10^5. This slight change in the bacterial counts could be due to the hygroscopic nature of the samples which provided the microorganisms with moisture for growth. This hypothesis was confirmed when the values reduced after the fourteenth day of storage because of moisture reduction due to packaging. Thus, a general reduction in the bacterial counts was observed with all forms of packaging for the stored solar dried fish samples. This study was used to develop the HACCP.

3.5 Conclusions

- The raw material quality has a direct impact on yield and production costs of *Siganus sutor*. There was an increase in filleting time and lower filleting yield during delayed processing.

- The levels of PV, TBARS, TVB-N, and TMA increased during delayed processing as a sign of quality deterioration. The pH values reduced during delayed processing.

- Further studies could be done to document all production costs. There is need to further link the developed QIM scheme for *Siganus sutor* to precisely characterize quality with storage time.

- During actual solar drying, PV and TBARS decreased because of unstable nature of hydroperoxides and aldehydes interacting with proteins and TVB-N and TMA decreased owing to less bacterial action.

- Solar tunnel drying reduced biochemical spoilage indicators to a minimum before storage starts.

- During storage of the solar dried fish fillet for 75 days, the moisture content and water activity change was at a minimum in the vacuum-packed fish compared to those in normal polythene and packaging and those kept in the open.

- Peroxide value, a lipid oxidation indicator increased the least in the vacuum packed *Siganid* fish fillet compared to those stored in the open and in normal polythene.

- TBARS, another lipid oxidation indicator and TVB-N decreased during storage and the decrease was lowest in the vacuum-packed fish compared to those stored in normal polythene and in the open.

- Vacuum packaging of fish products should be recommended in the Kenyan Fisheries Act.

CHAPTER 4

CHAPTER 4

4.0 Lipid oxidation in solar dried fish with and without antioxidants

4.1 Introduction

Sea fish have high nutritional value due to readily digestible protein, mineral and vitamins and high levels of polyunsaturated fatty acids. These are the omega 3 group of fatty acids namely eicosapentaenoic acid (EPA) and docosohexaenoic acid (DHA) (Ackmann, 1999).
Based on epidemiological studies, a marine rich diet containing fish is of benefit to the consumer because of the presence of peptides and the highly unsaturated fatty acids that have been associated with lowering the risk of cardiovascular diseases including lowered plasma lipids and decreased blood pressure (Shekelle et al, 1985, Kromhout et al (1985)

However, whether fish is preserved by frozen storage or drying, there is still deterioration of the fish muscle due to the oxidation of polyunsaturated fatty acids (Taheri and Motallebi, 2012). The high rate of deterioration of the polyunsaturated fatty acids by lipid oxidation requires control. Lipid oxidation products are harmful if accumulated in a large quantity and consumed. The lipid oxidation products also cause protein denaturation which will affect texture and nutritional value of the fish proteins negatively (Saeed and Howell, 1999).

Lipid oxidation in fish tissues is controlled by an efficient antioxidant system. This is made up of α-tocopherol or vitamin E which stabilizes the oxidation of the high content of unsaturated fatty acids when fish is still alive (Decker et al, 2000; Jia et al, 1996). The efficiency of the antioxidant mechanism in the post mortem or post-harvest state is lost due to the consumption of the intrinsic antioxidant in the oxidative processes and there is no source of fresh antioxidants (Petillo et al 1998).

Post-harvest lipid oxidation control in fish requires utilization of antioxidants. The available antioxidants in the market are synthetic ones like butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and BHQ which are facing resistance in the market because they are suspected to be carcinogenic. There is increasing interest in the identification of plant extracts that can retard or minimize lipid oxidation in lipid containing foods (Sanchez-Alonzo et al, 2007) and for use *in vivo*. Fish with a high content of polyunsaturated fatty acids is highly

susceptible to lipid oxidation, resulting in rancidity and undesirable changes in flavor, texture, colour and nutritional value (Alghazeer et al, 2008; Pereira de Abreu et al, 2011).

Successful utilization of antioxidants from natural sources will highly depend on constant supply of raw material for sustainability. There are aquatic plants in Kenya that are abundant in supply and are rich in natural antioxidants especially phenolic compounds that can be useful sources of antioxidants. The water hyacinth which is an alien aquatic plant has been tested for phenolic compounds that have antioxidant activity (Shanab and Salaby, 2012; Lalitha and Jayanthi, 2012; Surendrarai et al 2013).

At the south coast of Kenya, the seaweed -*Eucheuma denticulatum* and *Kappahyccus alverezi* (cottoni) are now farmed with the whole seaweed harvested, dried and sold to middlemen at low prices because it is not processed further into value added products (Bolton et al, 2007.; Wakibia et al, 2011, KMFRI Bulletin 2018). Seaweeds contain other higher value products like polysaccharide stabilizers including carrageenan and alginates, bioactive compounds and lately phenolic antioxidants (Matanjun et al, 2008; Moubayed et al, 2017). Turmeric has been used in foods over time for flavor and coloration of foods and is reported to have strong antioxidant properties (Priya and Prabhu, 2006).

Fish drying is a preservation method that is used to control postharvest losses in fish, however, the dried fish undergo further deterioration during storage due to lipid oxidation. The lipid oxidation products affect the texture and cause denaturation of protein thus lowering the eating quality and nutritional value.

4.1 Materials and Methods

The sources of the natural antioxidants used were water hyacinth, seaweeds (*Euchema denticulatum*) and turmeric. Butylated hydroxy anisole (BHA) were used as synthetic antioxidant controls and were purchased from Sigma.
The methods described are those used to determine total phenolics and antioxidant activity.

4.1.1 Preparation of plant antioxidant samples

The seaweeds (*Euchema denticulatum*) were purchased from Kibuyuni in the south coast of Kenya from local women community members involved in seaweed farming. The plants were

transported to the laboratory in KMFRI 2 hr drive away (90 km). They were washed thoroughly in freshwater and rinsed by letting them hang to drip for 1 hour. They were then cut into smaller pieces and placed in an oven set at 45^{o}C to dry to a constant weight. The drying period took 3 days. The dried seaweed samples were reduced to fine powder using a kitchen blender. The ground samples were placed in polythene bags and kept in a freezer at -20^{o}C until analysis.

Turmeric samples were purchased from the local market in Mombasa, brought to KMFRI, chopped into smaller pieces and dried in an oven at 45^{o}C for 4 days to a constant weight. The dried turmeric samples were homogenized into fine powder using a kitchen blender, placed in polythene seal lock bags and stored freezer at -20^{o}C until analysis.

Water hyacinth was collected from Lake Victoria in Kisumu and separated into roots, stem and leaves. Each of these parts was dried in an oven at 45^{o}C for 3 days to a constant weight still in Kisumu. The separated parts were placed in black polythene bags, then placed in an insulated Styrofoam box and transported by air to Mombasa. The samples were then transferred to a deep freezer set at -20^{o}C till analysis.

4.1.2 Extraction of plant polyphenols

Portions (100 g) of each of the 3 plants (seaweed, turmeric and water hyacinth) were each separately hydrolyzed with a solution of 3% H_2SO_4 (de Abreu et al, 2011) for 15 min at 130^{o}C at a liquid/solid ratio of 8:1 g/g. The residue was de-lignified with 6.5% NaOH for 1 hr at 130 ^{o}C, at a liquid solid ratio of 10:1 g/g. From the above liquid phase, phenolic compounds were obtained (ratio water phase/organic phase, 1:3), using ethyl acetate. A vertical evaporator was used to separate the organic phase.

4.1.3 Total phenolic content (TPC)

4.1.3.1 Sample preparation for TPC.

TPC was determined using Folin-Ciocalteu assay according to Henríquez et al, (2010). Ten grams of each of the dried and ground samples were mixed with 70% acetone. The mixture was homogenized for 1 min and shaken in a water bath at 20 ^{o}C for 1 hr. Three 1.5 ml aliquots were centrifuged at 2500 x g for 15 min at 4^{o}C. The supernatants was retained for analysis (1st extraction). After that the residue was re-extracted under similar conditions and the supernatant

was kept for analysis (2nd extraction). TPC levels in each supernatant, (1st and 2nd extraction) were measured. The total of the 1st and 2nd extraction for all TPC results were recorded.

4.1.3.2 Total Phenolic Content (TPC) determination

Folin-Ciocalteu reagent was reduced by phenolic compounds, to form a blue complex. Each extract (0.5 ml) and 3 ml of distilled water and 0.25 ml Folin-Ciocalteu reagent were mixed. Next 0.75 ml of saturated sodium carbonate and 0.95 ml of distilled water was added to the mixture and left for 30 min at 37°C. The absorbance was read by spectrophotometry at 765 nm. The phenolic content was calculated from a gallic acid standard curve and expressed as milligrams of gallic acid equivalents per gram of fresh weight (mg GAE/g FW).

4.1.3.3 Application of the antioxidant samples in fish muscle and storage trials

Fish (Siganids) samples were purchased from local fishermen and iced immediately. All fish were purchased fresh and their quality assured. The sources of antioxidants were turmeric, water hyacinth, seaweed and commercial BHA.

Fish samples were filleted, and skin removed. The filleted samples weighing 25 kg were then divided into five equal portions each weighing 5 kg. Each portion was then placed on a stainless-steel tray. The portions of 5kg each were separately treated with 2.5% w/v of seaweed, 2.5% w/v of water hyacinth, 2.5% w/v turmeric; and 1.0 % w/v of BHA solutions in distilled water with the latter acting as the control (Table 4.1). The fillets with the extracts were each placed in a plastic container with ice overnight for drying the following day.

Table 4.1: Incorporation of the natural antioxidant extracts from water hyacinth, seaweed, turmeric and BHA (positive control) in the Siganid fillet for drying then storage.

Treatment	Details	Drying	Storage
Turmeric	• Purchased from market, dried in oven, ground to fine mesh • 75g taken and mixed in 3000 mls water • 5kg Fillet then soaked in the mixture and left to settle for 12 hours (overnight) • Fillet removed and placed on drying trays of the dryer at an angle for 30 mins to drip • Removed and transferred to the drying chamber of solar hybrid windmill dryer	a) Drying continued and weight loss taken every 2 hours until a constant weigh obtained. b) Samples wrapped in aluminium foil each time of sampling and placed in ice. c) Temperature, water activity determined. d) Data collected for drying characteristics	End of drying, samples transferred to the lab in KMFRI for storage in (i) Open air (ii) Ordinary polythene (iii) Vacuum packs Samples being analysed for shelf-life evaluation
Water hyacinth	• Collected from Lake Victoria in Kisumu • Dried in an oven at 45^{o}C then ground to fine mesh like turmeric • 75g of dried ground water hyacinth mixed with 3000 mls water in plastic container • 5kg Fillet was soaked in the mixture and left to settle for 12 hours (overnight) • Fillet removed and placed on drying trays of the dryer at an angle for 30 mins to drip • Removed and transferred to the drying chamber of solar hybrid windmill dryer		
Seaweed	• Collected from seaweed farm in Gazi, south coast of Kenya • Dried in an oven at 45^{o}C for 3 days • Ground into a fine mesh • 75g of dried ground seaweed mixed with 3000mls water in plastic container • 5kg Fillet then soaked in the mixture and left to settle for 2 hours • Fillet removed and placed on drying trays of the dryer at an angle for 30 mins to drip • Removed and transferred to the drying chamber of solar hybrid windmill dryer		

BHA	• Purchased from Sigma • 10g of Butylated hydoxyanisole mixed well in 1000 mls of water in a plastic container • 5 kg fish fillets were immersed in the mixture for 12 hours, removed, placed on trays to drip for 30 min. and then transferred to the drying chamber of solar hybrid windmill dryer		
CONTROL	• 5 kg fish fillets were immersed in water mixture for 12 hours, removed, placed on trays to drip for 30 min. and then transferred to the drying chamber of solar hybrid windmill dryer		

After drying, the fish fillets were placed in the drying trays outside the dryer to cool and were wrapped in aluminium foil, labelled and kept in polythene bags and transferred to the laboratory in KMFRI, 240 km away for storage trials. The parameters analysed during the 90 day storage were TBARs, PV, total phenolic content and % inhibition of free radical formation.

4.2 Results and discussion

Table 4.2 and Figure 4.1 show that water hyacinth leaves and turmeric have a higher phenolic content that reflect greater antioxidant activity. In this study however, the water-soluble extracts were used for turmeric, water hyacinth and seaweeds.

Table 4.2: Total phenolic content in water hyacinth stem, leaves and roots and in seaweeds and Turmeric as plant sources for natural antioxidants

Sample	Concentration in µg/ml
Hyacinth stem (E. crassipes)	0.026
Hyacinth leaves	0.028
Hyacinth roots	0.018
Sea weed (Euchema)	0.006
Tumeric (C. longa)	0.027

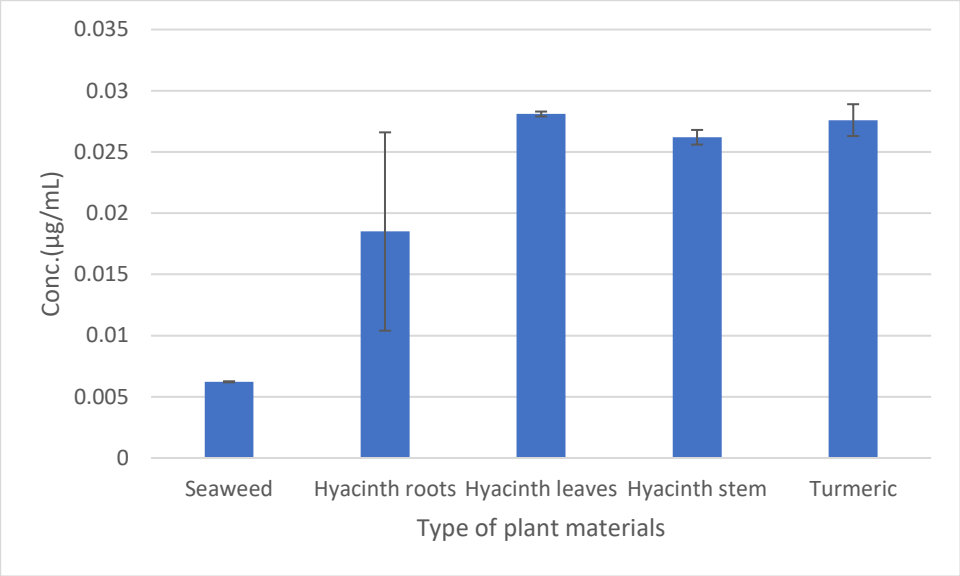


Figure 4.1: Total phenolic content in graphic form for extracts of water hyacinth, turmeric and seaweed

4.3 Storage trial results with antioxidants incorporated in fish muscle

4.3.1 Lipid oxidation indicators during storage of dried siganids

Lipid oxidation was measured as peroxide value (PV) and expressed as as MilliEquivalents O_2/kg and thiobarbituric acid reducing substances (TBARS) expressed as mg malondialdehyde /kg.

4.3.1.1 Peroxide value in fresh and stored *Siganids*

On day 0, the PV values in treated *Siganid* muscle were control 0.05±0.02 MilliEquivalents O_2/kg, BHA 0.066±0.003 MilliEquivalents O_2/kg; seaweed 0.006±0.011 MilliEquivalents O_2/kg, turmeric 0.06±0.02 MilliEquivalents O_2/kg and water hyacinth 0.04±0.001 MilliEquivalents O_2/kg (Figure 4.2).

After 90 days of storage of treated Siganid muscle, the PV in the control was 1.83±0.98 MilliEquivalents O_2/kg, BHA 0.14 ±0.03 MilliEquivalents O_2/kg; seaweed 0.39±0.18 MilliEquivalents O_2/kg; turmeric 0.23 ±0.11 MilliEquivalents O_2/kg and in the water hyacinth treated muscle the PV was 0.40±0.15 MilliEquivalents O_2/kg. The control had higher levels of PV after 90 days of storage compared to the muscle treated with BHA and antioxidant extracts from turmeric, seaweed and water hyacinth indicating the protection offered against lipid peroxidation in dried stored *Siganids*. The PV levels increased by 97.16% between day 0 and

day 90 in the control. The increase in PV in treated Siganid muscle between day 0 and 90 was BHA 58.84%; turmeric 73.92%, seaweed 85.08% and water hyacinth 89.09%. In order of % increase of PV, BHA <Turmeric<Seaweed<Water hycinth<Control.

The PV increased steadily in all treatments until day 60. Between day 0 and day 30, there is suppressed increase in PV in all the treatments reaching a maximum of 0.05 MilliEquivalents O_2/kg for BHA treated, 0.06 ±0.03 MilliEquivalents O_2/kg for turmeric treated, 0.07±0.02 MilliEquivalents O_2/kg and 0.07 ±0.004 MilliEquivalents O_2/kg for hyacinth treated Siganid muscle. The increase in PV in the control was significantly different on day 15 compared to day 0 ($p<0.05$) Table 4.3. In the BHA, turmeric, seaweed and water hyacinth treated Siganid muscle there was no significant difference in PV ($p<0.05$) between day 0 and 30. The antioxidants suppressed PV until day 30.

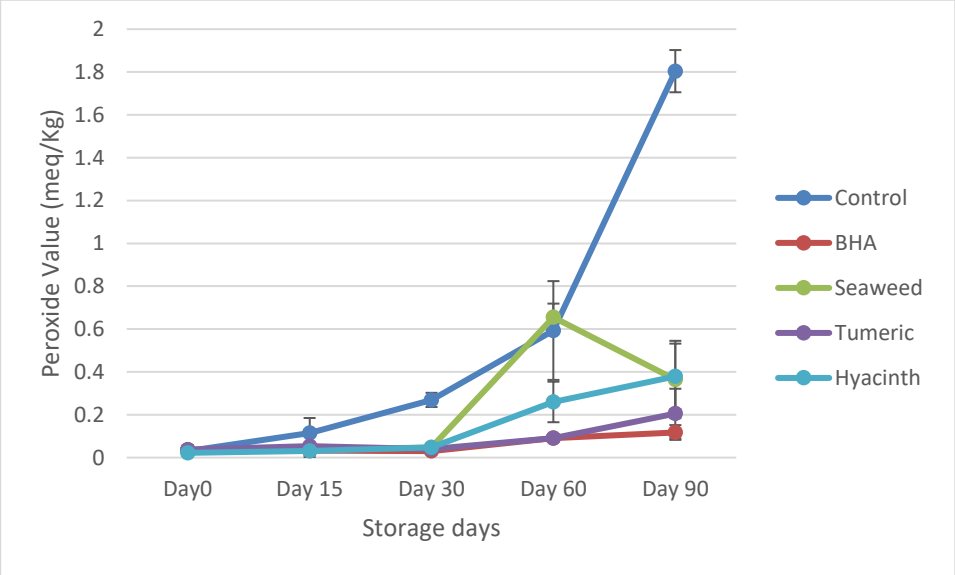


Figure 4.2: Change in peroxide value (PV) levels during 90 day storage of Siganid fillets treated with BHA (1% w/v in distilled water), seaweed (2.5% w/v in distilled water), turmeric (2.5% w/v in distilled water) and water hyacinth (2.5% w/v in distilled water without antioxidants.

4.3.1.2 TBARS

The TBARS value was 0.0±0.006 mg MDA/kg in the control at the start of storage on day 0 (Figure 4.3). The level of TBARS was 0.034± 0.001 mg MDA/kg on day 30 and increased to 0.64±0.011 mg MDA/kg on day 90 of storage. In the BHA treated Siganid muscle the level of TBARS was 0.027±0.000 mg MDA/kg on day 0, decreased to 0.0012±0.000 mg MDA/kg on day 30 and then increased to 0.051±0.006 mg MDA/kg on day 90. The TBARS in the turmeric treated Siganid muscle was 0.086±0.006 mg MDA/kg on day 0 and decreased to 0.002 ± 0.002 mg MDA/kg on day 30 and increased to 0.113±0.006 mg MDA/kg on day 90. In the seaweed treated Siganid muscle the TBARS value on day 0 was 0.086±0.006 mg MDA/kg. This

decreased to 0.002±0.001 mg MDA/kg on day 30 and then it increased to 0.183± 0.006 mg MDA/kg. In the water hyacinth treated Siganid muscle, TBARS levels were 0.090 ±0.011 mg MDA/kg on day 0, decreased to 0.0016± 0.0006 mg MDA/kg on day 30 and increased to 0.2067± 0.0055 mg MDA/kg on day 90. Between day 30 and day 60 there was a large increase in TBARS in the control from 0.0343 to 0.4173±0.011 mg MDA/kg equivalent to 91.7% compared with a lower increase observed in the BHA, turmeric, seaweed and water hyacinth treated Siganid muscle.

The TBARS for BHA treated muscle was 0.0017±0.006 mg MDA/kg in day 30 and increased to 0.03± 0.016 mg MDA/kg on day 60 which was the lowest level of TBARS observed indicating that BHA was the most effective antioxidant. In the turmeric treated Siganid muscle the TBARS increased from 0.0016± to 0.031±0.006 mg MDA/kg in day 60, followed by seaweed treated muscle where the TBARS increased from 0.0016±0.0005 on day 30 to 0.039±0.0055 mg MDA/kg on day 60; water hyacinth treated Siganid muscle where TBARS values were was 0.0016±0.0005 on day 30 and increased to 0.035±0.00 mg MDA/kg on day 60. The TBARS in the control and BHA treatment were significantly different on day 0, 15, 30 and 60 of storage ($p<0.05$).

In turmeric, seaweed and water hyacinth treated Siganid muscle the level of TBARS reduced and were significantly different between day 0 and day 15 only. The decrease in TBARS values in the first 30 days may be due to the cycling effect as peroxides (primary products) form and are converted to secondary products TBARS. After 30 days storage TBARS in treated samples increased significantly ($p<0.05$) in all samples as most peroxides are converted to TBARS. . However, both peroxides and TBARS continued to increase up to 90 days of storage in the untreated control samples. The protection offered by the treatments can be related to the phenolic contents of turmeric, seaweed and water hyacinth as described below.

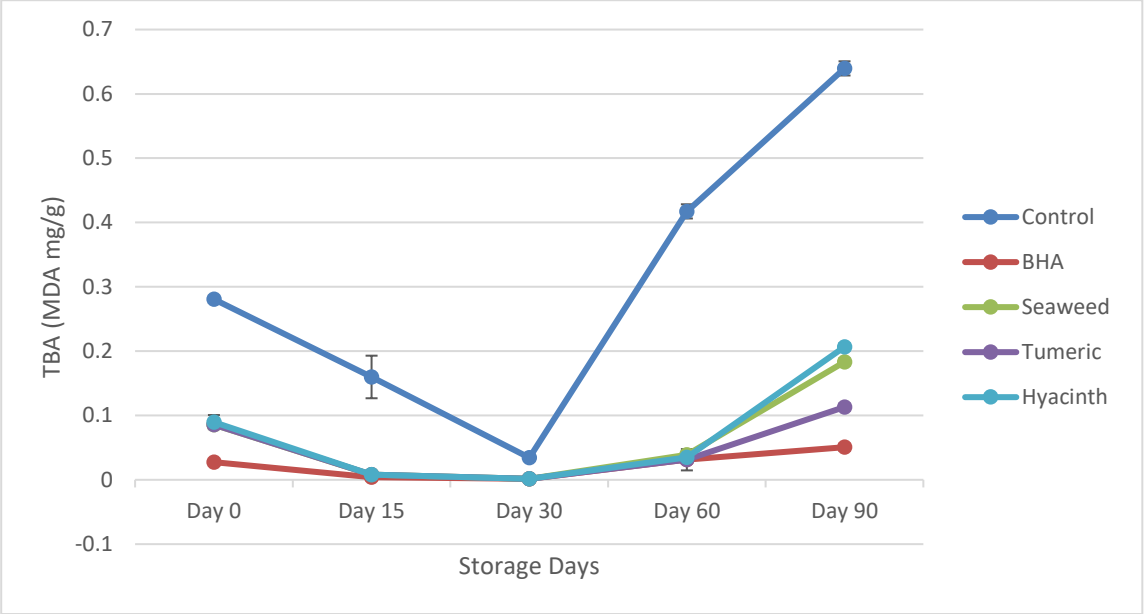


Figure 4.3: Change in TBARS levels during 90 day storage of Siganid fillets treated with BHA (1% w/v in distilled water), seaweed (2.5% w/v in distilled water), turmeric (2.5% w/v in distilled water) and water hyacinth (2.5% w/v in distilled water) water soluble antioxidant extracts. The control sample was treated with distilled water without antioxidants.

4.3.1.3 Total phenolic content in Siganid muscle

The total phenolic content in the control (fish muscle) was 0.002 µg/ml ±0.000 on day 0 and 0.0014 µg/ml± 0.001 µg/ml on day 90 (Figure 4.4). The highest phenolic content was in *Siganid* fish muscle treated with BHA at 0.012 µg/ml±0.001 on day 0 which decreased to 0.006 µg/ml±0.000 in day 90 of storage (45.70%). In turmeric treated fish, the TPC was 0.0082 µg/ml±0.0004 on day 0 which decreased to 0.004 µg/ml± 0.0001 on day 90 (52.38%). The total phenolic content in seaweeds was 0.005µg/ml±0.000 on day 0 that decreased to 0.003µg/ml±0.000 on day 90 (44.32%).

In the case of water hyacinth the decrease in total phenolic content was from 0.004 µg/ml±0.0004 to 0.00004µg/ml on day 90 (42.84%). There was a significant difference ($p<0.05$) in reduction of TPC on day 30 in the control (table 4.3) compared to day 0. In the BHA treated muscle there was no significant difference ($p<0.05$) in TPC in the first 30 days of storage. There was a significant difference by day 15 ($p<0.05$) in the turmeric treated *Siganid* fish muscle while in seaweed and water hyacinth treatments there was no significant difference during storage in the first 30 days (Table 4.3). The TPC in turmeric was twice the amount found in seaweed and water hyacinth at the beginning and during storage which reflected the higher antioxidant effect of turmeric. The TPC was lowest in the water hyacinth during storage and they showed the highest PV and TBARS values during storage.

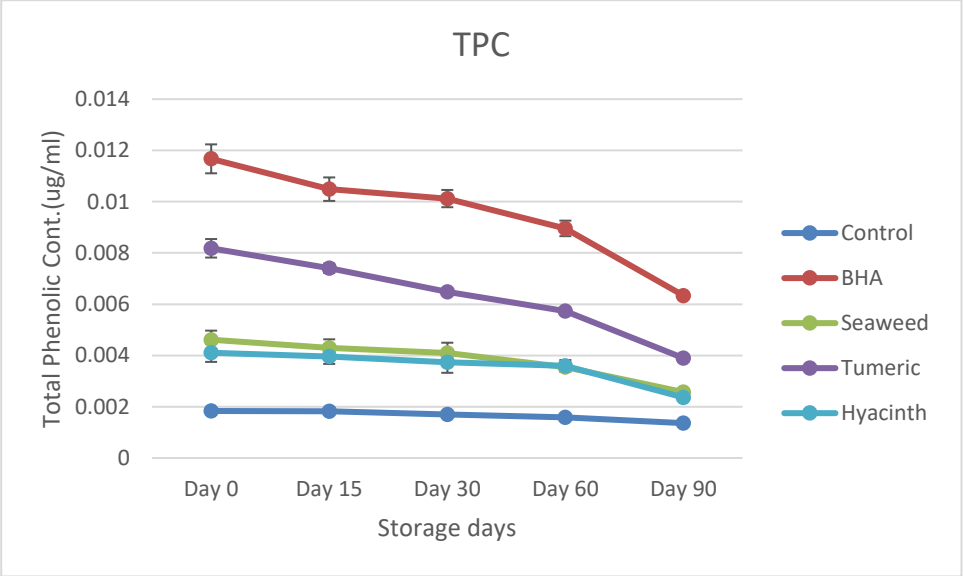


Figure 4.4: Change in total phenolic content levels during 90 day storage of Siganid fillets treated with BHA (1% w/v in distilled water), seaweed (2.5% w/v in distilled water), turmeric (2.5% w/v in distilled water) and water hyacinth (2.5% w/v in distilled water without antioxidants

4.3.1.4 % Inhibition of free radical formation during storage

The % free radical inhibition was lowest in the control at 16.24 % ± 0.50 followed by seaweed treated *Siganid* muscle at 31.70 % ±1.28, water hyacinth at 43.67±1.65 %, turmeric at 61.4046±0.14% and BHA at 88.01±0.79% on day 0 (Figure 4.5). The % inhibition decreased in all the 4 treatments and on day 90 it was still highest in the BHA treated muscle at 72.92%±0.21, 33.72±0.21 in turmeric treated muscle, 11.49±3.50 in seaweed and 11.77±0.21 in water hyacinth treated muscle.

The % inhibition of free radical formation during storage was lowest in control and highest in BHA treated *Siganid* followed by turmeric and water hyacinth and seaweed (Figure 4.5). The effectiveness of antioxidants is shown in table 4.3 over the 30-day period. The % inhibition was significantly different on day 15 in the control compared to day 0 of storage (Table 4.3). In the BHA, turmeric and seaweed treated siganid muscle, the reduction in % inhibition was significant ($p<0.5$) on days 0, 15 and 30. In the water hyacinth treated Siganid muscle the % inhibition was significantly different on day 15 compared to control and seaweed treated Siganid.

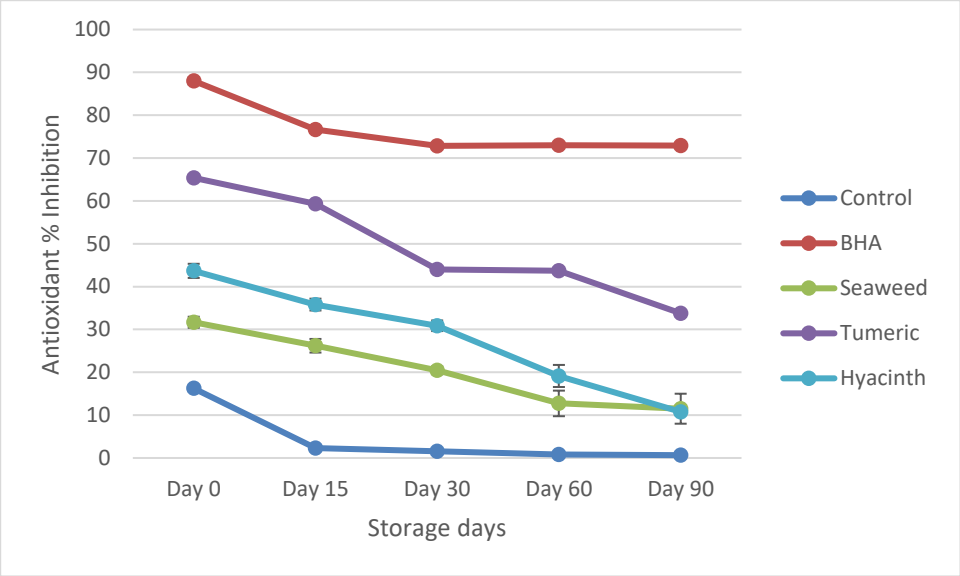


Figure 4.5: Change in % inhibition of free radical formation during 90 day storage of Siganid treated with BHA (1% w/v in distilled water), seaweed (2.5% w/v in distilled water), turmeric (2.5% w/v in distilled water) and water hyacinth (2.5% w/v in distilled without antioxidants

Table 4.3: Statistical analysis for TBARS, PV, TPC and % Inhibition of Siganid fillets treated with BHA (1% w/v in distilled water), seaweed (2.5% w/v in distilled water), turmeric (2.5% w/v in distilled water) and water hyacinth (2.5% w/v in distilled water soluble antioxidant extracts. The control sample was treated with distilled water without antioxidants.

Treatment	Parameters	Day 0	Day 15	Day 30
CONTROL	TBARs	0.2808 ± 0.0055^{a}	0.1599 ± 0.0331^{b}	0.0343 ± 0.0006^{c}
	PV	0.0519 ± 0.0229^{a}	0.1359 ± 0.0704^{b}	0.2908 ± 0.0331^{b}
	TPC	0.0018 ± 0.0000^{a}	0.0018 ± 0.0000^{a}	0.0017 ± 0.0000^{b}
	Inhibition%	16.245 ± 0.5010^{a}	2.3410 ± 0.1380^{b}	1.5280 ± 0.5150^{b}
BHA	TBARs	0.0273 ± 0.0000^{a}	0.0039 ± 0.0000^{b}	0.0016 ± 0.0010^{c}
	PV	0.0571 ± 0.0028^{a}	0.0545 ± 0.0225^{a}	0.0518 ± 0.0007^{a}
	TPC	0.0117±0.0006^{a}	0.0105 ± 0.0005^{a}	0.0101 ± 0.0003^{a}
	Inhibition%	88.006 ± 0.7870^{a}	76.634 ± 0.2070^{b}	72.853 ± 0.5150^{c}
TURMERIC	TBARs	0.0858 ± 0.0055^{a}	0.0078 ± 0.0055^{b}	0.0016 ± 0.0017^{b}
	PV	0.0591 ± 0.0211^{a}	0.0755 ± 0.0522^{a}	0.0618 ± 0.0026^{a}
	TPC	0.0082 ± 0.0004^{a}	0.0074 ± 0.0002^{b}	0.0065 ± 0.0000^{b}
	Inhibition%	65.385 ± 0.1430^{a}	59.317 ± 0.2760^{b}	44.032 ± 0.7200^{c}
SEAWEED	TBARs	0.0858 ± 0.0055^{a}	0.0078 ± 0.0055^{b}	0.0016 ± 0.0006^{b}
	PV	0.0576 ± 0.0115^{a}	0.05778 ± 0.019^{a}	0.0695 ± 0.0177^{a}
	TPC	0.0046 ± 0.0004^{a}	0.0042 ± 0.0003^{a}	0.0041 ± 0.0004^{a}
	Inhibition%	31.680 ± 1.2850^{a}	26.195±1.5570b	20.451 ± 0.9260c
WATER HYACINTH	TBARs	0.0897 ± 0.0110^{a}	0.0078 ± 0.0055^{b}	0.0016 ± 0.0006^{b}
	PV	0.0437 ± 0.0018^{a}	0.0521 ± 0.0135^{a}	0.0689 ± 0.0044^{a}
	TPC	0.0041 ± 0.0004^{a}	0.0040 ± 0.0003^{a}	0.0037 ± 0.0004^{a}
	Inhibition%	43.674 ± 1.6460^{a}	35.805 ± 1.3800^{b}	30.859 ± 1.2350^{b}

Different superscript (a b c) across the table indicates significance difference using Tukeys Pairwise comparison ANOVA.

4.4 Discussion

The control Siganid fillets showed the highest levels of peroxides, TBARS and free radical formation over a storage period of 90 days. Fish fillets treated with synthetic BHA indicated the best antioxidant activity as shown by significantly lower lipid oxidation products due to its high polyphenol content. The concentration of BHA was however high as it is used in very small quantities in foods. Here it was 10 g per 1000 mls. BHA is a known antioxidant and was

used as a positive control. The natural antioxidant plant materials used to treat the Siganid fillets were not as effective as BHA, but significantly reduced lipid oxidation compared to the control, Turmeric was more effective in reducing lipid oxidation compared with seaweed and water hyacinth; this was probably due to their relatively high polyphenol content.

The increase in PV to day 60 and eventual drop was due to propagation of free radicals and formation of peroxides initially followed by TBARS secondary and tertiary products.
It is likely that the polyphenolic antioxidants caused a delay in oxidation but with time, owing to light penetration of packaging material and heat, and depletion of antioxidant level, the process of propagation in lipid oxidation increased towards the end of the storage period. The increase in PV is slightly higher in seaweed and water hyacinth treated Siganid muscle as their concentration of antioxidants was lower than in turmeric and BHA. Between day 0 and 30, the total phenolic content in the muscle was lower in the seaweed and water hyacinth throughout the storage followed by turmeric and BHA (Figure 4. 4) which had higher phenolic compounds. The natural intrinsic phenolic content in control was the lowest and reflected the high oxidation level. The total phenolic content was low in day 0 to 30 for water hyacinth and seaweed giving a concomitant rise in PV. Finally, the total phenolic content continued to drop meaning its electron donor capacity was decreasing and reducing antioxidant activity that suppressed oxidation. The control was 0.29mEq/kg on day 30. This means that the natural antioxidants from turmeric, seaweed and water hyacinth exerted some antioxidant activity in these early stages of storage. The natural antioxidants of turmeric, seaweed and water hyacinth have antioxidant activity, but their effectiveness varied. BHA, the synthetic antioxidant at the concentrations used was effective.

The reduction in the TBARS in days 0 to 30 corresponds to reduction in total phenolic content in that period. However, the total phenolic content decreased further in days 60 to 90 with an increase in TBARS in the control followed at a lower rate in treatments with BHA, turmeric, seaweeds and water hyacinth. Inhibition of free radical formation was also related to the strength of the antioxidants. In this study BHA has a higher % inhibition than turmeric and seaweed in the 30-day storage period and these were in turn higher in % inhibition than control and water hyacinth. The total phenolic content was highest in BHA treated Siganid muscle and this was related to its higher antioxidant activity.
All treatments were more effective than the control in suppressing lipid oxidation.

Antioxidants can reduce lipid oxidation by competitively binding active forms of oxygen, interfering with the initiation and propagation steps, binding free radicals and break the chain by stabilizing peroxides and form antioxidant radicals that are too unreactive for further reactions or form non-radical products (Halliwell and Gutteridge, 1995).

The natural antioxidants used in this study and synthetic antioxidant BHA all contain phenolic groups. The mechanism of action of phenolic groups relies on the resonance stabilisation of the phenoxy radicals that occurs at the ortho and para position on the aromatic ring. In addition, it can be influenced by the size of the substituting group. Further, many phenolic compounds contain carbonylic and carboxylic groups that can chelate metals and thereby inhibit lipid oxidation and rancidity (Howell and Saeed, 1999).

Synthetic phenolic compounds like BHA and BHT have been widely used; however, there is a concern that they may be toxic or carcigonegic. Therefore, natural antioxidants like turmeric, gallic acid and plant extracts like water hyacinth and seaweeds can provide effective alternatives.

4.4.1 Conclusions

The natural antioxidants in water hyacinth, seaweeds, turmeric suppressed lipid oxidation just like the synthetic antioxidant- BHA. The Total Phenolic content (TPC) in turmeric was twice the amount found in seaweed and water hyacinth at the beginning and during storage which reflected the higher antioxidant effect of turmeric. The TPC was lowest in the water hyacinth during storage and they showed the highest PV and TBARS values during storage. The order of strength of the antioxidants was BHA>Turmeric>Seaweeds>Water hyacinth. BHA, used in lower concentrations in most foods was at a much higher concentration in this study and because synthetic antioxidants are now considered unsafe, it is advantageous to replace them with natural antioxidant sources like water hyacinth, turmeric and seaweed plant phenolics.

CHAPTER 5

CHAPTER 5

5.0 Physicochemical properties of stored Siganid fish muscle treated with natural antioxidants

5.1. Introduction

There is an increase in the consumption of aquatic products preserved by hot air drying. Most drying methods cause quality changes in fish protein amino acid residues which result in the modification of structural, digestibility and functional properties of the protein (Deng et al., 2014).

It is important to maintain functionality of myofibrillar proteins in muscle foods to enhance the eating quality of meat products. It is also essential to better understand the thermal stability of fish proteins particularly the myofibrillar proteins during processing including drying (Ahmed et al 2009). Loss of functionality of proteins is caused by degradation and denaturation of proteins resulting in texture changes.

The texture of meat products is also influenced by the major protein actomyosin which plays an important role during heat processing. (Skipnes et al 2008; Ahmed et al 2009). The process of heat gelation involves the solubility of the myofibrillar proteins in salt solution, denaturation of protein molecules on heating and thirdly aggregation of proteins by non-covalent electrostatic, hydrophobic and hydrogen bonds and covalent disulfide linkages (Esturk and Park 2014, Stones and Stanley, 1992; Lefevre et al, 1999). Most changes in protein properties during drying take place in myosin and actin which denatures at temperatures of 43 to 60 ^{o}C (Dang et al 2014, Skipness et al 2008) Changes in protein structure during heat processing and subsequent storage can be monitored by measurement of thermodynamic properties by differential scanning calorimetry and viscoelastic properties by large and small deformation rheology. Proteins are modified by thermal processing and further changes may occur during storage due to the oxidation of proteins and lipids.

In this study, the effect of four antioxidants on the structural and physicochemical properties of dried fish muscle during storage at ambient temperature, were investigated. First, the viscoelastic properties were measured as the elastic modulus G’ and viscous modulus G’’ by

small deformation rheology. Secondly, the thermodynamic properties including transition temperature (Tm) and enthalpy change ΔH were obtained from differential scanning calorimetry scans.

5.2. Materials and methods

5.2.1. Materials

The rabbit fish 'Siganus sutor or Siganids (warm water species found in the Western Indian Ocean region) were caught by artisanal fishermen, iced, gutted, then dried in the hybrid windmill solar tunnel dryer located in Kipini as described in Chapter 2.

Antioxidants water hyacinth (Eichronia), seaweeds (Eucheuma), turmeric (Curcuma) and synthetic antioxidant BHA were obtained from Mombasa, Shimoni and Kisumu as described in Chapter 4.

5.2.2. Fish muscle preparation

The solar dried fish, with or without natural antioxidants, were cut into small pieces with a kitchen knife and transferred to a blender where the muscle tissue was homogenized to a fine powder. The samples were transferred to seal-lock bags, labelled and transported by air to the University of Surrey where they were stored in a freezer at -80^{o}C until analysis.

5.2.3. Rheological properties

Small deformation rheological studies were performed on a rheometer (Rheometrics -Dynamic stress rheometer Model SR 200, made in the USA).

5.2.3.1 Setting up the rheometer for analysis

The pressure gauge of the rheometer was set to 60. Water was filled in the cooling unit for the rheometer and the temperature allowed to rise to 45^{o}C. The rheometer was then switched on and the probe was unlocked and rotated. The rheometer was then calibrated by resetting the calibration button to 0 (zero) and the knob rotated to create a gap of -2.000 mm. The probe was lowered again, and the reading recorded when it was approximately 2.000. The computer was switched on and the rheology software opened. The temperature on the computer was set at

20^{o}C and the start button on the screen activated. Next, the function Edit geometry was activated in the window and the calibration value of approximately 2 found earlier entered in the appropriate box. The options icon was activated, followed by "Tool Cal" and the calibration process started. When calibration ended, "OK" was clicked then "OK" clicked again. At the end of calibration, the probe was taken back to the upper position before lowering at the start of the testing. The sample for analysis was prepared.

5.2.3.2 Sample loading on rheometer

Solar dried ground muscle (4 g) was weighed into a beaker and 8 mls of deionized water added (the weight was based on initial protein and moisture content) to make a paste. The sample was then spread on the plate making it as thin as possible. The Rheometer probe knob was switched on to lower the clean probe. Silicone oil was applied around the plate edge to prevent drying out of the sample during heating. The rheometer was run for about 1.5 hrs. The rheology reading on the computer screen was saved in the folder to give the excel version of the G and G'' readings.

5.2.4 Thermodynamic properties

Differential Scanning Calorimetry (DSC VII) is a unique technique that measures the thermodynamic properties of materials particularly the transition temperature and enthalpy change with time and temperature. DSC is a thermal analysis instrument that measures the temperature and heat flow related to transitions of materials. On heating, DSC indicates the amount of heat that is radiated or absorbed by the sample, based on a temperature difference between the reference material and the sample. Both the sample and reference in sealed containers or pans are in contact with a thermoelectric disk which transfers heat from the surrounding furnace to the pans at a linear heating rate.

5.2.4.1 Sample preparation

The sample containers (pans) were cleaned and handled with tissue or tweezers throughout the experiment. Based on the moisture and protein content estimates on the solar dried fish, 0.25g of fish muscle was weighed in a plastic weighing boat and using a micropipette, 0.75ml of deionized water added. The samples were transferred carefully to the weighing pans for sample (S). An equal amount of water was carefully transferred to the reference pan (R). The pans

were then transferred to the DSC which was switched on and operated according to the manual, overnight. The results were read off the computer according to software instructions.

On the DSC PC, the icon SETSOFT was used. The readings obtained were for Temperature peak (Value example 30.79 ^{o}C) and Enthalpy change, example 0.02 (Endothermic). A table was then drawn up with the data for maximum temperature and Enthalpy.

Different sample treatments were compared. The table with Tm (Maximum temperature of denaturation) and Δ H (Heat enthalpy change) was drawn.

5.3 Results and Discussion

5.3.1 Rheological analysis

Results for rheological analysis are shown in Tables 5.1 to 5.4 and examples of representative rheograms in figures 5.1 to 5.12.

The rheological changes in the dried fish fillets were monitored during storage and are shown in Tables 5.1 to 5.4 The elastic modulus G' and viscous modulus G'' increased during the temperature sweep from 20 to 90 ^{o}C after which the muscle was cooled back to 20 ^{o}C (Figure 5.1 to 5.12). The increase in G' values is a measure of texture changes and occurs due to the denaturation of primarily myofibrillar proteins. Myosin denatured at around 41-43 ^{o}C and was seen in the rheology scan as the crossover point of G' and G''. This denaturation temperature and protein unfolding was confirmed by the first DSC transitions Tm which occurred at around 42-43 ^{o}C. (Tables 5.6 to 5.10. and Figure 5.2). The second main transition due to unfolding of the actin protein occurred at around 58-60 ^{o}C, as confirmed by the DSC scans (Figures 5.13 to 5.15 and Tables 5.6 to 5.10).

Table 5.1: Rheological properties of solar dried Siganus sutor muscle treated with water as control during ambient storage in normal polythene for up to 90 days. G and G'' are mean score in Pa units

CONTROL							
Treatment	20°C at the beginning		≥20°≤90°C		20°C after cooling		
	G'	**G"**	**G'**	**G"**	**G'**	**G"**	**Crossover point ^{o}C**
Control day 0	7916	2680	6963	1514	16013	4377	None
Control day 60	4017	1969	20699	2994	36112	10586	None
Control Day 90	13.7	41	27669	4741	75240	33619	66.27

Table 5.2: Rheological properties of solar dried Siganus sutor muscle treated with natural antioxidant water extracts of water hyacinth (Eichronia) during ambient storage in normal polythene for up to 90 days. G and G'' are mean score in Pa units

WATER HYACINTH							
Treatment	20°C at the beginning		≥20°≤90°C		20°C after cooling		
	G'	**G"**	**G'**	**G"**	**G'**	**G"**	**Crossover point ºC**
Control day 0	7916	2680	6963	1513	16013	4377	None
Water hyacinth Day 0	522	750	22915	3161	43453	14380	21.55
Water hyacinth Day 15	494	787	22739	3304	36741	11285	42.54
Control day 60	4017	1969	20699	2994	36112	10586	None
Water hyacinth Day 60	3117	1317	6671	1535	12220	3572	0.00

Table 5.3: Rheological properties of solar dried Siganus sutor muscle treated with natural antioxidant water extracts of seaweed (Eucheuma) during ambient storage in normal polythene for up to 90 days. G and G'' are mean score in Pa units

SEAWEED							
Treatment	20°C at the beginning		≥20°≤90°C		20°C after cooling		
	G'	**G"**	**G'**	**G"**	**G'**	**G"**	**Crossover point ºC**
Control day 0	7916	2680	6963	1513	16013	4377	None
Seaweed Day 0	6190	3331	9266	2452	44807	16436	67.86
Control day 60	4017	1969	20699	2994	36112	10586	None
Seaweed day 60	1505	670	10.6	101	18428	6634	71.22
Control day 90	13.7	40.7	27669	4741	75241	33619	66.27
Seaweed day 90	65505	18881	25046	3415	38720	12324	0.00

Table 5.4: Rheological properties of solar dried Siganus sutor muscle treated with natural antioxidant water extracts of Turmeric (Curcuma) during ambient storage in normal polythene for up to 90 days. G and G'' are mean score in Pa units

TURMERIC							
Treatment	20°C at the beginning		≥20°≤90°C		20°C after cooling		
	G'	**G"**	**G'**	**G"**	**G'**	**G"**	**Crossover point ºC**
Control day 0	7916	2680	6963	1513	16013	4377	None
Turmeric Day 0	87.83	57.84	281.56	592	6483	2513	75.38
Control day 60	4017	1969	20699	2994	36112	10586	None
Turmeric day 60	919	1192	26639	4427	69470	26444	51.92
Control day 90	13.77	40.73	27669	4741	75241	33619	66.27
Turmeric day 90	49.90	58.96	10715	1869	23985	8706	52.56

Table 5.5: Rheological properties of solar dried Siganus sutor muscle treated with synthetic antioxidant BHA during ambient storage in normal polythene for up to 90 days. G and G'' are mean score in Pa units

BHA							
Treatment	20°C at the beginning		≥20°≤90°C		20°C after cooling		
	G'	**G"**	**G'**	**G"**	**G'**	**G"**	**Crossover point ºC**
Control day 0	7916	2680	6963	1513	16013	4377	None
BHA Day 0	12.02	45.97	10883	2357	40555	13511	76.83
Control day 60	4017	1969	20699	2994	36112	10586	None
BHA day 60	11.39	41.11	14812	2504	37447	13464	65.61
Control day 90	13.77	40.73	27669	4741	75240	33619	66.27
BHA day 90	1124	563.4	31014	4655	70557	27383	49.94

Further heating to 90 ºC on the rheometer resulted in a rapid increase in G' values and caused gelation via covalent bonds, but mainly covalent links like disulphide bonds. Upon cooling to 20 ºC the G' and G'' values increased gradually due to the formation of non-covalent hydrophobic and electrostatic interactions.

The rheological and DSC methods allowed the monitoring of changes that occurred in storage of the dried fish. G' values after heating and cooling of the untreated dried fish increased with time of storage from 16013 Pa at day 0 to 36112 Pa after 60 days and 75240 Pa after 90 days (Table 5.1). Increases in G' values have been reported for cod (Badii and Howell, 2001) and Atlantic mackerel fish (Saeed and Howell, 2002) stored at -20 ºC mainly due to the denaturation of the myofibrillar proteins. For both studies it was shown that the addition of antioxidants like vitamin C, vitamin E and butylated hydroxytoluene (BHT) reduced the rate of increase of G' values, keeping the fillet close to the original control in terms of texture and biochemical composition. This trend was also evident in the present study on stored solar dried Siganid fish.

5.3.2 Addition of antioxidants

In this study the effect of antioxidants namely water hyacinth, turmeric, seaweed and BHA, on the muscle integrity and texture during storage for 0, 15, 60 and 90 days was investigated in terms of the rheological and thermodynamic properties. Results for the rheological temperature sweep scans for solar dried fish samples in the presence of antioxidants are reported in Tables 5.1 to 5.5.

The addition of all antioxidants lowered the G' values, which reflects desirable texture qualities that are closer to the control dried fish at 0 day. Water hyacinth gave the lowest values after

heating and cooling to 20 ^{o}C, followed by turmeric, seaweed and BHA, which were all lower than for the control stored for a similar length of time. The thermodynamic properties Tm and enthalpy change values did not alter significantly with storage (Figures 5.13 to 5.15).

For water hyacinth the G' values at day 0, 15 and 60 days were 43453, 36112 and 12220 Pa respectively (Table 5.2). The DSC scan for water hyacinth showed that Tm decreased slightly but not significantly. The enthalpy change remained the same as the control initially but decreased after 60 days of storage, indicating denaturation of the proteins on prolonged storage (Table 5.7, Figures 5.13 and 5.14).

Similarly, G' values of fish fillets stored with turmeric were 36112, after 60 days and 23985 Pa after 90 days (Table 5,4) and were significantly lower than the control samples after storage (Table 5.1). The Tm for myosin and actin did not change significantly, although the enthalpy change decreased after 60 days, indicating denaturation (Table 5.9, Figures 5.13 to 5.15).

Similarly, seaweed addition (Table 5.3) to the dried fillets resulted in 36, 112 Pa after 60 days and 23985 Pa after 90 days indicated lower values than the controls without antioxidants (Table 5.1). In the presence of seaweed, the Tm for the myosin peak increased with storage time indicating changes in myosin possible through seaweed –myosin interaction or lipid-protein interactions. The Tm for actin was like that of the control. The enthalpy change was also reduced with storage for 60 days indicating denaturation during storage (Table 5.8 Figure 5.13 to 5.15).

The positive control BHA had high values 37447 and 70557 Pa at 60 and 90 days (Table 5.5) which were higher than those for the other samples and closer to the control without antioxidant (Table 5.1). The Tmax for myosin and actin increased with time of storage in the presence of BHA. After 60 days the enthalpy change was reduced compared to the control (Table 5.10, Figure 5.14 and 5.15).

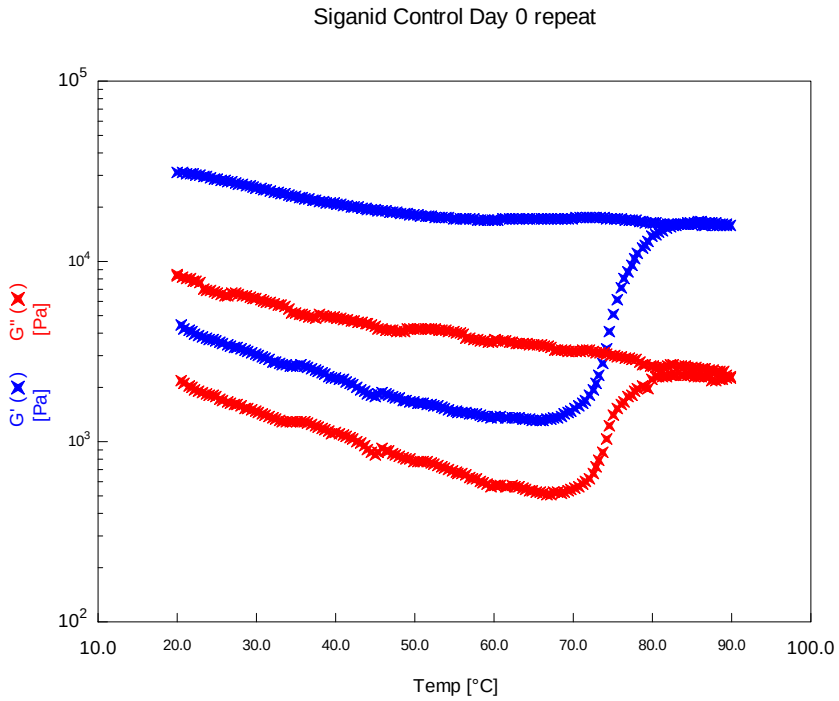


Figure 5.1: Small deformation rheology temperature sweep (20ºC to 90ºC and cooled back to 20ºC) showing G' and G'' values for stored (day 0) solar dried Siganid fillets the control sample was treated with distilled water without antioxidants.

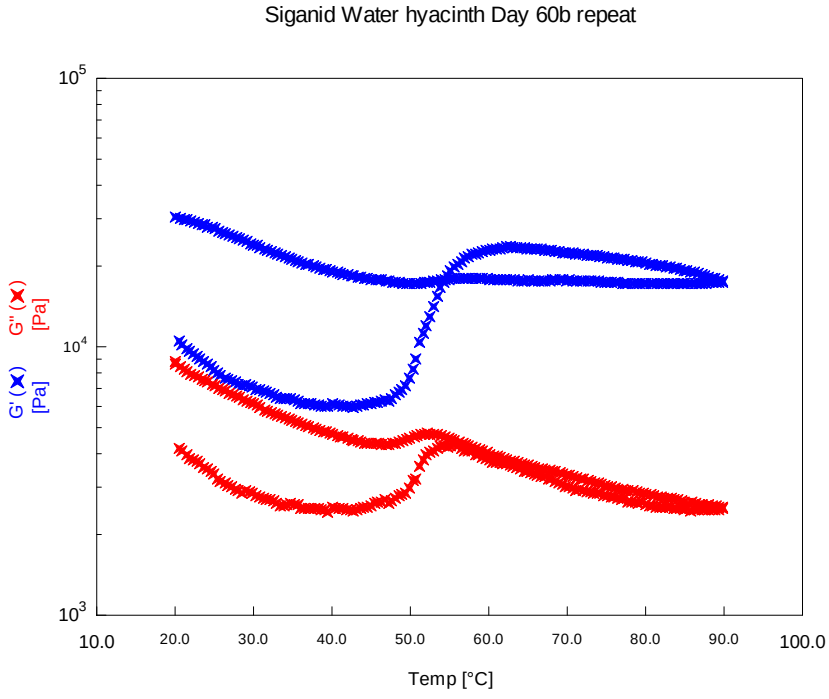


Figure 5.2: Small deformation rheology temperature sweep (20ºC to 90ºC and cooled back to 20ºC) showing G' and G'' values for stored (day 60) solar dried Siganid fillets treated with water hyacinth (2.5 % w/v in distilled water). The control sample was treated with distilled water without antioxidants

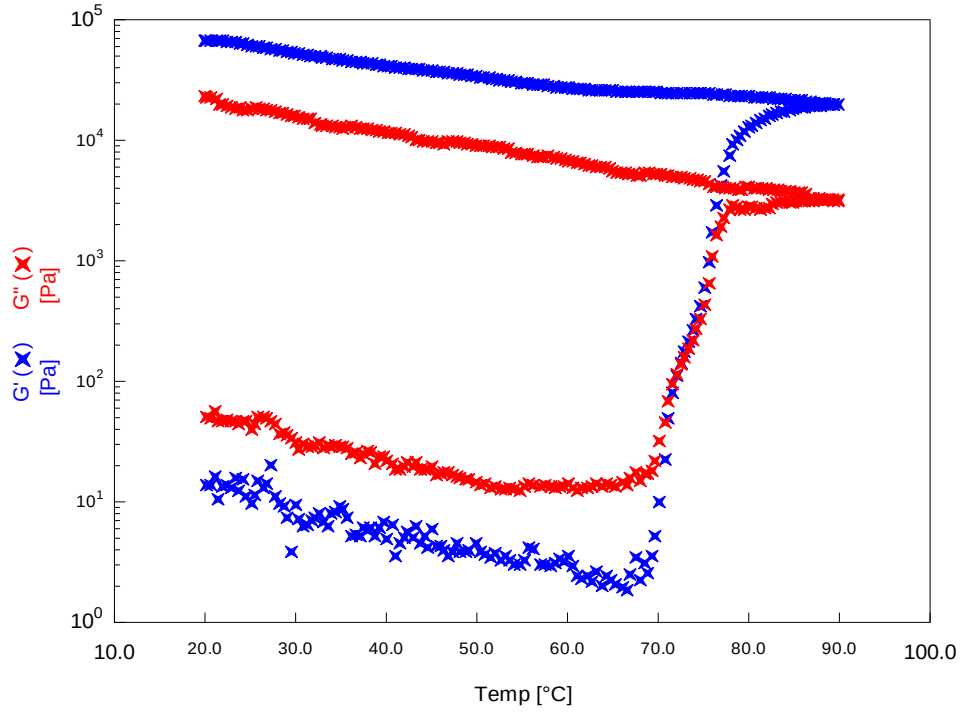


Figure 5.3: Small deformation rheology temperature sweep (20ºC to 90ºC and cooled back to 20ºC) showing G' and G'' values for stored (day 90) solar dried Siganid fillets. The control sample was treated with distilled water without antioxidants.

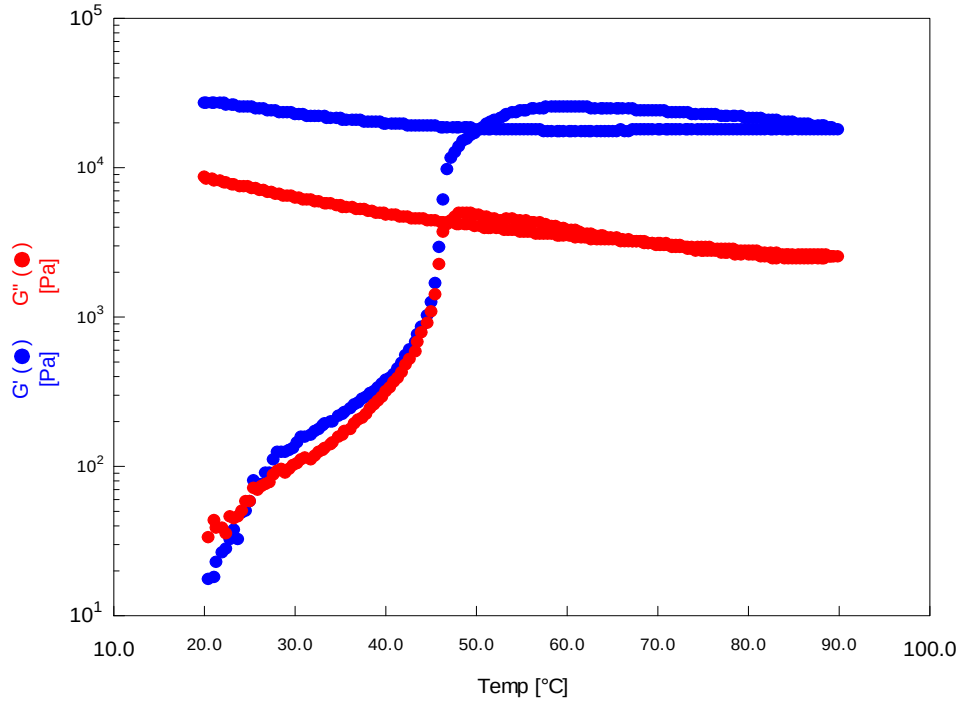


Figure 5.4: Figure 5.4 Small deformation rheology temperature sweep (20ºC to 90ºC and cooled back to 20ºC) showing G' and G'' values for stored (day 0) solar dried Siganid fillets treated with water hyacinth (2.5 % w/v in distilled water). The control sample was treated with water without antioxidants.

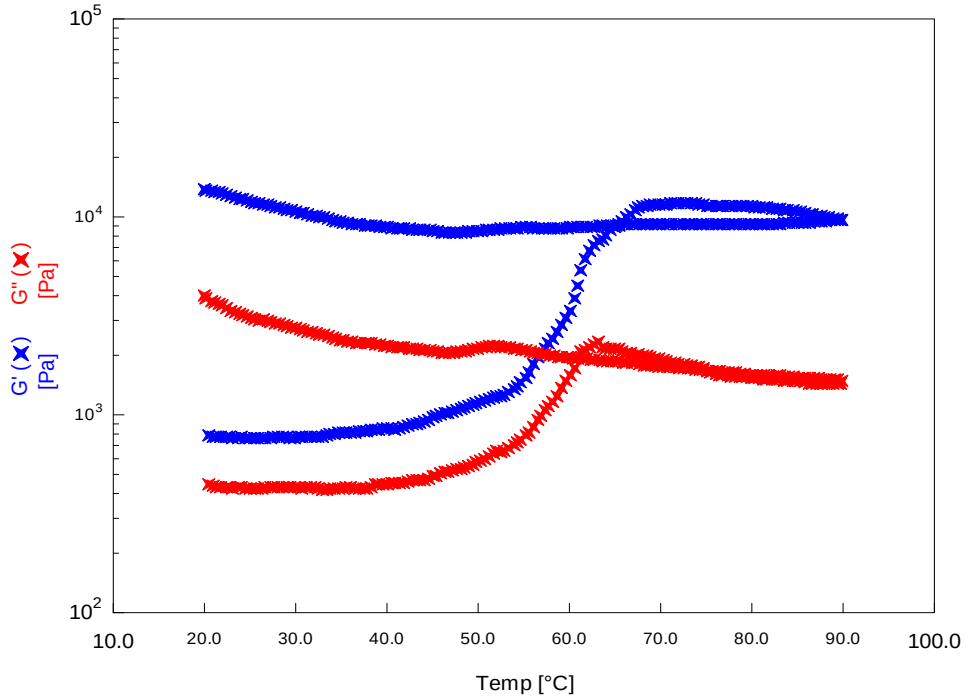


Figure 5.5: Small deformation rheology temperature sweep (20ºC to 90ºC and cooled back to 20ºC) showing G' and G'' values for stored (day 60) solar dried Siganid fillets treated with water hyacinth (2.5 % w/v in distilled water). The control sample was treated with distilled water without antioxidants.

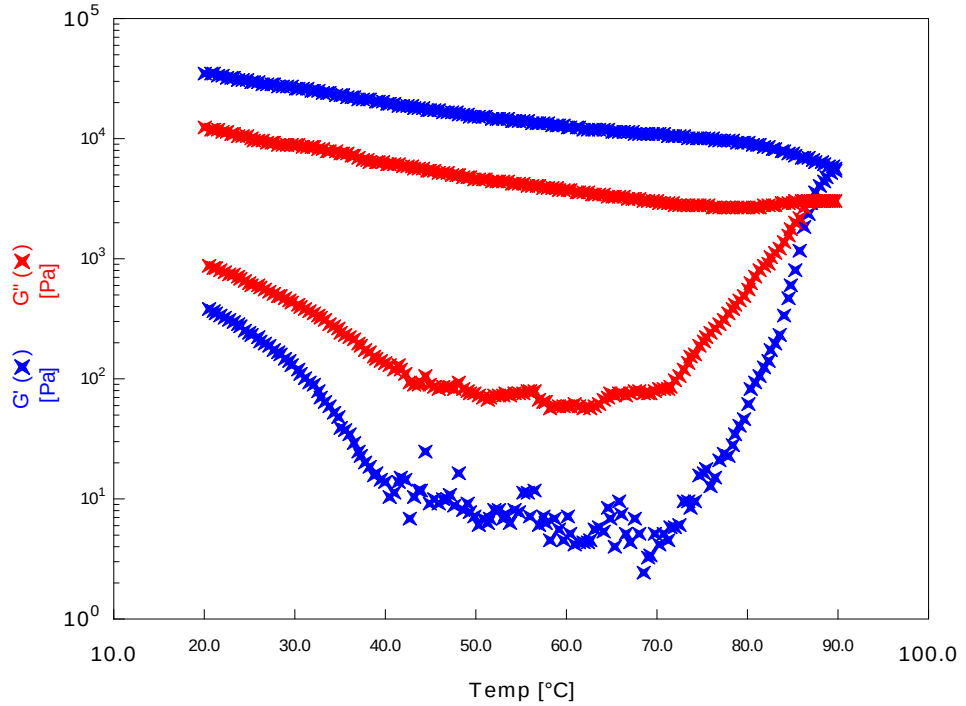


Figure 5.6: Small deformation rheology temperature sweep (20ºC to 90ºC and cooled back to 20ºC) showing G' and G'' values for stored (day 0) solar dried Siganid fillets treated with seaweed (2.5 % w/v in distilled water). The control sample was treated with distilled water without antioxidants.

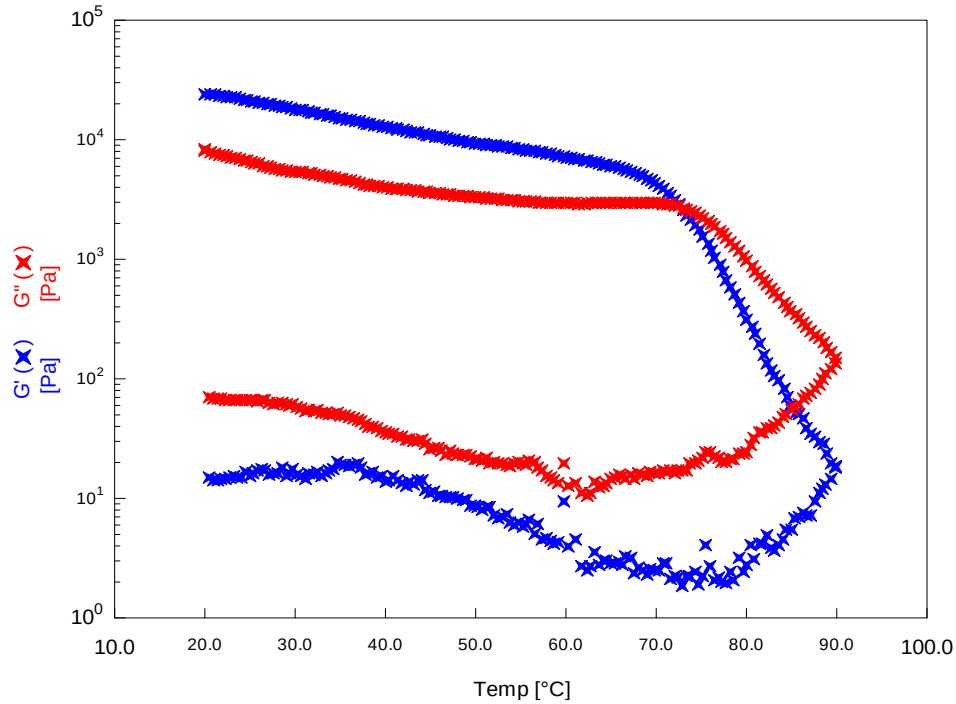


Figure 5.7: Small deformation rheology temperature sweep (20ºC to 90ºC and cooled back to 20ºC) showing G' and G'' values for stored (day 60) solar dried Siganid fillets treated with seaweed (2.5 % w/v in distilled water). The control sample was treated with distilled water without antioxidants.

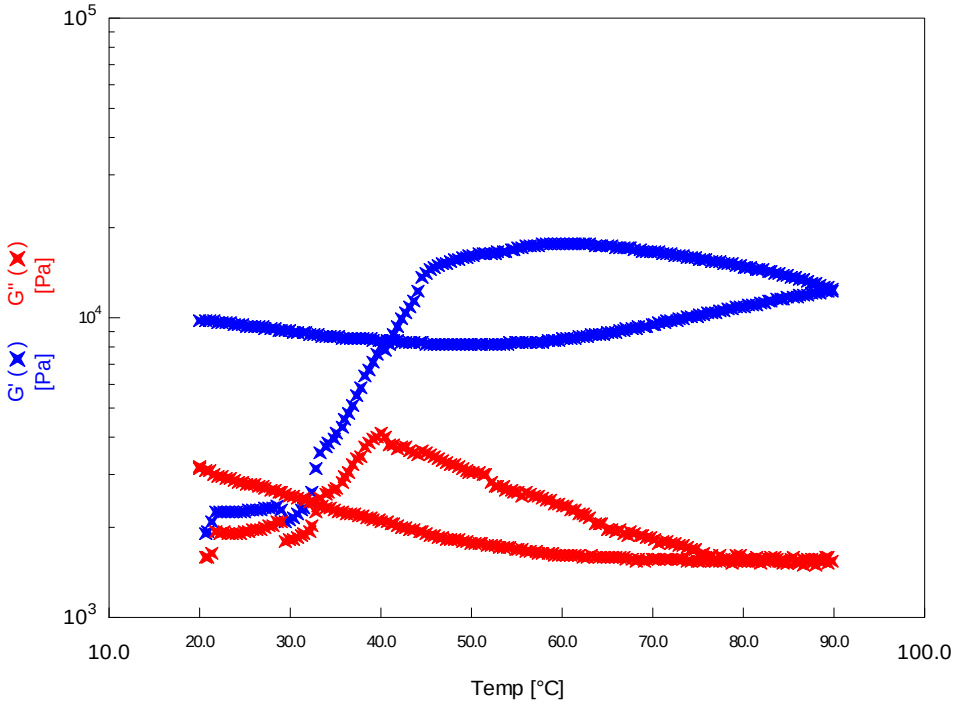


Figure 5.8: Small deformation rheology temperature sweep (20ºC to 90ºC and cooled back to 20ºC) showing G' and G'' values for stored (day 90) solar dried Siganid fillets treated with seaweed (2.5 % w/v in distilled water). The control sample was treated with distilled water without antioxidants.

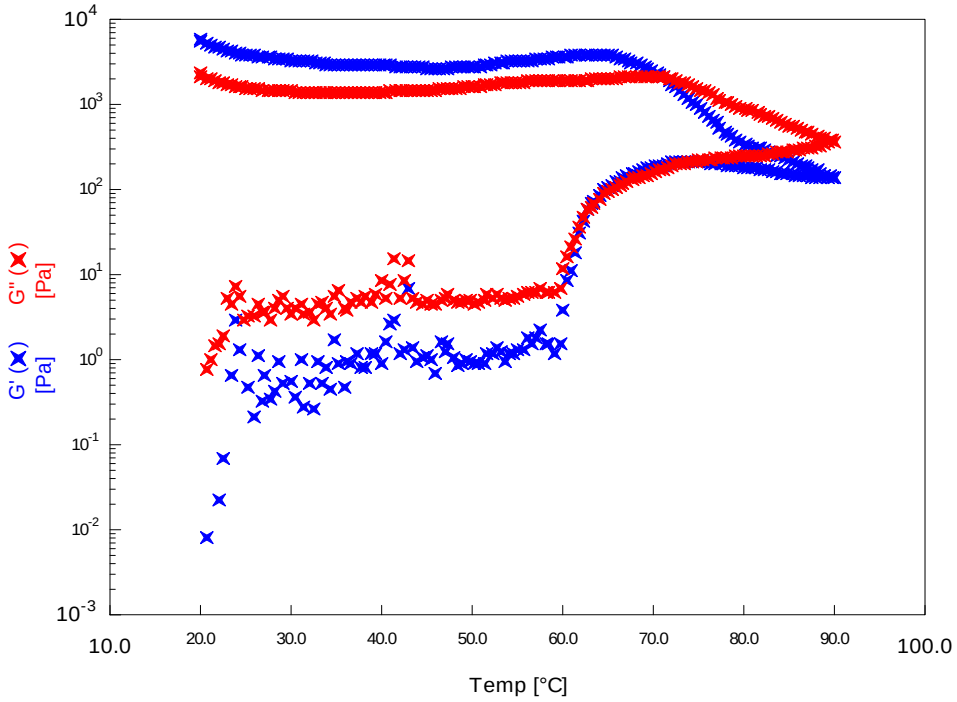


Figure 5.9: Small deformation rheology temperature sweep (20ºC to 90ºC and cooled back to 20ºC) showing G' and G'' values for stored (day 0) solar dried Siganid fillets treated with Turmeric (2.5 % w/v in distilled water). The control sample was treated with distilled water without antioxidants.

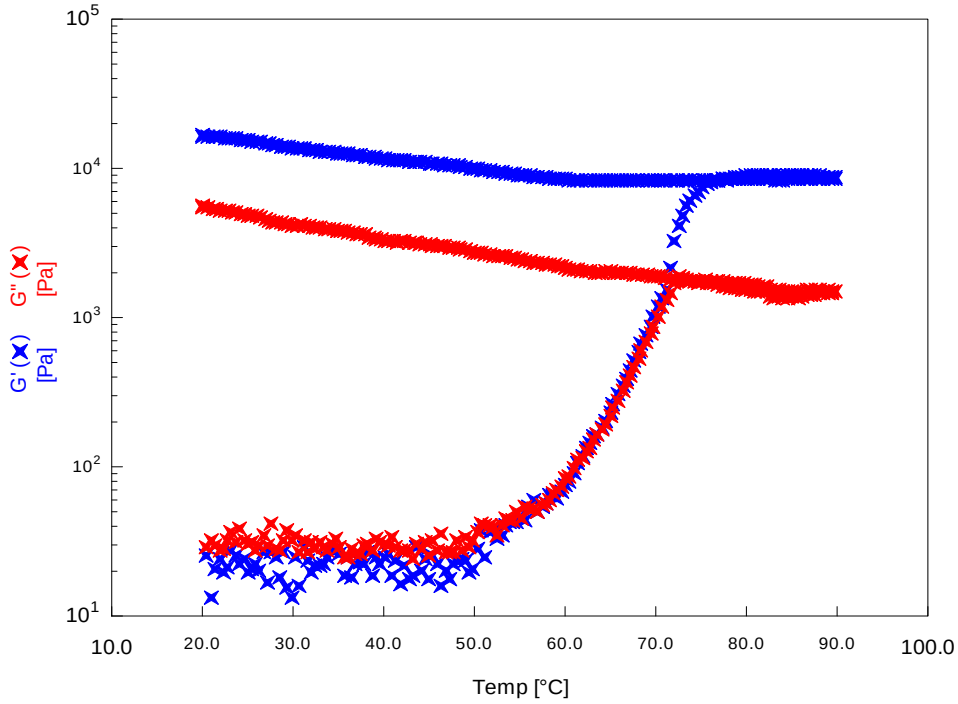


Figure 5.10: Small deformation rheology temperature sweep (20ºC to 90ºC and cooled back to 20ºC) showing G' and G'' values for stored (day 90) solar dried Siganid fillets treated with Turmeric (2.5 % w/v in distilled water). The control sample was treated with distilled water without antioxidants.

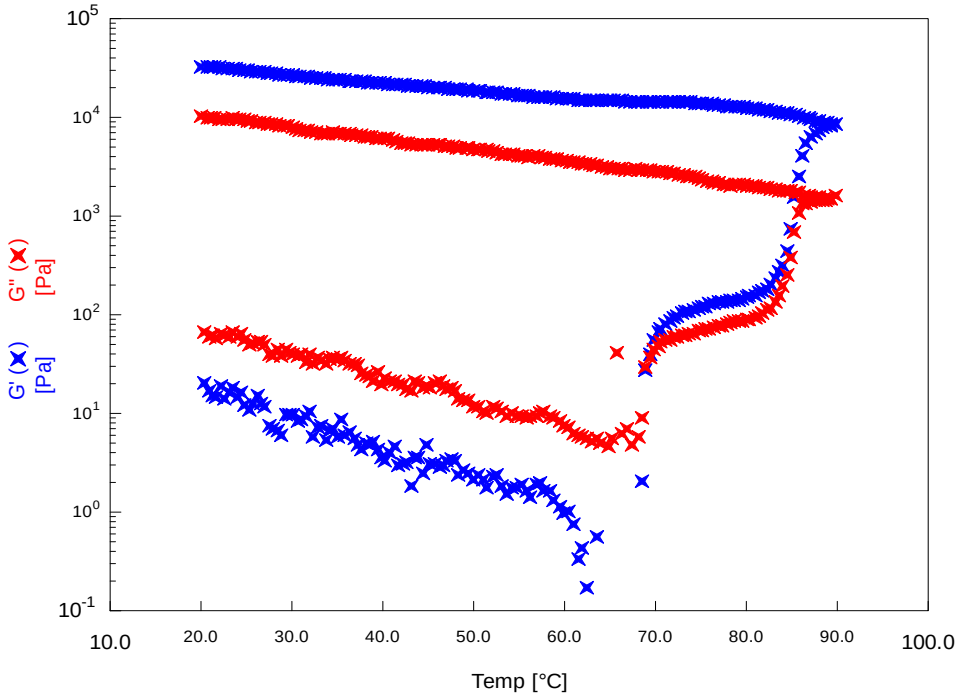


Figure 5.11: Small deformation rheology temperature sweep (20ºC to 90ºC and cooled back to 20ºC) showing G' and G'' values for stored (day 0) solar dried Siganid fillets treated with BHA (1% w/v in distilled water). The control sample was treated with distilled water without antioxidants.

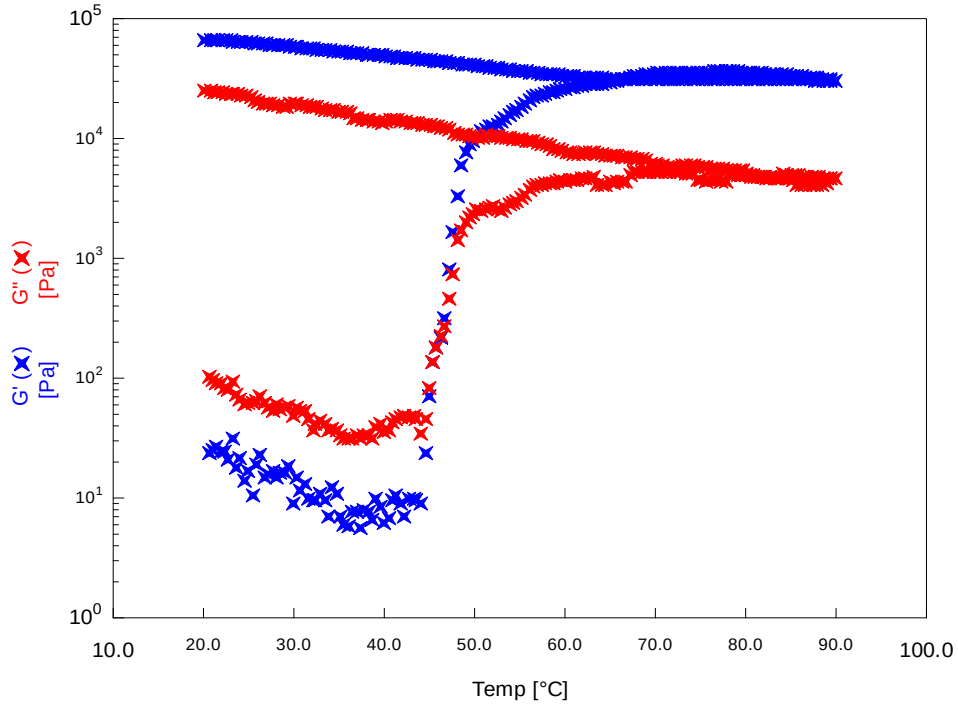


Figure 5.12: Small deformation rheology temperature sweep (20ºC to 90ºC and cooled back to 20ºC) showing G' and G'' values for stored (day 90) solar dried Siganid fillets treated with BHA (1% w/v in distilled water). The control sample was treated with distilled water without antioxidants.

5.3.3 Differential Scanning Calorimetry (DSC) Analysis

The results for DSC analysis are as shown in figures 5.13 to 5.15 and tables 5.2 to 5.6

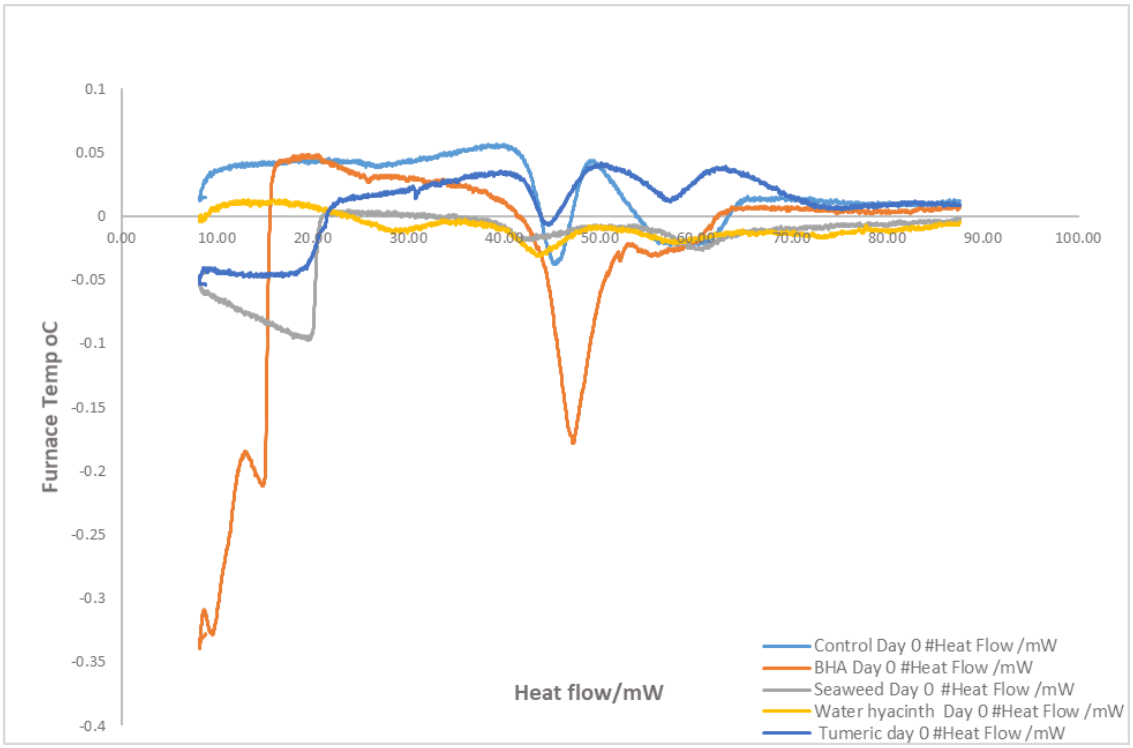


Figure 5.13: Differential Scanning Calorimetry thermogram for fish muscle showing denaturation temperature (Tmax) and heat enthalpy change values (ΔH) for stored (day 0) solar dried Siganid fillets treated with BHA (1% w/v in distilled water), seaweed (2.5% w/v in distilled water), turmeric (2.5% w/v in distilled water) and water hyacinth (2.5% w/v in distilled water) water soluble antioxidant extracts. The control sample was treated with distilled water without antioxidants.

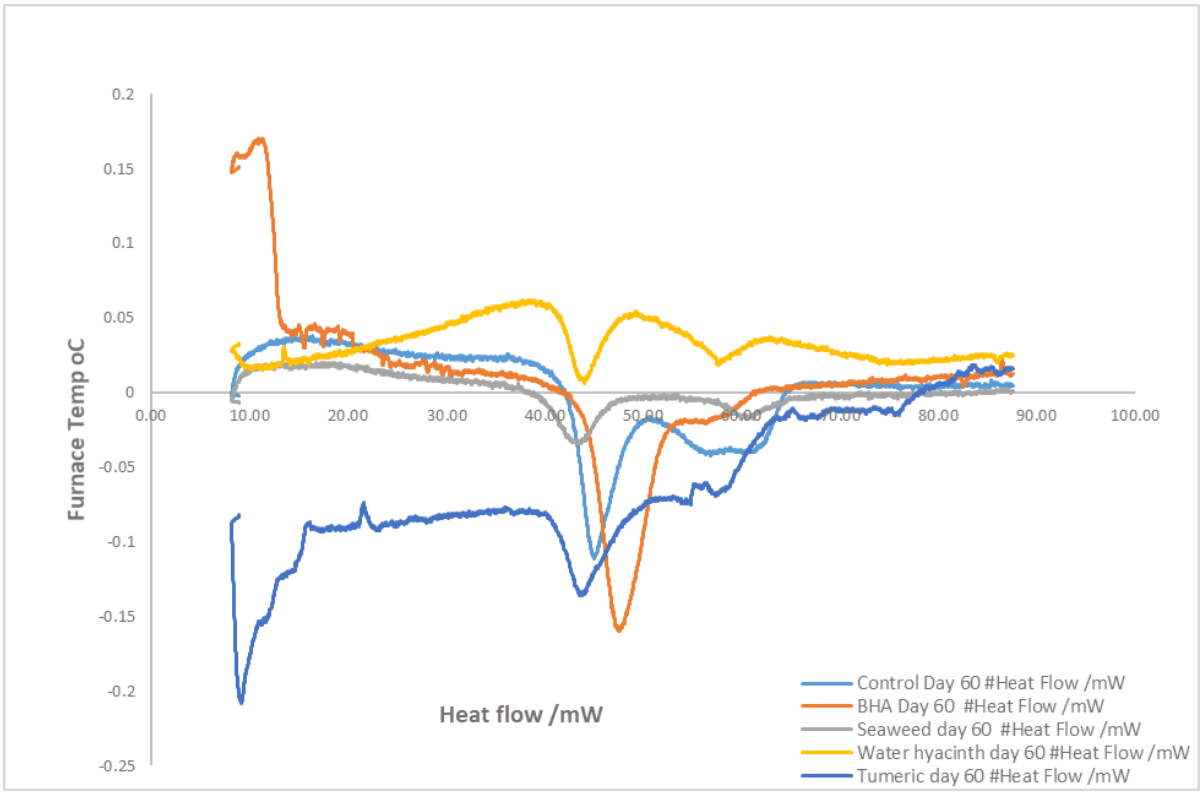


Figure 5.14: Differential Scanning Calorimetry thermogram for fish muscle showing denaturation temperature (Tmax) and heat enthalpy change values (ΔH) for stored (day 60) solar dried Siganid fillets treated with BHA (1% w/v in distilled water), seaweed (2.5% w/v in distilled water), turmeric (2.5% w/v in distilled water) and water hyacinth (2.5% w/v in distilled water) water soluble antioxidant extracts. The control sample was treated with distilled water without antioxidants.

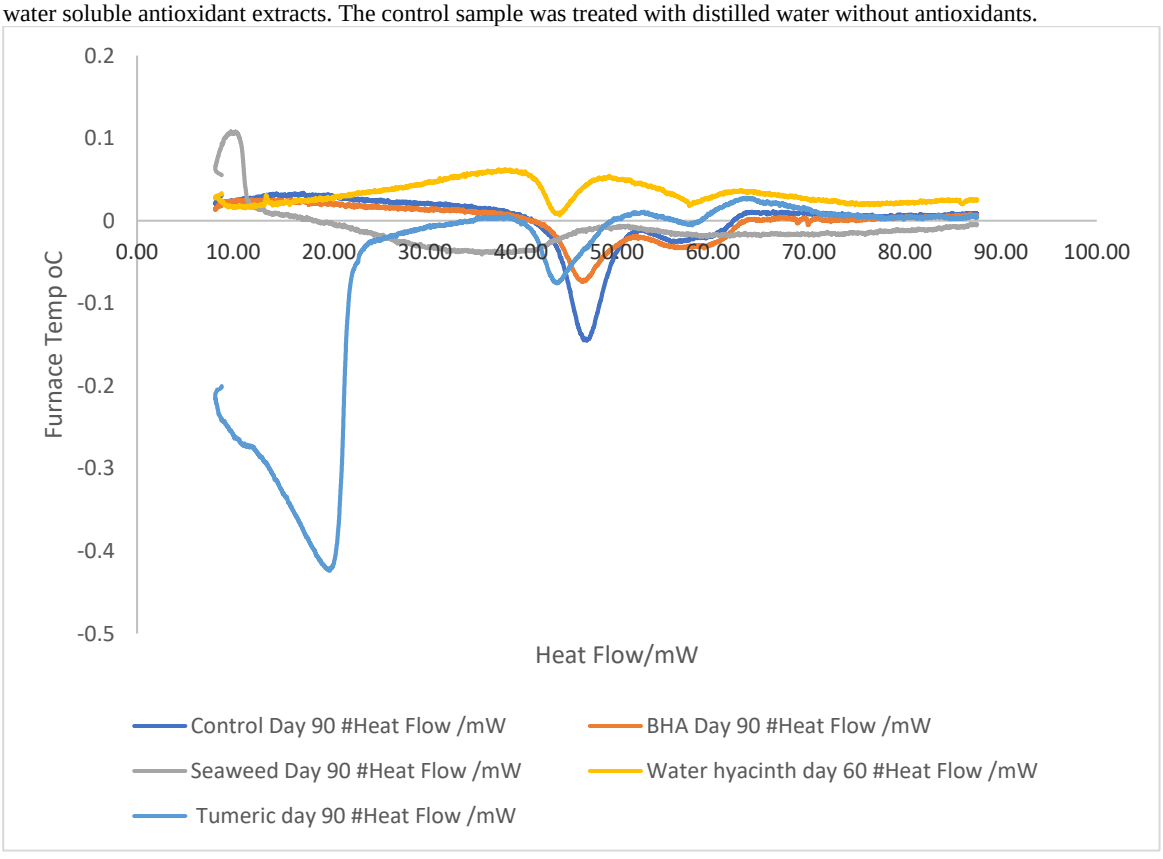


Figure 5.15: Differential Scanning Calorimetry thermogram for fish muscle showing denaturation temperature (Tmax) and heat enthalpy change values (ΔH) for stored (day 90) solar dried Siganid fillets treated with BHA (1% w/v in distilled water), seaweed (2.5% w/v in distilled water), turmeric (2.5% w/v in distilled water) and water hyacinth (2.5% w/v in distilled water) water soluble antioxidant extracts. The control sample was treated with distilled water without antioxidants.

Table 5.6: Transition denaturation temperatures Tonset, Tmax ^{o}C and Heat enthalpy change (ΔH) for myosin and actin proteins obtained by differential scanning calorimetry (DSC) for stored solar dried Siganid fish muscle (control)

	Myosin			Actin		
Treatment	Denaturation temperature ^{o}C		Enthalpy ΔH	Denaturation temperature ^{o}C		Enthalpy ΔH
	(T_{onset})	(T_{max})	Endothermic Effect/J/g	(T_{onset})	(T_{max})	Endothermic Effect/J/g
Control Day 0	42.17	44.91	0.11	50.46	57.52	0.27
Control Day 15	43.21	45.72	0.20	52.31	59.93	0.30
Control Day 60	42.97	45.09	0.07	52.58	60.53	0.24
Control Day 90	43.63	46.81	0.44	53.47	59.25	0.09

Table 5.7: Transition denaturation temperatures Tonset, Tmax ^{o}C and Heat enthalpy change (ΔH) for myosin and actin proteins obtained by differential scanning calorimetry of stored solar dried Siganid fish muscle treated with water extracts of water hyacinth as an as antioxidants. Comparisons only with control for each treatment

	Myosin			actin		
Treatment	First transition Denaturation temperature ^{o}C		Enthalpy ΔH	Second transition Denaturation temperature ^{o}C		Enthalpy ΔH
	(T_{onset})	(T_{max})	Endothermic effect/J/g	(T_{onset})	(T_{max})	Endothermic effect/J/g
Control Day 0	42.17	44.91	0.11	50.46	57.53	0.27
Water hyacinth Day 0	41.00	43.66	0.06	53.98	57.58	0.03
Control Day 15	43.21	45.72	0.20	52.31	59.93	0.30
Water hyacinth Day 15	41.13	45.04	0.05	55.93	57.67	0.02
Control Day 60	42.97	45.09	0.07	52.58	60.54	0.24
Water hyacinth Day 60	41.70	44.03	0.12	56.68	57.56	0.07

Table 5.8: Transitions denaturation temperatures Tonset, Tmax ^{o}C and Heat enthalpy change (ΔH) for myosin and actin proteins by differential scanning calorimetry (DSC) of stored solar dried Siganid fish muscle treated with water extracts of seaweed (Eucheuma) as antioxidants. Comparisons only with control for each treatment

Treatment	Myosin First Transition Denaturation temperature ^{o}C		Enthalpy change ΔH	Actin Second transition Denaturation temperature ^{o}C		Enthalpy change ΔH
	(T_{onset})	(T_{max})	Endothermic effect/J/g	(T_{onset})	(T_{max})	Endothermic effect/J/g
Control Day 0	42.17	44.91	0.11	50.46	57.53	0.27
Seaweed Day 0	40.45	42.05	0.05	58.07	60.71	0.04
Control Day 60	42.97	45.09	0.07	52.58	60.54	0.24
Seaweed Day 60	40.55	43.35	0.10	58.15	59.95	0.03
Control Day 90	43.63	46.81	0.44	53.47	59.25	0.09
Seaweed Day 90	48.65	48.89	0.001	55.69	58.41	0.004

Table 5.9: Transition denaturation temperatures Tonset, Tmax ^{o}C and Heat enthalpy change (ΔH) of myosin and actin protein s by differential scanning calorimetry (DSC) of stored solar dried Siganid fish muscle treated with water extracts of Turmeric as antioxidants. Comparisons only with control for each treatment

Treatment	Myosin transition Denaturation temperature ^{o}C		Enthalpy change ΔH	Actin transition Denaturation temperature ^{o}C		Enthalpy change ΔH
	(T_{onset})	(T_{max})	Endothermic effect/J/g	(T_{onset})	(T_{max})	Endothermic effect/J/g
Control Day 0	42.17	44.91	0.11	50.46	57.53	0.27
Turmeric Day 0	42.48	44.57	0.11	54.61	57.36	0.10
Control Day 60	42.97	45.09	0.07	52.58	60.54	0.24
Turmeric Day 60	41.15	43.80	0.17	56.40	58.68	0.03
Control Day 90	43.63	46.81	0.44	53.47	59.25	0.09
Turmeric Day 90	41.42	43.72	0.23	54.80	57.85	0.06

Table 5.10: Transition denaturation temperatures Tonset, Tmax ^{o}C and Heat enthalpy (ΔH) of myosin and actin proteins by differential scanning calorimetry (DSC) of stored solar dried Siganid fish muscle treated with BHA as antioxidant. Comparisons only with control for each treatment

Treatment	Myosin Transition Denaturation temperature ^{o}C		Enthalpy change ΔH	Actin transition Denaturation temperature ^{o}C		Enthalpy change ΔH
	(T_{onset})	(T_{max})	Endothermic effect/J/g	(T_{onset})	(T_{max})	Endothermic effect/J/g
Control Day 0	42.17	44.91	0.11	50.46	57.53	0.27
BHA Day 0	43.99	47.12	0.45	53.62	58.54	0.07
Control Day 60	42.97	45.09	0.07	52.58	60.54	0.24
BHA Day 60	44.55	47.46	0.59	55.30	58.88	0.02
Control Day 90	43.63	46.81	0.44	53.47	59.26	0.09
BHA Day 90	43.45	46.36	0.20	58.34	59.07	0.13

5.4 Discussion

This study shows that antioxidants particularly the natural antioxidants water hyacinth, followed by turmeric and seaweed can be used to minimize texture changes during the storage of dried fish. Fish particularly fatty fish comprises triglycerides (75%) and phospholipids (25%) Most fish oils contain saturated fatty acids and an abundance of polyunsaturated fatty acids like docosapentaenoic acid (DHA) and eicosapentaenoic acid (EPA). Whilst the PUFAs are implicated in lowering atherosclerosis and lowering blood cholesterol, these unsaturated fatty acids and cholesterol are readily oxidized leading to rancidity and formation of chemicals including mutagens and carcinogens such as hydroxides, endoperoxides and epoxides (Halliwell and Gutteridge, 1995) (See chapter 3).

Lipid peroxidation is initiated in the presence of oxygen and metal ions, when hydrogen is abstracted from a methylene group in the PUFA by a reactive species. The primary products of lipid oxidation are peroxides which are converted to secondary products like aldehydes (TBARS). The free radicals and aldehydes produced during lipid peroxidation can combine with each other or more likely with protein molecules to end the chain reaction. This can cause severe denaturation of proteins and undesirable changes in nutritional properties including loss of amino acids, protein cross-linking and DNA damage. (Saeed, Fawthrop and Howell, 1999). Aldehydes can react with amino groups forming intramolecular bonds and cross-links with other protein molecules (Saeed et al., 1999) and provided evidence by ESR spectroscopy that lipid oxidation damages proteins through the transfer of free radicals to amino acids and proteins to form protein free radicals. These radicals can react with each other, for example tyrosine free radicals can react to form dityrosine (Saeed et al., 2006). It is this cross-linking that causes aggregation in proteins and toughening of fish muscle during processing like drying and freezing and on storage.

It is well known that antioxidants can minimize lipid oxidation via several mechanisms including binding oxygen, chelating metal ions, binding free radicals or stabilizing hydroperoxides. The antioxidants used in this study were water hyacinth, turmeric and seaweed which contain polyphenols, as well as a synthetic BHA with phenolic rings. These phenolic groups are known to bind free radicals especially through their aromatic ring structure which stabilizes the lipid free radicals by resonance (Halliwell, 1994). In addition, metal chelation is possible by hydroxyl groups in the phenolic compounds.

5.5 Conclusions

Because synthetic antioxidants are now considered unsafe, it is advantageous to replace them with natural foods like water hyacinth, turmeric and seaweed plant phenolics.

These natural antioxidants minimized lipid oxidation in solar dried fish during storage and thereby reduced protein oxidation and cross-linking, leading to lower G' values and reduced toughening compared to dried fish without antioxidants.

CHAPTER 6

CHAPTER 6

6.0 Carbon footprint for solar dried fish

6.1. Introduction

In fisheries worldwide, there is technological advancement from catch to processing to obtain good quality fish (Parker and Tydmers, 2015, Gosh et al 2014). The whole value chain starts from the construction of vessels through to maintenance, provision and loss of fishing gear, how combustion fuel is used during fishing, transportation of catch to various markets, as well as further processing like drying and waste discharge at sea. All these processes have an impact on the environment and one of the ways of quantifying this is through Life Cycle Assessment (LCA) or "Eco balance" (Gosh et al, 2014; Okoko et al 2017). Whereas focus in the fisheries has been on the stocks and utilization, it is important to note that the extraction and processing of the resource has impacts on the environment which has not received much attention in the past.

In LCA, the environment impact is studied from "cradle to the grave". This simply means from the resource extraction, up to the disposal of the product and to production of the waste. A simpler form of estimation of LCA is through the carbon footprint. The advantage of the carbon footprint is that it produces a single numerical index of environmental performance that can be readily understood; it contributes to the ecological footprint and is defined as "the total amount of carbon dioxide and other greenhouse gases emitted over the full life cycle of a product". The unit of measurement of the carbon footprint is "equivalent tonnes of CO_2".

Although fisheries is not a major contributor of the carbon footprint, (Shubhadeep et al 2014), it is estimated that on average the ratio of CO_2 emissions for marine fisheries is about 3 teragrams (10^{12}) million tonnes of fuel combustion. Carbon footprints are related to climate change and its impacts (Okoko et al 2017) and it is therefore important to identify the carbon footprint of different unit processes.

In the marine fisheries, the carbon footprint is traced through three phases:

i) The first phase is the pre-harvest phase which consists of construction of vessels all through to maintenance of the vessels and provision of fishing gear.

ii) The second phase is the harvest phase whose components are traditional, mechanized and motorized craft.

iii) The third phase is the post-harvest phase which includes fish transportation and processing. The selected functional unit used is 1 kg of fish to the consumer. Studies have shown that it is the harvest phase that consumes the highest amount of energy and with the highest emission of CO_2 (Gosh et al 2014). In the near future, carbon footprint of aquatic products mainly fish, will be integrated in assessments just like in eco-labelling. This is because the process of "catch" to "product" is growing in fuel consumption and equipment needs. The distances now covered by fleet to catch fish, the production of fish through aquaculture where feeds contribute to high consumption of fuel will lead to the need for monitoring of the carbon footprint in the fishery industry. This is also applicable to the labelling of the products (Madin & Macreadie, 2015).

Refrigeration and fishing distance also consume substantial energy and therefore the carbon footprint is bound to be high. In Kenya, long distance fishing vessels are common in Kenyan territorial waters. Farmed Tilapia and mackerel are now imported in Kenya frozen from China. All these require high energy expenditure not comprehensively recorded in Kenya. These is a lack of incorporating these high carbon footprints into the assessment of their sustainability by eco-labels, sustainability certification or seafood consumer sustainability guides ((Madin & Macreadie, 2015).

Dried fish is a common cuisine in Kenya and the sun is the main source of energy for the drying process (Oduor-Odote, 2010). In the dried fish chain however the pre-harvest energetics and carbon footprint (vessel construction, maintenance and gears), harvesting (mechanized and motorized) and the post-harvest stages especially transportation and marketing to waste management are the sources of carbon footprint. Cooling and refrigeration are the main consumers of fuel and emissions are reduced to a minimum, making drying a process that has a lower carbon footprint (Gosh et al 2014). Whereas the standard LCA involves reporting on goal and scope, life cycle inventory, life cycle assessment and interpretation of results, this study focused only on Greenhouse Gas Emissions (GHG)/Global Warming Potential (GWP).

This study gives an opportunity to understand and estimate the contribution of solar dried fish to climate change by determining the carbon footprint. Data from fuel consumption and subsequent CO_2 emission may help to understand the reasons for rising fishing costs and prices.

This information may be used in future to help formulate policy on regulation of fishing efforts, fuel subsidies, and addressing climate change mitigation. The data around the functional unit of 1 kg solar dried Siganid encompasses all the major industrial activities required to catch, process, and deliver the solar dried product to the consumer. The estimation of Carbon and Carbon emission from fuel (diesel) is calculated by converting the diesel consumed using the standard conversion factor that 1 litre of fuel (diesel) produces 10.7 kWh of heat, and the C emitted from 1 KWh of heat is 0.68 kg and for CO_2 is 0.25 kg.

6.2 Materials and Methods

The first step was to define the goal and scope according to (ISO 14041 2006), the life cycle inventory, the life cycle assessment and the interpretation of results. The goal of the study was to produce solar dried fillets of Siganids and determine the carbon footprint. The study was carried out to determine the environmental impact of solar dried Siganid fillet from harvesting to final product using the hybrid windmill-solar tunnel dryer. The information is useful to stakeholders who will be able to label and show indicative CO_2 equivalent on their products.

The functional unit was the CO_2 equivalent from dried Siganid unit/kg and quantity, quality and period formed the basis. The results were expressed in terms of 1 Kg of dried product (filleted unit). The method calculated the CO_2 emissions of all processes until the dried product was obtained from 'cradle to gate'-meaning from harvest at sea to final processed product like the solar dried fillet Other sources of carbon such as markets, transportation, were not considered. The boundaries of this production system started with fishing or raw material extraction (cradle) and ended at the production of 1kg of dried product (gate) of filleted solar dried fish. The ISO -14040-14042 standards were used for environmental impact studies as reference with MS Excel software. The sources of data were from existing databases, any reports and previous studies on raw materials. The system studied here only included fish production at the processor level and related processes like fishing and basic equipment manufacturing.

The Siganids were purchased from Kipini landing upon landing using motorized and non-motorized canoes. They were gutted, filleted and kept in ice. The fish were then transferred to the drying trays of the hybrid windmill solar tunnel dryer (Chapter 2).

Data was collected within the system boundaries (Figure 6.1) and relevant environmental inputs and outputs then analysed later.

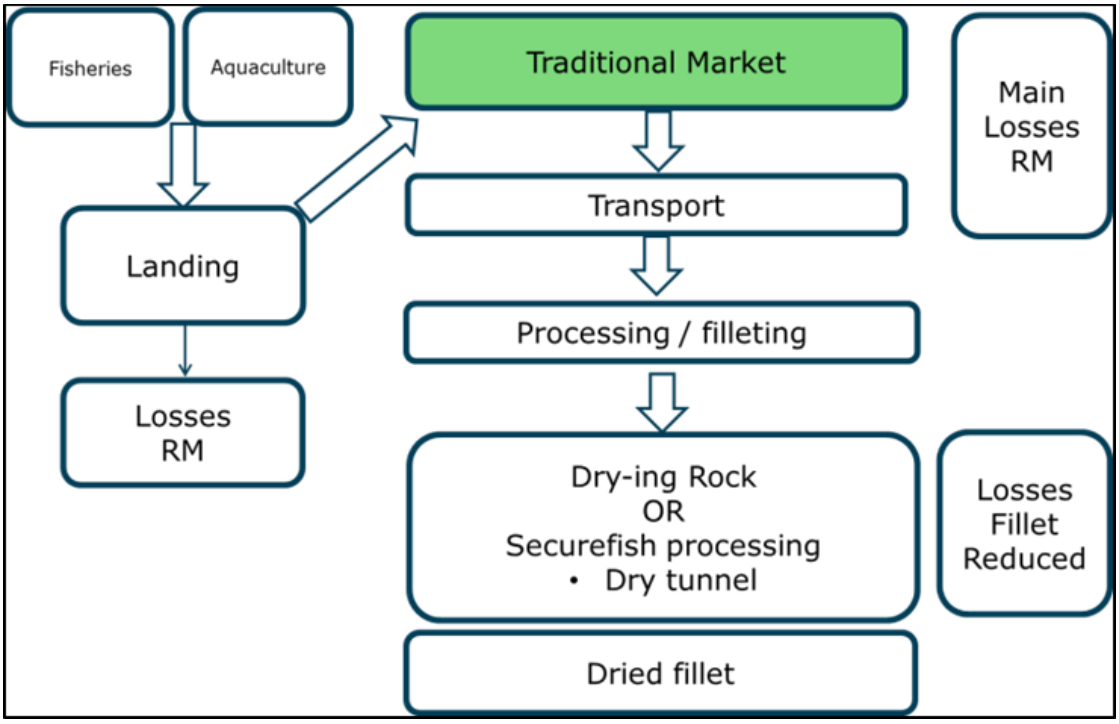


Figure 6.1: System boundary for solar dried Siganids (RM is Raw Material).

CO_2 foot printing uses database values for CO_2 emissions of combustion and utilisation of fossil fuels. The data base values are presented in Annexes 1 to 9 and Tables 6.1 and 6.2. The values were derived from peer reviewed data and are primarily for fossil fuel combustion and energy generation outputs. A generic tool for carbon footprint was developed to include easy access data primarily targeting low technological end process chains and enabling some newer technologies for dried fish products. Provisions are made for data that can be entered in the current study or in the industry in future for studies with a wider scope.

Any data that was not available and not expected to contribute to carbon footprint to a large extent was left out because many inputs and outputs do not have GHG-emissions. This was not a full LCA which would require additional data inputs. Economic allocation was used in this tool since this makes an adequate distinction in sea food derived products, and sea food waste derived products. The designed tool consisted of four separate steps/ data entry steps to complete the full product life cycle: These are

1) Raw material sections for data on actual harvesting
2) Landing or sorting on board
3) Transport of raw material to the processing facility
4) Processing; energy input of filleting; processing and losses in processing

The data was collected during harvesting of fish (cradle), landing and sorting on board, transport of raw material to the processing facility and processing which includes energy use during filleting and losses during processing (gate). The data for energy consumption and fishing data was obtained through interviews with fishermen and recorded in pre-prepared and designed data sheets (Annexes 1-6). The specific forms which were used under field conditions are presented in Annexes 7- 9. The tool was based on MS Excel calculations.

The targeted moisture content for solar dried fish was 10-20% (Chapter 3). The full capacity of the hybrid windmill solar tunnel dryer was 150 kg and considering that fish is about 70% moisture, 60% decrease in moisture content was required to get the final 10-20 % moisture. This required 30 to 40 sun hours on a sunny day for fish 1 inch or less thick, weighing between 300 to 500 g,

To achieve the targeted moisture content, 120 kg water had to be evaporated. The area of the collector surface of the dryer and area which was exposed to the sun was 9m x 2.5m = 22.5 m². The energy collection per day was therefore 22.5 x 5.3 kWh/m² = 2 107 J/m² day (assuming 100% efficiency). This gives a maximum daily drying capacity of 120 kWh or 430 MJ. This is equal to the evaporation of about 170 - 200 kg water daily. With an efficiency of about 65% it is possible to dry the fish in one day.

The drying temperatures in the drying cabinet varied between 40 ºC to 55 ºC sometimes reaching 60 ºC. On a good sunny day, when all the fans were working, the humidity ranged between 22-50% Some leakages, cloud cover etc. made drying time extend to 3 days to achieve the moisture content of 10-20%. All the data which were needed for carbon footprint could not be addressed from the data sourced. Where available, data from the site was collected and reported in annexes 1- 9 which are quite elaborate and whose use is for more data collection in future. The reference data for CO_2 emissions and combustions of fossil fuels was obtained from summaries of peer reviewed journals, reports and other publications (SKAO 2014). Data collection for carbon footprint analysis took the form of live interviews with the fishermen involved in going to sea. A structured form was used to collect the data as per Annexes 1 to 9. The data around the functional unit of 1 kg solar dried Siganid encompassed all the major industrial activities required to catch, process, and deliver the solar dried product to the consumer. The estimation of Carbon and Carbon emission from fuel (diesel) was calculated by

converting the diesel consumed using the standard conversion factor that 1 litre of fuel (diesel) produced 10.7 kWh of heat, and the C emitted from 1 KWh of heat is 0.68 kg and for CO2 was 0.25 kg.

6.3 Results

Based on the input parameters (Annexes 6.1 – 6.9) a carbon foot print calculation was made for fillet dried using the hybrid windmill solar drying system, with a total output of 2.9 CO_2 / kg and fossil energy-based drying with a total output of 3.2 kg CO_2 Eq / kg dried fillet (table 6.1 and 6.2), and figures 6.2 and 6.3.

The Carbon footprint data was generated during the raw material (RM) harvesting, landing, transport and processing. The sources for data for carbon footprint in raw material harvesting are during fishing because fuel is used, the fuel types used as different fuels generate different amounts of carbon footprint (Annex 6.1); stripping of fish on board, quantity of landed target species, quantity of the product landed, the waste from the byproducts. The total CO_2 for raw material when calculated from the Carbon footprint tool is 2.87 CO_2 Equivalent per kg of whole fish and it is mainly from fuel use (Table 6.1, 6.2 and Figures 6.2 and 6.3). At landing of fish the factors considered are landing procedures (if landed and transported), calculation on fuel used-(diesel for processing), transport and electricity used at landing site, loss at landing site (spoilage), use of waste product and use of waster product in another production chain, loss at landing site, economic value of by products and carbon footprint at the processing step; In transport as a source of carbon footprint, what is considered is distance between landing and processing. Transport method, fuel use during transportation. In the case of local processing the score is "zero" due to no movement (Table 6.1 and 6.2). Then there is the element of processing where energy total or CO_2 is not known then we refer to Annex 1. When there is electricity consumption per unit use form solar system and windmill, the carbon equivalent is 0.02 and when energy is from fossil fuels the carbon equivalent generated is 0.37 Kg CO_2/kg for whole fish. The total CO_2 Equivalent for whole fish production using fossils fuel becomes 3.23 CO_2 equivalents /Kg while for solar dryer it 2.87 plus 0.02 giving a total of 2.87 CO2 equivalents /Kg (Tables 6.1 and 6.2; Figures 6.2 and 6.3).

Table 6.1: Carbon footprint contribution in different steps of the production chain. Represented are the solar assisted dried fish process, and the drying process using fuel-based energy inputs.

Production step	**Production unit**	**CO_2 Eq/kg fillet dried Solar Assisted**	**CO_2 Eq/kg fillet dried Fossil fuel based**
Raw material	Total CO_2 footprint raw material fisheries	2.87	2.87
Landing total	Total CO_2 footprint at landing	0.00	0.00
Total Transport Landing to processing	Total transport to processing	0.000	0.000
Total Processing	Total processing	0.02	0.37
Total product	**Total product**	**2.89**	**3.23**

Table 6.2: Carbon footprint contribution in different steps of the production chain. Represented are the solar assisted dried fish process, and the drying process using fuel-based energy inputs.

		CO_2 Eq/kg fillet dried Fossil fuel based	**CO_2 Eq/kg fillet dried Solar Assisted**
Raw material	Fuel use fishing	2.87	2.87
Landing	Calculated based on total fuel / landed product	0.00	0.00
Landing	Calculated based on total energy / landed product	0.00	0.00
Landing	Spoilage	0.00	0.00
Transport	Additional fuel consumption do to cooling, freezing	0.00	0.00
Transport	Additional fuel consumption do to cooling, freezing	0.00	0.00
Processing	Use of electricity from solar energy	0.00	0.02
Processing	Use of electricity from wind energy	0.00	0.00
Processing	Use of electricity from fossil sources	0.37	0.00
Processing	Use of fuel per unit	0.00	0.00
Processing	Use of coolant per unit	0.00	0.00
Total		**3.23**	**2.89**

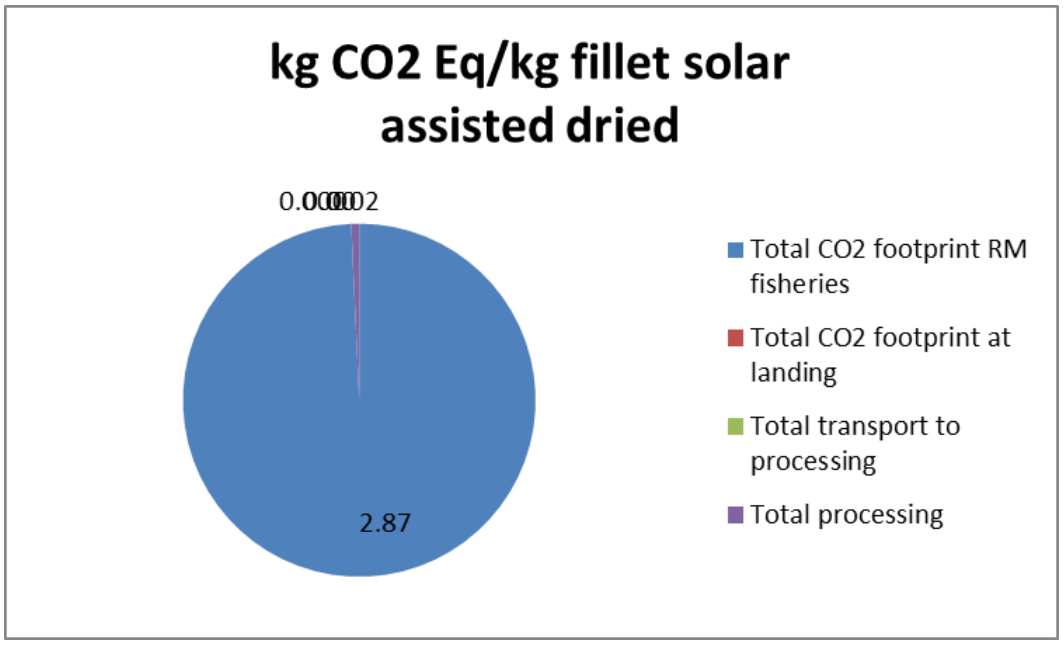


Figure 6.2: Carbon footprint of solar assisted dried fillet and dried fillet based on fossil fuel.

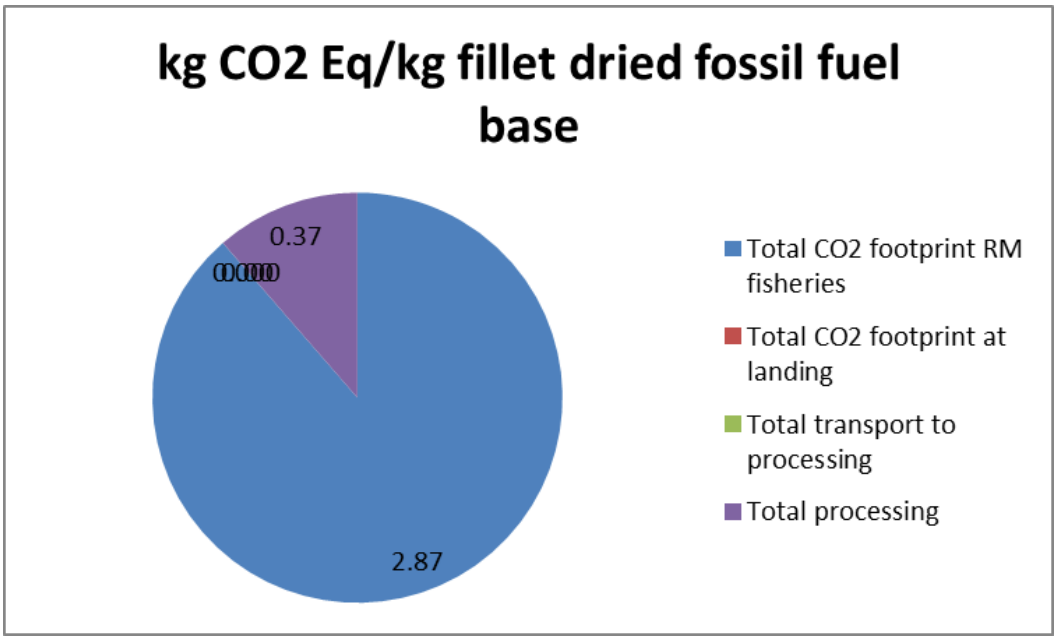


Figure 6.3: Carbon footprint of solar assisted dried fillet and dried fillet based on fossil fuel.

6.4 Discussion

Dried fish is a common dish in Kenya and the sun is the main source of energy for the drying process (Oduor-Odote, 2010). In the dried fish chain however the pre-harvest energetics and carbon footprint (vessel construction, maintenance and gears), harvesting (mechanized and motorized) and the post-harvest stages especially transportation and marketing to waste management are the sources of carbon footprint. Cooling and refrigeration, the main consumers of fuel and emissions were reduced to a minimum making drying as a process that has a lower

carbon footprint (Gosh et al 2014). Whereas the standard LCA involves reporting on goal and scope, life cycle inventory, life cycle assessment and interpretation of results, this study focused only on Greenhouse Gas Emissions (GHG)/Global Warming Potential (GWP). This study gave an opportunity to understand and estimate the contribution of solar dried fish to climate change by assessing the carbon footprint. Data from fuel consumption and subsequent CO_2 emission could help gain insight into increasing fishing costs and prices. This may be used in future to help formulate policy on regulation of fishing efforts, fuel subsidies, and giving proposals on suitable climate change mitigation measures.

6.5 Conclusions

The carbon dioxide emissions were low in this study due to low energy use (direct solar energy). The main emissions were caused by fisheries activities. However, due to the low fuel use in the fisheries practice (use of dhows) the total energy use is very low. The chosen processing chains were low in carbon emissions in comparison to other production cycles. The main reason for low carbon emissions was due to the primary production of the species as they do not have a high energy input for fishing, due to the artisanal conditions.

Due to the substantial influence of the primary production of the species, it is advisable to perform additional environmental impacts in different case studies. These dried fish case studies are relatively low in energy consumption. Considering that dried fish is also a preferred cuisine in many countries, paying more focus to it and emphasising on eco-friendly processing methods like the hybrid windmill solar tunnel will help reduce carbon footprint and improve food and energy security.

CHAPTER 7

CHAPTER 7

7.0 Quality safety tool -Generic HACCP for solar dried fish

7.1 Introduction

The success in the fishery processing and marketing industry relies on the safety of the product. The entire process of safety includes risk assessment and management as opposed to inspection of the product once it is about to reach the market or when it is already in the market. Inspection of products (Huss et al 2004) though useful in the earlier years for food safety are costly and retrospective and results may take several days to come out after the product has been sold. It means therefore that there are lower chances of detecting a food safety hazard.

Methods that control the hazard before they happen are therefore more important. Hazard Analysis Critical Control Point (HACCP) is one such method. HACCP has been developed for various fresh fish products. Before HACCP, Food Safety Assurance and Quality Control systems were the methods used. Food safety assurance involved removal, prevention or minimization of a hazard to acceptable levels whereas quality control systems were used after the product was manufactured or produced when a safety analysis for defects was carried out (Sperber 2005). However, the above methods could not detect hazards that do not occur often. The HACCP system had advantages because it was a more preventive system (Quinn & Marriot, 2002). It is now agreed internationally that the HACCP approach should be the one to use for food safety (Codex Committee on Food Hygiene, 1993; Codex Committee on food hygiene 2009; NACMCF 1997; EU Regulations 852/2004 2004; USDA (1996); Synder 1991; National Seafood Alliance 2011). There are 7 principles (steps) or tasks that guide food safety approach. They are listed as follows:

1. Conduct a Hazard Analysis & Risk Assessment (RA)
2. Identify Critical Control Points
3. Establish Target Levels (TLs) and Critical limits (CLs) for each CCP
4. Establish monitoring methods at each CCP
5. Identify Corrective Actions
6. Identify verification procedures
7. Organise a record keeping system

Steps that should be taken prior to HACCP include Good Manufacturing Practices and Sanitation Control Procedures.

In the fishery industry, HACCP has been developed for fresh fish products. Attempts have been made to develop generic HACCPs in the smoked fish industry. In the solar dried this is the first attempt to develop an HACCP system. Solar dryers so far fabricated in Kenya by KMFRI in collaboration with JKUAT have produced dried fish of superior quality compared with those available in the Kenyan markets. However, no quality handling standards are in place for dried fish in Kenya. As quality assurance of the final dried product is important for the market, an HACCP plan was designed for processing of solar dried fish in Kenya

7.2 Materials and Methods

The objective of the HACCP was defined, and the application and standard definitions were outlined. The people responsible for implementation of the HACCP and their roles were recorded, the possible hazards were defined, and the nature of the products described as well as the product usage. The HACCP plan was then put in place.

7.2.1 **Defining the objectives**

As the hazard analysis and monitoring scheme was carried out for solar dried fish, the possible microbiological, physical and chemical food safety hazards, CCP's and CP's per process step were determined.

7.2.2.1 Application

All process steps from purchasing to sales (from reception to transport to the consumer) including production, quality and engineering support were described.

7.2.2.2 Definitions (taken from the literature and references cited)

Hazard: ''A biological, chemical or physical contamination or conditions leading thereto, that makes a food product unsafe for consumption'' (from CAC, 2009).
Risk: ''A risk is a function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard in food(s) '' (from Sumner et al., 2004).

Hazard analysis: The process of listing and assessing hazards and which conditions cause them, identifying hazards that influence food safety, and therefore need to be incorporated in the HACCP plan.

CCP: Critical control point. Important points, step or procedures which require specific control measure to lower potential public health hazards to a very low level.

Control measures: Each action or activity used to remove hazards or limit them to an acceptable minimal level.

GCM/GM: General (Control) Measures to control a specific part of the plan.

CP: Control point.

7.2.2.3 Responsibility

The quality manager of hygiene is responsible for the approving each procedure and enact the following: Re-assess any changes in product or production process in consultation with others in the HACCP or production team and review the flow diagram and hazards.

- Be the contact point for liaising with food safety regulatory bodies.

7.2.2.4 Possible Hazards

All the delivered raw material and dispatched final product had to be at or below regulatory safe limits. Hazards can be divided into three areas:

Physical hazards

Physical hazards include fishhooks, wood splinters, metals, stones, glass etc. which are small enough to be visible but hard/sharp enough to hurt consumers.

In the processing facility we take measures to reduce the chances of contamination to as low as possible by receiving the raw material from certified suppliers/ trusted suppliers. Other mitigations measures are discussed later

Microbiological hazards

Microbiological hazards comprise living and dead organisms which are hazardous and are either intrinsic or extrinsic of the product. To ensure that the control of the microbial safety level, bacteriological, yeast and mould tests are carried out on:

Mycotoxins:

Poisons produced by fungi which may be because of toxin production by mould in inadequately dried fish stored at a relatively high-water activity. Of great concern are aflatoxin, ochratoxin and deoxynivalenol.

Chemical Hazard

These are chemical components which are hazardous for the consumer. Included are substances which are formed (from the product) by poor treatment of the product and non-product substance such as cleaning products, migration of packaging etc. Poisons can also include the presence of higher concentrations of PCBs, dioxin and heavy metals in fish products.

Allergens:

These are components which can lead to allergic reactions and all the consequences thereof. These could be reactions from consumption of fish and products from fish, soya and products based on soya and celery and products based on celery.

7.2.2.5 Products

Product description

Split/filleted S*iganids* locally known as "Tafi'' were solar dried. The fish was processed from fresh fish through solar drying to a reduced water activity while maintaining organoleptic quality and longer shelf life of 6 months in a cool dry place.

The solar dried *Siganid* fillet was processed and packaged by the Kipini Tuna fisherfolk of Kipini BMU. The *Siganid* fish was caught in the Western Indian Ocean region (WIO region)

Product usage

The hybrid windmill solar tunnel dryer produced dried fish product for consumption. The dried fish product should be soaked and boiled prior to use. The directions for use are on every packaged product.

7.2.2.6 HACCP team

The HACCP team is assembled. There are regular meetings to discuss defects, new products, process changes, hazards, improvements, facility audits, internal complaints are handled. Meetings considering validation, risks, monitoring and verification with regulatory agencies are recommended to be done.

Table 7.1: The HACCP team composition

Participants	**Contribution**
QA/QC in charge	Monthly summary report, defects per line at any processing batch. Verification and evaluation once per year
Production in charge	Line report (production report, defects)
Overall manager	Chairman, strong support for QA/QC in HACCP

7.2.2.7 Verification

The following points are considered in the yearly quality report:

a. All procedures be kept safely by the guardian (QA)
b. Completeness of forms checked yearly
c. Analysis of recalls and internal and external complaints
d. Reviewing specific and general control measures, defects and corrective measures for confirmation and effective monitoring of critical control measures
e. Consistency of the existing documentation

7.2.2.8 Flow chart for solar dried fish

The flow chart for the solar dried Siganid fish i shown in Figure 7.1

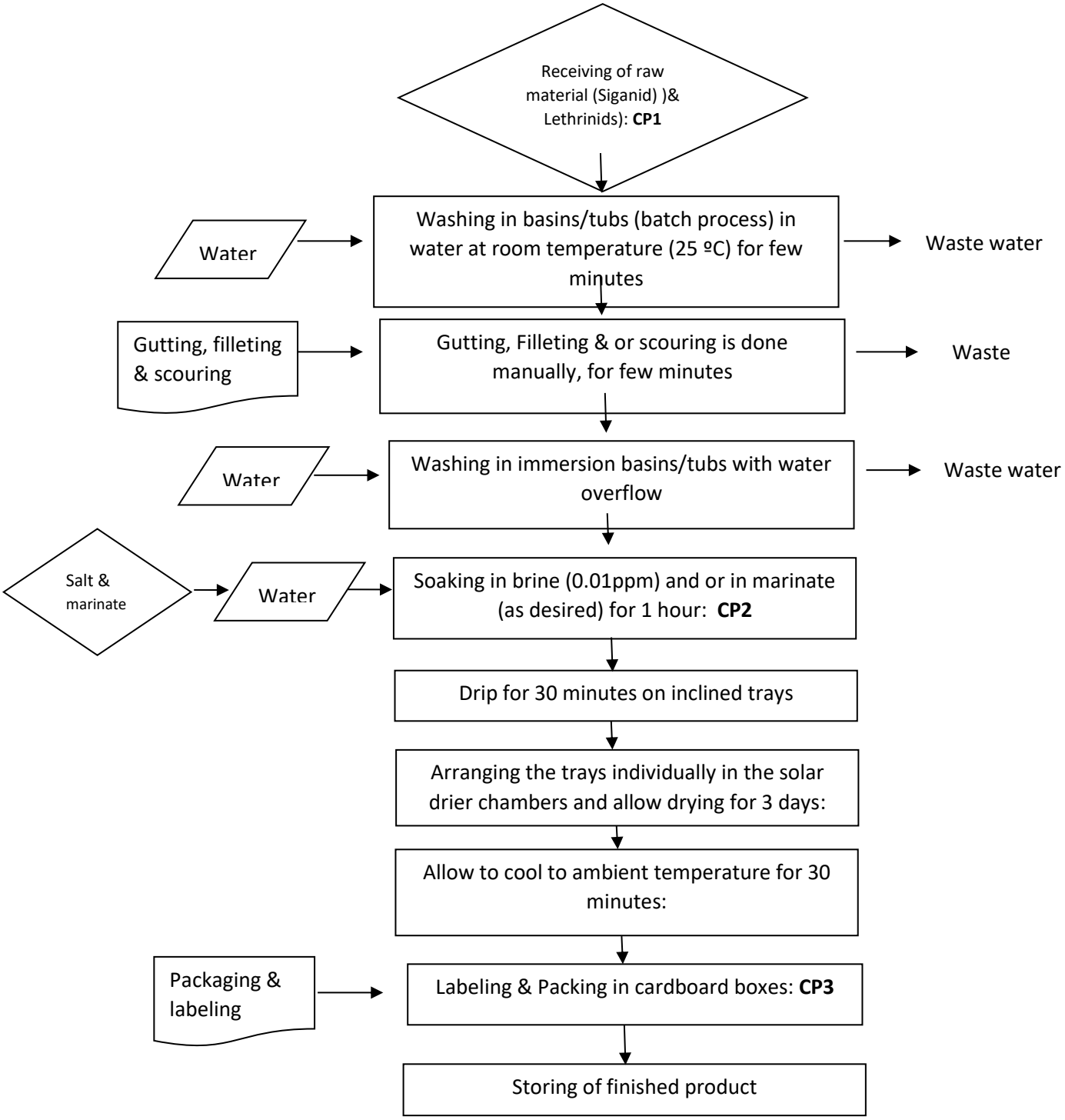


Figure 7.1: Schematic flow sheet for solar dried Siganids

7.4 Discussion

There has been a lot of emphasis on safety of fresh fish. Kenya took the matter seriously and started adopting HACCP when in 1997 the EU banned fish exports from Kenya (Abila, 2003). There have been no efforts however to introduce or adapt HACCP for the dried fish chain in Kenya. This is because dried fish is made from left over fish that has started to spoil and it is a belief that dried fish has "no moisture" hence safe because it dry and therefore should not deteriorate.

Dried fish on the contrary has been shown here to undergo deterioration through lipid oxidation and measures like vacuum packaging and using fresh product for drying improves quality. According to FAO (2004) and in this study, critical control points is when the drying process is delayed which can create room for bacterial growth and in handling equipment which may reintroduce pathogens the quality of the initial product notwithstanding. In general, dried fish products are considered shelf stable. Consequently, they are often stored and distributed unrefrigerated.

The characteristic of a dried fish product, which makes it shelf stable, is their low water activity (aw). Water activity measures of 'free water' in a food that is available for the growth of microorganisms, including pathogens. Below water activity of 0.85 the growth and toxin production of all pathogens like Staphylococcus aureus and Clostridium botulinum is prevented and this is important for a shelf-stable dried product (FDA, 2001, Abbas et al, 2009). At higher water activities the product should be stored refrigerated. The flow chart for solar drying of rabbit fish is presented in Figure 7.1 and an overview of the HACCP plan is given in Table 7.1. In practice, HACCP systems can be adapted, as necessary. The timescale for adaptation differs from procedures such as monitoring of CCPs and equipment used for this. An example of timescales and frequencies to perform procedures is presented in Table 7.2.

Another issue that is of interest is the international trade of fish products. Standardization of the HACCP concept may be required soon in Kenya. Therefore, aspects like management responsibility and compatibility between HACCP and other Quality Systems commonly used must be considered.

For harmonization of HACCP systems, a new International Standard, ISO 22000, is available which was published on 1 September 2005, as an Internal Standard for food safety management

to ensure safe food supply chains worldwide (Frost, 2005). A generic Risk analysis handbook for solar dried Siganids is proposed in (Tables 7.3 to 7.10) and is being used together with HACCP to help eliminate hazards thereby reducing critical control points. Risk analysis focuses on Pre-requisite Programmes (PRPs) eliminating unnecessary risks prior to and during processing. The scores to possible hazards are indicated in the Hazards handbook.

This was a scientific way of assigning scores to probable hazards in the drying chain. Overall, it is used to identify whether the step in the chain is a CCP or a CP or just a PRP (Prerequisite Program). Normally non CCP are management issues that do not need very close monitoring; however, they can become CP or CCP with neglect.

(The letters P, C M in Tables 7.3 to 7.10 stand for the nature of the different hazards. P stands for physical, C chemical and M is Microbiological). P x E = R in the table are P: Probability of risk, E is Exposure and R is the risk as a result of P and E (Jacxsens et al, (2009). If R is low the Risk is low.

7.5 Conclusions

Starting quality of fish before drying helps ensure safety and quality of the products. Risk analysis and establishment of HACCP in the dry fish will boost the consumer confidence in the dry fish chain and create a wider market. Assurance of adequate drying to low moisture activity and subsequent hygienic and sanitized handling reduces risk in the dry fish chain. Even though the final product is cooked, maintenance of proper handling and hygiene is crucial for business, income stability for wider product circulation.

Table 7.2: Overview HACCP plan Solar Dried Siganid fillet (Tafi or Rabbit fish)

STEP IN THE PROCESS	HAZARD	SOURCE	PREVENTIVE MEASURES	CCPS	CRITERIA		MONITORING				CORRECTIVE ACTIONS	VERIFICATION	RECORDS
					m	M	What	How	Frequency	Who			
RECEIVING OR BUYING FISH				1									
	Biological: Spoilage, Pathogens	Improper storage prior to sale	Purchase from qualified or certified supplier	Presence of document/ certificate	Here, m=M	Here, m=M	Assess whether or not document/ certificate is present	Establish whether or not document/ certificate is available upon receipt of fish	Document or certificate for each purchased lot	Quality manager	Select another supplier	Document from supplier with data from laboratory analysis	Monitoring records
	Chemical: Heavy metals, POPs	Environment			Document/ certificate is available	Document/ certificate is available							Document/ certificate of supplier
	Physical: Foreign objects	Adulteration on board or at supplier											Procedures
													Work instructions
STORAGE OF BOUGHT FISH				2									
	Biological: Spoilage, Pathogens	Improper storage	Instruction on proper use of flake ice	Control of time and T during storage	1 day	2 days	Presence of sufficient flake ice	Visual inspection	At beginning and en of a shift	Quality manager	Add flake ice	Assess that employees know and understand work instructions	Monitoring records
		No pest control	Pest control	Sufficient flake ice present	T of the room 1°C	T of the room 2°C	Measure T of storage room & storage time fish	Thermometer	Measure T twice a day		Train employees		Procedures
	Chemical: Heavy metals, POPs	N/A											Work instructions
	Physical: Foreign objects	Filth, foreign bodies present	Cleaning of storage facilities	Prerequisite program									
GUTTING AND FILLETING				3									
	Biological: Spoilage, Pathogens	Water used, premises and tools used for gutting and filleting	Water of safe and sanitary quality	Control of time and T during gutting and filleting	25 min for filleting	30 min for filleting	Inspect that employees follow work instructions	A laminated work instruction is available	Establish at the start of each shift that instructions are available	Quality manager	Train employees	Assess that employees know and understand work instructions	Monitoring records
					Each fillet immediately in bag and stored in ice	Each fillet immediately in bag and stored in ice	Measure T of fish at start of processing						Procedures
	Chemical: Heavy metals, POPs												Work instructions
	Physical: Foreign objects	Filth, foreign bodies present	Cleaning of storage facilities	Prerequisite program									
STEP IN THE PROCESS	HAZARD	SOURCE	PREVENTIVE MEASURES	CCPS	CRITERIA	MONITORING	CORRECTIVE ACTIONS	VERIFICATION	RECORDS	STEP IN THE PROCESS	HAZARD	SOURCE	PREVENTIVE MEASURES
				4									

SOAKING OF FILETS IN BRINE OR MARINATE	Biological: Spoilage, Pathogens	Water	Proper work instructions	Time and amount of salt or marinate used	Here, m=M	Here, m=M	Inspect that employees follow work instructions	A laminated work instruction is available	Establish at the start of each shift that instructions are available	Quality manager	Train employees	Measure salt content	Monitoring records
	Chemical: Heavy metals, POPs	*N/A			Employees follow instructions	Employees follow instructions	Measure salt content at the start of a shift					Start of a shift	Procedures
	Physical: Foreign objects	Filth, foreign bodies present	Cleaning of storage facilities	Prerequisite program								Once a week	Work instructions
SOLAR DRYING OF FISH				5									
	Biological: Spoilage,Pathogens	Molds	Control of drying process by use of working instructions	Employees use work instructions	Here, m=M	Here, m=M	Inspect that employees follow work instructions	A laminated work instruction is available	Establish at the start of each shift that instructions are available	Quality manager	Train employees	Verify the drying is controlled with respect to temperature, time and air velocity	Monitoring records
	Chemical: Heavy metals, POPs	*N/A			Employees follow instructions	Employees follow instructions							Procedures
	Physical: Foreign objects	Filth, foreign bodies present	Cleaning of storage facilities	Prerequisite program									Work instructions
STORAGE OF DRIED FISH				6									
	Biological: Spoilage,Pathogens	Improper packaging material, no pest control	Purchase from qualified or certified supplier	Presence of document/ certificate	Here, m=M	Here, m=M	Assess whether or not document/ certificate is present	Establish whether or not document/ certificate is available upon receipt of packaging material	Document or certificate for each purchased lot	Quality manager	Select another supplier	Document from supplier with data from laboratory analysis by supplier	Monitoring records
	Chemical: Heavy metals, POPs	*N/A			Certificate is available	Certificate is available							Procedures
	Physical: Foreign objects	Filth, foreign bodies present	Cleaning of storage facilities	Prerequisite program	In cool dry place T< 20°C	In cool dry place T< 25°C							Work instructions
*** N/A: NOT APPLICABLE**													

Table 7.3: Verification Stages and Their Minimum Frequency in a HACCP Plan (from Sperber, 1998)

Verification Procedures	Minimum Frequency
Verification schedule	Annually
Prerequisite program verification	In the beginning and annually thereafter
Validation of critical limits and HACCP system	Before and during HACCP system implementation
Revalidation of HACCP plan	Annually or upon significant change
Observation and interview of CCP monitor	Quarterly/monitor/CCP
CCP monitoring record review	Daily
Equipment calibration	According to HACCP plan
Other records review	Monthly
HACCP system verification	Annually

Table 7.4: HACCP-Hand book- Receiving of raw material

NAME Company	HACCP-HANDBOOK		Page: 1
	RISK ANALYSIS TABLE HACCP – Step 7 & 8		Date: 06/2018
	Product: Solar dried fish	Flow chart: TUNA FISHER-FOLK	

PROCESS STEP: Receiving of raw material

Concerning	Potential danger	Type	Preventive measure	P x E = R	CCP	CP	PRP
	1. Insufficient microbiological quality: total count too high; presence of pathogens	M	• Purchase from pre-qualified suppliers and incoming raw material quality control	**2 3 4**	-	**1**	-
	2. Presence of heavy metals	C	• Purchase from pre-qualified suppliers and incoming raw material quality control	2 2 3	-	-	9
	3. Presence of foreign objects	P	• Purchase from pre-qualified suppliers and incoming raw material quality control	1 3 3	-	-	9
	4. Chemical hazards (POPs)	C	• Purchase from pre-qualified suppliers and incoming raw material quality control	2 2 3	-	-	9

Concerning	Remarks/Motivation
Hazard 1	Drying is a step further in the process that will eliminate vegetative micro-organisms, weekly some samples are analysed on the microbial quality on *Salmonella* spp., and *Listeria monocytogenes*
Hazard 2	Controlling heavy metals in the company itself is almost impossible and gives little additional information. The suppliers are expected to comply with set limits of heavy metals and therefore controlled by PRP.
Hazard 3	Slitting, scouring and filleting are steps further in the process that can help in elimination of foreign objects like fishing hook through visual inspection.
Hazard 4	Fish will be purchased from suppliers who demonstrate fishing in permitted grounds.

Table 7.5: HACCP-Hand book- Washing in basins and tubs

NAME Company	HACCP-HANDBOOK		Page: 2
	RISK ANALYSIS TABLE HACCP – Step 7 & 8		Date: 06/2018
	Product: Solar dried fish	Flow chart: TUNA FISHEFOLK KIPINI	

PROCESS STEP: Washing in basins/tubs

Concerning	Potential danger	Type	Preventive measure	P x E = R	CCP	CP	PRP
	1. Microbial and chemical contamination through insufficient water quality	**M/C**	• Portable water is used. Regular water quality checks	**2 2 3**	-	-	**3**
	2. Additional contamination through insufficiently cleaned and disinfected tanks	**M**	• Proper cleaning and disinfection procedures	1 2 2	-	-	1
	3. Chemical contamination through residuals of cleaning and disinfecting agents	**C**	• Approved food grade disinfectants used.	1 2 2	-	-	1

Concerning	Remarks/Motivation
	A drying process is included in the process. This process can destroy vegetative and other pathogens due to reduced water activity, but more resistant microbes will survive resulting in an insufficient microbial quality.

Table 7.6: HACCP-Hand book- Gutting, scouring and filleting

NAME Company	HACCP-HANDBOOK		Page: 3
	RISK ANALYSIS TABLE HACCP – Step 7 & 8		Date: 06/2018
	Product: Solar dried fish	Flow chart: TUNA FISHERFOLK	

PROCESS STEP: Gutting, Scouring & or Filleting

Concerning	Potential danger	Type	Preventive measure	P x E = R	CCP	CP	PRP
	Microbiological quality: total count too high; multiplication of pathogens	**M**	Gutting, scouring and/or filleting to be done within 30 minutes per batch	**1 2 2**	-	-	**14**

Concerning	Remarks/Motivation

Table 7.7: HACCP-Hand book- Washing in immersion basins/tubs

NAME Company	HACCP-HANDBOOK		Page: 4
	RISK ANALYSIS TABLE HACCP – Step 7 & 8		Date: 06/2018
	Product: Solar dried fish	Flow chart: TUNA FISHERFOLK	

PROCESS STEP: Washing in immersion basins/tubs

Concerning	Potential danger	Type	Preventive measure	P x E = R	CCP	CP	PRP
	1. Microbial and chemical contamination through insufficient water quality	**M/C**	• Portable water is used. Regular water quality checks	**2 2 3**	-	-	**3**
	2. Additional contamination through insufficiently cleaned and disinfected tanks	**M**	• Proper cleaning and disinfection procedures	1 2 2	-	-	1
	3. Chemical contamination through residuals of cleaning and disinfecting agents	**C**	• Approved food grade disinfectants used.	1 2 2	-	-	1

Concerning	Remarks/Motivation
	A drying process is included in the process. This process can destroy vegetative and other pathogens due to reduced water activity, but more resistant microbes will survive resulting in an insufficient microbial quality.

Table 7.8: HACCP-Hand book- Soaking in brine or marinating

NAME Company	HACCP-HANDBOOK		Page: 5
	RISK ANALYSIS TABLE HACCP – Step 7 & 8		Date: 06/2018
	Product: Solar dried fish	Flow chart: TUNA FISHERFOLK	

PROCESS STEP: Soaking in brine and or marinating

Concerning	Potential danger	Type	Preventive measure	P x E = R	CCP	CP	PRP
	1. Microbial growth due to insufficient brining	M	Time and adequate salt used	**2 3 4**	-	**2**	-
	2. Allergens from marinate especially soy	C	Right concentrations of the marinates used and clear packaging information	3 2 4	-	3	-

Concerning	Remarks/Motivation
Hazard 1	Insufficient brining may lead to growth of halophilic microbes
Hazard 2	In case of wrong concentration (marinates), subsequent process cannot eliminate the allergens.

Table 7.9: HACCP-Hand book- Dripping

NAME Company	HACCP-HANDBOOK		Page: 6
	RISK ANALYSIS TABLE HACCP – Step 7 & 8		Date: 06/2018
	Product: Solar dried fish	Flow chart: TUNA FISHERFOLK	

PROCESS STEP: Dripping

Concerning	Potential danger	Type	Preventive measure	P x E = R	CCP	CP	PRP
	1. Additional contamination through insufficiently cleaned and disinfected racks & cross contamination through drip	M	• Work instruction: Avoid mounting of dripping racks on top of another	**1 2 2**	-	-	**14**

Concerning	Remarks/Motivation

Table 7.10: HACCP-Hand book- Drying

NAME Company	HACCP-HANDBOOK		Page: 7
	RISK ANALYSIS TABLE HACCP – Step 7 & 8		Date: 06/2018
	Product: Solar dried fish	Flow chart: TUNA FISHERFOLK	

PROCESS STEP: Drying

Concerning	Potential danger	Type	Preventive measure	P x E = R	CCP	CP	PRP
	1. Microbial growth	M	• Controlled temperature and humidity	**3 3 5**	**1**	**-**	**-**

Concerning	Remarks/Motivation
	Insufficient drying will give optimal growing conditions for pathogenic microorganisms. This also marks the end of a major processing stage for microbial control as subsequent stages relies on the product quality and safety at this stage

Table 7.11: HACCP-Hand book- Labelling and packaging in cardboard boxes

NAME Company	HACCP-HANDBOOK		Page: 8
	RISK ANALYSIS TABLE HACCP – Step 7 & 8		Date: 06/2018
	Product: Solar dried fish	Flow chart: TUNA FISHERFOLK	

PROCESS STEP: Labelling & Packing in cardboard boxes

Concerning	Potential danger	Type	Preventive measure	P x E = R	CCP	CP	PRP
	1. If the integrity of the packaging materials is breached dried fish can gain moist and thereby result in an increase in water activity	M	• Packaging from pre-qualified suppliers • Correct handling and packaging procedures followed.	**2 2 3**	**-**	**4**	**-**
	2. Migration of printing ink from packages	C	• Packaging from pre-qualified suppliers and proof of certificate of analysis	1 2 2	-	-	9

Concerning	Remarks/Motivation

CHAPTER 8

CHAPTER 8

8.0 DISCUSSION

8.1 Introduction

Food security is an important issue globally and particularly in sub-Saharan Africa due to a shortage of food, water and energy supplies; these shortages are predicted to increase with population increase. It is necessary to improve methods of processing and utilize all the by catch and by-products in fisheries and agriculture. Fisheries is the major livelihood of coastal people in Kenya. Therefore the objectives of this study were to improve the preservation of fish by sun drying in a fabricated hybrid windmill solar tunnel dryer, to monitoring the quality during drying and storage, to reduce lipid oxidation using natural antioxidants and to examine the effect of antioxidants on the rheological and thermal properties of protein during storage; to monitor the carbon footprint during processing and to produce HACCP guidelines and measure quality cost.

8.2 The hybrid windmill solar tunnel dryer

In this study, hybrid windmill solar tunnel dryer was fabricated successfully, and its performance was measured by drying salted and unsalted fish, using solar energy during the day and wind energy at night and on cloudy days. The moisture content of salted and unsalted *Siganid* fish reduced exponentially as the drying time increased. A similar trend was reported by Guhnan *et al*. (2005), Alibas (2012) and Kituu *et al*. (2014). The moisture ratios for the unsalted fish reduced relatively fast compared to the salted fish, which agrees with the observations made by Graivier et al. (2006) and Jittinandana (2002). The reduced drying associated with the salting of fish could be attributed to the binding of moisture by salt in the fish flesh.

The ambient humidity was high throughout the drying period which is common along the Kenyan coast and is a cause of the slower drying rate and prolonged drying period considering the cloudy atmosphere that was prevailed. Drying during the first 18 hours showed that the ambient and drying

chamber relative humidity values were almost indistinct. However, beyond the 18th drying hour, the ambient temperatures increased, and subsequently, the drying chamber temperature increased. However, the increase in drying chamber temperature was much more than the increase in ambient temperature, which demonstrated better energy harnessing by the dryer hence improving the ability of the system to dry the fish to a better quality. This is because lower air humidity results in increased moisture absorption potential (Mujaffar and Sankat, 2005). A two-way statistical Student's *t*-test showed a significant difference between ambient and drying chamber relative humidity (t_{stat}=*7.2543*, $t_{crit,\ 5\%}$=2.0687).

The high solar dryer temperatures resulted in significant reduction in the relative humidity in the dryer compared to the ambient humidity resulting in increased drying potential. This is in agreement with the observation by Sahoo (2012) who noted that relative humidity reduced exponentially as air temperature increased. The safe moisture content for fish brined in 0 to 15% salt are 0.15 and 0.35 kg/kg, d.b respectively (Brugay *et al*, 2003). The final moisture content of 0.2kg/kg, db was shelf stable for dried fish. The drying of the fish to stable values of moisture content was due to higher chamber temperatures in the dryer.

The drying rate constant (k) values ranged from 0.037-0.05 per hour for the salted and unsalted *Siganid* fish. However, the values decreased with increase in brine concentration. This implies that an increase in salt concentration binds water to the fish flesh, and this makes it unavailable for drying. Based on the equation 6 (Chapter 2), the drying rate constants and the thickness of the materials, values of the effective diffusivity (D_f) were 6.53 x 10^{-12} and 6.63x 10^{-12} m^2/s for the salted and unsalted *Siganid* fish, respectively. These values further augment the observation that the unsalted samples dried much faster than the salted samples.

The best model for the describing the drying of the salted (5%) and unsalted *Siganid* fish in the hybrid windmill solar tunnel dryer was model 2, the Page model (table 2.2) which had the highest R^2, and the least RMSE and reduced χ^2, A two way statistical analysis did not show a significant difference between modelled and actual moisture ratio for salted (t_{stat}=8.0267×10^{-2}, $t_{crit,\ 5\%}$=2.0687) and for unsalted (t_{stat}=2.8740×10^{-5}, $t_{crit,\ 5\%}$=2.0687) at 5% level of significance.

8.3 Quality, Biochemical and Microbiological Properties of *Siganus Sutor* fillets

8.3.1 Quality scores during delayed icing

The quality scores were based on the QI scheme. The scoring scale was designed in QIM format such that score 0 meant the freshest, score 1 was slightly fresh while score 3 meant the least fresh. The results showed that the freshness quality of the fish assessed decreased with time and those assessed after 4 hours had the poorest quality. From the score, the fish were still of good quality after 4 hr delayed processing with a QI maximum of 1.2 for the gills.

8.3.1.1 Quality index

There was a strong correlation of $R^2 = 0.9735$ observed that were significantly different ($p < 0.05$). The noted difference showed premium quality grade 1 scoring lower than good quality grade 2 and marginal quality grade 3 accordingly.

The results indicated that attributes gradually deteriorated with time and obeyed the QIM scheme; fresh fish just after catch was given lower scores which increased with time, coming close to a maximum score at the end of shelf life (Martinsdottir*et al*., 2001). It is therefore worth noting that the QIM was successfully used to categorize samples into quality grades. Five pieces of fish used in the current study was in accordance with the guidelines for evaluation of freshness of whole fish (Martinsdottir*et al.* 2001). A minimum of three (large fish) to 10 (small fish) random samples is recommended in the guidelines as this should be taken to cover the biological differences in spoilage rate of fish. All the quality grades were within the consumption limits as the QI for the analyzed fish had a maximum score of 21 whereas, grade three that was scored higher had QI of 6.

8.3.1.2 Individual quality parameters in QIM scheme

The average scores for the individual quality descriptors varied considerably within the groups However, subsequent quality grouping recorded consistently higher scores throughout except for eyes form. Figure 3.3 further depicts that skin mucus and skin odour should be excluded from the

scheme as they were either difficult to evaluate or did not reflect on quality for *Siganid* fish and recorded minor changes in the groups (Sveinsdottir*et al,* 2003).

8.3.1.3 Influence of raw material quality on yield and labour productivity

The yield diminished with reduction in fish quality as grade 1 recorded higher fillet yield of about 54% compared to grade 2 and 3 that were 50% and 45% respectively. However, the yield was inversely correlated with production time reported during filleting as longer time (minutes) were observed with poorer grades. Earlier studies in cod filleting showed the relationship between raw material quality and productivity and lack of icing on board (Zugarramurdi et al., 1995; Zugarramurdi et al., 2004). Longer storage time produced lower yields and required slightly longer time for filleting. The difference looks insignificant for few samples but in an industrial set up it would translate to big economic losses in terms of time and yield. Because freshly caught fish is still in rigor, the muscles are easily cut than when the fish loses rigor. Fillets tend to stick to the bones and therefore give low yields and increases time to fillet.

8.3.2. Biochemical parameters during delayed processing

During the 4-hour delayed icing period there was increase in T-VBN, PV and TBARS. pH decreased and TMAO was not detected.

8.3.3 Water activity and pH

The water activity was constant at values of 0.88 for the three grades meaning that the delayed processing of grade 2 and 3 did not have effect on water activity. Dehydration of foods helps to extend shelf life by reduction in water activity (a_w) as a low water activity inhibits microbial growth and enzymatic reactions. The pH ranges of 6.03 to 7.1 have been recorded for most fresh fish. The low pH levels are because of stress at the time of death which causes depletion in energy reserves especially glycogen being converted to lactate. It could also be due to increased production of free fatty acids during hydrolysis of fats (Özogul et al 2005).

8.3.4 Lipid oxidation

Peroxide Value (PV) increased during the 4 hr delayed processing period from 21.65 $mEqO_2$/kg at 0 hr to 106 $mEqO_2$/kg after 4 hr. Peroxide value measures the breakdown of hydroperoxides, the primary lipid oxidation products. A value of 20$mEqO_2$/kg is proposed as the maximum limit for fish oil but there is no maximum standard value agreed for PV as higher PV values have been obtained (Alghazeer et al, 2008), even in this study without detection of spoilage. There are no levels for dried fish reported.

The secondary lipid oxidation products TBARS increased from 0.367 mg malondialdehyde /kg at 0 hr to 3.011 mg malondialdehyde/kg after 4hr of delayed processing storage. The delayed processing showed a linear increase with time from 0 to 4 hours. TBARS are formed from unstable hydroperoxides during lipid oxidation and contribute to rancidity. The increase in TBARS corresponded to the formation of hydroperoxides which was slower in breakdown than formation of TBARS. The value of TBARS in this short storage period was less than the upper limit set for most fish oils of 7 to 8 (Gimenez et al, 2011; Boran et al, 2006) and 27 mg malondialdehyde for mackerel fish (Özogul et al 2005).

8.3.5 TVB-N

The T-VBN levels rose from 13.8 to 42 mg N per 100 g of fish muscle from 0 to 4 hr of delayed processing. T-VBN release depends on bacteria decomposition of fish flesh. Freshly caught fish have TVB-N values of 5 and 20 mg N per 100g of muscle and 30-3 mg N per 100 g of muscle is the level of acceptability for iced stored fish in cold regions (Ozogul et al 2005 and Huss 1998) although in warm water fish, this could be higher.

Raw material quality had a direct impact on yield and production costs of *Siganus sutor*. There was an increase in filleting time and lower filleting yield during delayed processing. The levels of PV, TBARS, TVB-N, TMA increased during delayed processing as a sign of quality deterioration. The pH values reduced during delayed processing. Further studies could be done to document all production costs. There is a need to further link the developed QIM scheme for *Siganus sutor* to precisely characterized quality with storage time.

8.4 Drying characteristics during delayed processing (0, 2, 4, 6, 8 and 10 hr delayed processing)

This was covered in chapter 2. The main interest was to dry the fish for storage to acceptable moisture content of 15%

8.4.1 Moisture content in fish samples

Despite delayed icing, all the fish were dried to about 15% moisture, which is the shelf stable moisture content that does not favour bacterial or mould growth.

8.4.2 Biochemical changes during drying (0, 2, 4, 6, 8 and 10 hr delayed processing)

8.4.2.1 Water activity and pH in fish samples undergoing delayed filleting

The water activity reduced during drying although the fish that was filleted at 0 h, had a faster loss in water activity than the one that was filleted last after 10 h delayed processing. Water activity is the available water for microbial growth and is unbound water. In fish stored for 0 hr the water available for microbial growth is more readily available and is lost easily than fish undergoing delayed processing.

The pH values for the fish processed after 0 h and 2 h were lower than those processed after 6, 8 and 10 hr. The pH values were higher because as the delay continued, bacteria also metabolized various substrates in the fish to produce volatile basic compounds that are alkaline, hence the increased pH (Huss, 1995; and Susanto et al, 2011, Makawa et al, 2014).

8.4.2.2 Peroxide value and TBARS

Peroxide value was highest in the fish processed after 10 h and lowest in the fish processed after 0 h (Figure 3.8). The lower PV at the end of drying was because the hydroperoxides are unstable at higher temperatures and break down further (Frankel 2005) to volatile and none volatile compounds and secondary products with limits being 20mEq O_2/kg (Weber et al 2008, Haque et al 2013). No limits are known for PV in solar dried fish.

TBARS

The increase in TBARS at 0, 2, 4, 6, 8 and 10 h at the beginning was due to the initial formation of hydroperoxides which break down to aldehydes and related compounds. However, during the process of drying, the TBARS decreased either due to presence of more volatile tertiary compounds and due to formation of adducts with proteins (Weber et al 2008; Özogul et al 2005; Frankel 2005), which can cause toughening of fish. The limits for TBARS in fish oil is 7 to 8 mg MDA/kg but this may not apply to solar dried fish because the fish at the end of drying and at the beginning of storage is still suitable for human consumption despite the higher levels of TBARS.

8.4.2.3 TVB-N and TMA

There was an increase in both TVB-N and TMA during delayed processing at time 0, 2, 4, 6, 8 and 10 hr at the start of drying for the fresh samples. As the drying continued, the trends from TVB-N and TMA-N were different though the net effect was a reduction in TVB-N and TMA-N levels.

The lower TVB-N values can be explained by lower microbial loads in the fish throughout the initial drying periods. The peak period when the TVB-N is highest is due to microbial effects with bacteria causing the release of TVB-N up to a maximum when the population of bacteria start to go down again. There seemed to be a maximum increase in TVB-N between 12 to 16 hr of the net drying period. This was the period preceding the fish having been stored overnight to resume drying the following day. Bacterial growth would continue to occur. Increased TVB due to increase in microbial loads has been reported (Anderson, 2008; Orban et al 2011). The TVB-N values finally reached lower values at the end of drying.

The trend is slightly different for TMA-N in that there was no peak increase before a decrease although there was still a net reduction in TMA-N at the end of the drying period. The TMA levels were not detected in the fish fillet that were filleted after 0, 2 and 4 hours for delayed processing and did not show any TMA throughout the drying period. The TMA levels started to be noticed in the fish that was processed after 6, 8 and 10 hr delayed processing.

It is important to note the levels of TMA in the fish at the start of drying irrespective of the delay are still within the allowable limits of spoilage of 5mg TMA/100g (Orban et al 2011). The

pathways and rates for formation of TMA and TVB-N could be different involving different types of bacteria with different capacities to form volatile bases (Orban et al 2011) though at the end both are reduced after the drying process.

8.5 Biochemical changes during storage of solar dried fish only at 0 h of delayed icing

8.5.1 Moisture during storage

Moisture content above 25% can support bacterial growth and mould growth can take place at moisture content above 16%. The fish was still within safe limits for microbial attack despite an increase in moisture content. The absorption of moisture is due to the hygroscopic nature of the fish muscle.

8.5.2 Water activity

Water activities below 0.8 for beef products are common and are equivalent to about 23% moisture. The water activity achieved in this study during storage is still below the threshold of 0.8 hence the fish is still safe for consumption. There is however a complex relationship between moisture content and water activity. Moisture content is useful when looking at the time to end drying. Water activity is for information on storage. It is not always correct to assume that a higher moisture content will mean a higher water activity (Karel, 1975; Potter 1968; Oduor-Odote et al, 2010).

8.5.3 pH

The pH during storage of the *Siganid* fillet pH dropped in all forms of storage though it was lowest in vacuum packed. Changes in pH occur during storage but is normally faster in ambient storage i.e. equivalent to open storage. Although pH has been used to measure fish deterioration, the variation in pH is due to many factors like chemical composition, storage conditions in relation to air among others (Susanto et al, 2011).

Under normal circumstances, the pH is expected to drop then increase again during storage. In this case the pH decreased. This means that there is still some activity during storage that could be another factor other than glycogen and its effect is higher in the fish stored in the open than in packaged fish. The hydrolysis of lipid to fatty acids could be another reason for decrease in pH. During storage of fish meat in ambient conditions, El-Deen and El-Shemery (2010) noticed a decrease in pH.

8.5.4 Lipid oxidation

Hydroperoxides, determined as peroxide value (PV) rose during storage of *Siganids* (Figure 3.16) suggested that the propagation process on lipid oxidation was continuing until day 75 of storage. Lipid oxidation is influenced by light and oxygen. The higher value of PV in the fish stored in the open and those stored in normal polythene could be because of both light and oxygen while the increase in PV in the vacuum-packed fish could be due to light mainly because the packaging is permeable to light. Whereas PV normally rises, and peaks then drops due the formation of secondary products, as many studies have shown (Ghlaly et al 2010), other studies have also shown a continuous rise in PV of fish oil without peaking, and then dropping during storage (Boran et al, 2006). PV limits for fresh fish oil is 20mEqO_2/kg (Jinadassa 2014). Thus, one spoilage indicator cannot be used to conclude spoilage.

Secondary lipid oxidation products like malonaldehyde (TBARS) decreased during drying (Figure 3.9) and throughout the storage period (Figure 3.17). The limit for TBARS is 5 mg malonaldehyde/kg for good quality fresh fish and fish can be consumed even when TBARS are 8.0 mg MDA/kg (Shallam et al 2007). The fish for storage was therefore within consumption range for humans. The decrease in the level of TBARS is due to malonaldehyde breakdown to tertiary products as well as cross-linking with proteins particularly tyrosine, lysine and tryptophan amino acids (Saeed and Howell, 2002, Saeed et al, 1999, Shallam et al, 2007). The rate of formation of PV can also be higher than TBARS as the latter break down and interact with other compounds.

8.5.5 TVB-N

The level of TVB-N levels were all within the limits allowed for fresh fish though the limits for dried fish has not been developed. The allowable limits set by the EC 95/149 is 35mg TVB-N per 100g. Similar studies have given TVB-N values of 17 to 33 mg N /100g for dried fish (Pavakar et al, 2013; Jinadasa, 2014). The lowest increase in TVB-N was in the fish stored in a vacuum and the highest increase was for those stored in the open (Figure 3.20). TVB-N is considered a fish spoilage indicator rather than a fish freshness indicator. These levels apply to fresh fish (Orban et al, 2011). The TVB-N value is categorized in quality classes with TVB-N up to 25mg/100g considered "high quality" up to 30 mg/100 g considered "good quality" between 30-35 mg/kg falling in "level of acceptability" and above 35 mg/kg considered "spoilt" (Jinadassa 2014). There are no reported studies on dried fish indicator parameters for *Siganids*. This study is however very useful to dry chain fish processors to obtain data for spoilage of dried fish deterioration and to adopt proper storage conditions.

8.5.6 Temperature

The temperature during storage of fish ranged from 30.4^{o}C at the beginning of storage to 23^{o}C at the end of storage (Figure 3.19), due to weather changes over time. Temperature during storage of dried fish is as critical as temperature considerations in preservation of fresh fish as it influences bacterial growth (Jinadassa 2014). Temperatures of between 5^{o}C and 60^{o}C can easily favour microbial growth so storage under such conditions can lead to deterioration of fish. In the case of dried fish, the ones that are stored at temperatures (23-30^{o}C can result in microbial growth if other conditions are right. Vacuum packaging will help under such circumstances.

8.5.7 Microbiological analysis and evaluation

Bacterial growth is a cause of fish spoilage apart from causing of disease. Bacterial numbers can be used as an indicator of fish quality. In this study the total number of bacterial count for the freshly landed *Siganus* ranged from 0.90 x 10^{6}–2.50 x 10^{6} cfu g^{-1} of fish flesh for the TPC analysis whereas the levels of Specific Spoilage Organisms (SSO) ranged between 0.60 x 10^{6} – 2.90 x 10^{6}. These levels were however higher than the maximum limits recommended by both the International Standards Organization (ISO-4833) and the Food and Agricultural Organization

(FAO) of 5 x 10^5(ICMSF, 1980; FAO/WHO, 1976, 1978; WHO, 1974; and Codex, 1977). This variance in accepted limit numbers has in fact made the developed countries such as Denmark and United States of America (USA) to set their own accepted maximum limits in a gram of flesh at 1 x 10^5 (100,000) and 5 x 10^5 (500,000) respectively. However, these observed count numbers were within the accepted limits mentioned by the Sudanese Standards and Metrology Organization (SSMO) of 5×10^5 – 10^6cfug^{-1} for fresh fish products (SDS 357). The numbers were also in the normal ranges stated by Liston (1980) of between 10^2 – 10^7cfug^{-1} of fish flesh.

The existing wide range of bacterial flora counts on freshly caught fish, according to Shewan (1977), depends on the environment rather than the fish species. Thus, the considerable high microbial loads obtained from the freshly landed fish samples could possibly be due to poor handling by the fishermen at sea or the processors of different hygienic profile having regular skin contacts with the fishes. This aspect of cross contamination was effectively confirmed by the observed higher MPN values observed in the washing water during the study. Another possible explanation could be the unhygienic sanitary environment of the fish source (ocean) and the fishing vessel.

In cases of solar drying, the bacterial load counts were on average, within the normal ranges stipulated by ISO-4833 standards (Codex, 1977). However, the mean counts were found highest (8.50 x 10^5cfug^{-1}) for TPC and (9.90 x 10^5cfu g^{-1}) for SSO. Thus, the low microbial counts obtained from the dry fish samples could be attributed to the low moisture content impacted by drying. However, during storage the counted numbers slightly increased with the majority still being in the range of 10^5, probably due to the hygroscopic nature of the samples which provided the microorganisms with moisture for growth. The hypothesis was confirmed when the values reduced at the fourteenth days of storage because of moisture reduction due to packaging. Thus, a general reduction in the bacterial counts was observed with all forms of packaging for the stored solar dried fish samples. This study was used to develop the HACCP.

8.6 Lipid oxidation in solar dried fish with and without antioxidants

Synthetic phenolic compounds like BHA and BHT have been widely used as antioxidants; however, there is a concern that they may be toxic. Therefore, natural antioxidants, like turmeric and plant extracts like water hyacinth and seaweeds, provide alternatives that were assessed in this study.

The control *Siganid* fillets showed the highest levels of peroxides, TBARS and free radical formation over a storage period of 90 days. Fish fillets treated with synthetic BHA (positive control) afforded the best antioxidant activity as shown by significantly lower lipid oxidation products due to its high polyphenol content. The concentration of BHA in this study was higher than would normally be used in food products. The natural antioxidant plant materials used to treat the *Siganid* fillets were not as effective as BHA, but significantly reduced lipid oxidation compared to the control, Turmeric was more effective in reducing lipid oxidation compared with seaweed and water hyacinth; this was in line with the total polyphenol content, the highest being in turmeric and lowest in water hyacinth.

The increase in PV to day 60 and eventual drop was due to propagation of free radicals and formation of peroxides initially followed by TBARS secondary and tertiary products. It is likely that the polyphenolic antioxidants caused a delay in oxidation but with time, owing to light and heat, and depletion of antioxidant level, the process of propagation in lipid oxidation increased towards the end of the storage period. Between day 0 and 30, the total phenolic content in the muscle was lower in the seaweed and water hyacinth throughout the storage followed by turmeric and BHA (Figure 4.2) which had higher phenolic compounds. After 30 to 90 days, the fall in polyphenol content in fish muscle was accompanied by a concomitant rise in peroxides and TBARS. The control was 0.29mEq/kg on day 30. This means that the natural antioxidants from turmeric, seaweed and water hyacinth exerted some antioxidant activity in these early stages of storage.

Antioxidants can reduce lipid oxidation by competitively binding active forms of oxygen, interfering with the initiation and propagation steps, binding free radicals and break the chain by stabilizing peroxides and form antioxidant radicals that are too unreactive to form non-radical

products (Halliwell and Gutteridge, 1995). The mechanism of action of phenolic groups in the antioxidants used relies on the resonance stabilisation of the phenoxy radicals that occurs at the ortho and para position on the aromatic ring. In addition, it can be influenced by the size of the substituting group. Further, many phenolic compounds contain carbonylic and hydroxyl groups that can chelate metals and thereby inhibit lipid oxidation and rancidity (Howell and Saeed, 1999).

8.7 Rheological and thermal properties

This study showed that antioxidants particularly the natural antioxidants water hyacinth, followed by turmeric and seaweed can be used to minimize texture changes during the storage of dried fish. Fish particularly fatty fish comprises triglycerides (75%) and phospholipids (25%) Most fish oils contain saturated fatty acids and an abundance of polyunsaturated fatty acids like docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). Whilst the PUFAs are implicated in lowering atherosclerosis and lowering blood cholesterol, unsaturated fatty acids and cholesterol are readily oxidized leading to rancidity and formation of chemicals including mutagens and carcinogens such as hydroxides, endoperoxides and epoxides (Halliwell and Gutteridge, 1995) (See chapter 3).

Lipid peroxidation is initiated in the presence of oxygen and metal ions, when hydrogen is abstracted from a methylene group in the PUFA by a reactive species. The primary products of lipid oxidation are peroxides which are converted to secondary products like aldehydes (TBARS). The free radicals and aldehydes produced during lipid peroxidation can combine with each other or more likely with protein molecules to end the chain reaction. This can cause severe denaturation of proteins and undesirable changes in nutritional properties including loss of amino acids, protein cross-linking and DNA damage (Saeed, Fawthrop and Howell, 1999). Aldehydes can react with amino groups forming intramolecular bonds and cross-links with other protein molecules (Saeed et al., 1999). Saeed et al (1999) provided evidence by ESR spectroscopy that lipid oxidation damages proteins through the transfer of free radicals to amino acids and proteins to form protein free radicals. These radicals can react with each other for example tyrosine free radicals can react to form dityrosine (Saeed et al., 2006). It is this cross-linking that causes aggregation in proteins and toughening of fish muscle during processing like drying and freezing and on storage.

8.8 Carbon footprint and quality tool during drying of *Siganid* fish in the Hybrid Windmill Solar Tunnel Dryer

Dried fish is a common dish in Kenya and the sun is the main source of energy for the drying process (Oduor-Odote, 2010). In the dried fish chain however the pre-harvest energetics and carbon footprint (vessel construction, maintenance and gears), harvesting (mechanized and motorized) and the post-harvest stages especially transportation and marketing to waste management are the sources of carbon footprint. Cooling and refrigeration the main consumers of fuel and emissions is reduced to a minimum making drying as a process that has a lower carbon footprint (Gosh et al 2014). Whereas the standard LCA involves reporting on goal and scope, life cycle inventory, life cycle assessment and interpretation of results, this study focused only on Greenhouse Gas Emissions (GHG)/Global Warming Potential (GWP).

This study gave an opportunity to understand and estimate the contribution of solar dried fish to climate change by determining the carbon footprint. Data from fuel consumption and subsequent CO_2 emission could help gain insight into increasing fishing costs and prices. This may be used in future to help formulate policy on regulation of fishing efforts, fuel subsidies, and giving proposals on suitable climate change mitigation measures. The data around the functional unit of 1 kg solar dried *Siganid* encompassed all the major industrial activities required to catch, process, and deliver the solar dried product to the consumer. The estimation of carbon and carbon emission from fuel (diesel) was calculated by converting the diesel consumed using the standard conversion factor that 1 litre of fuel (diesel) produced 10.7 kWh of heat, and the C emitted from 1 KWh of heat is 0.68 kg and for CO_2 was 0.25 kg.

The carbon dioxide emissions were low in this study due to hand labour, and low energy use (direct solar energy). The main emissions were caused by fisheries activities. However, due to the low fuel use in the fisheries practice (use of dhow boats) and artisanal conditions, the total energy use is very low. Therefore, the chosen processing chains were low in carbon emissions in comparison to other production cycles. Due to the substantial influence of the primary production of the species, it is advisable to perform additional environmental impacts in different case studies. Considering that dried fish is also a preferred cuisine in many countries, paying more attention to

it and emphasising on eco-friendly processing methods like the hybrid windmill solar tunnel will help reduce carbon footprint and improve food and energy security.

8.9 Quality safety tool -HACCP for solar dried fish

In practice, HACCP systems can be adapted, as necessary. The timescale for adaptation differs from procedures such as monitoring of CCPs and equipment used for this. An example of timescales and frequencies to perform procedures is presented in Table 7.2. In general, dried fish products are considered shelf stable. Consequently, they are often stored and distributed unrefrigerated. The characteristic of a dried fish product, which makes it shelf stable, is their low water activity (aw). Below water activity of 0.85 the growth and toxin production of all pathogens like *Staphylococcus aureus* and *Clostridium botulinum* is prevented which is important for a shelf-stable dried product (FDA, 2001). Since the final product is cooked, only process guidelines are necessary.

Another issue that is of interest is the international trade of fish products. Standardization of the HACCP concept may be required in the near future in Kenya. Therefore, aspects like management responsibility and compatibility between HACCP and other Quality Systems commonly used must be considered. For harmonization of HACCP systems, a new International Standard, ISO 22000, is available which was published on 1 September 2005, as an Internal Standard for food safety management to ensure safe food supply chains worldwide (Frost, 2005).

8.10 CONCLUSIONS

8.10.1 Hybrid windmill solar tunnel dryer

- The results of this study indicate that the hybrid windmill solar tunnel dryer is capable of harnessing solar energy compared with open sun conditions, as the ambient temperatures were always lower than the plenum chamber temperatures.

- The Page model was established as the best model that describes thin layer drying of *Siganus Sutor* fish in the integrated wind-solar tunnel dryer.

- During drying, the moisture content of fish reduced with drying time, and the reduction was exponential both under salted and unsalted conditions.

- Drying temperatures of 35^{o}C achieved at night can prevent spoilage and facilitate drying.

8.10.2 Raw material quality

- The raw material quality has a direct impact on yield and production costs of *Siganus sutor*. There was an increase in filleting time and lower filleting yield during delayed processing.

- The levels of PV, TBARS, TVB-N, TMA increased during delayed processing as a sign of quality deterioration. The pH values reduced during delayed processing.

- Further studies could be done to document all production costs. There is need to further link the developed QIM scheme for *Siganus sutor* to precisely characterize quality with storage time.

- Solar tunnel drying reduced biochemical spoilage indicators to a minimum before storage starts. During actual solar drying, PV and TBARS decreased because of unstable nature of hydroperoxides and aldehydes interacting with proteins and TVB-N and TMA decreased owing to less bacterial action.

- During storage of the solar dried fish fillet for 75 days, the moisture content, water activity change and bacterial load was at a minimum in the vacuum-packed fish compared to those in normal polythene and packaging and those kept in the open. Peroxide value and TBARS lipid oxidation indicators were lowest in the vacuum packed *Siganid* fish fillet compared to those stored in the open and in normal polythene. Vacuum packaging of fish products should be recommended in the Kenyan Fisheries Act.

8.10.3 Effect of natural antioxidants on lipid oxidation

- The natural antioxidants in water hyacinth, seaweeds, turmeric suppressed lipid oxidation but not as effective as the synthetic antioxidant- BHA.

- The Total Phenolic content (TPC) in turmeric was twice the amount found in seaweed and water hyacinth at the beginning and during storage which reflected the higher antioxidant effect of turmeric. The TPC was lowest in the water hyacinth during storage and they showed the highest PV and TBARS values during storage.

- The order of strength of the antioxidants was BHA>Tumeric>Seaweeds>Water hyacinth. BHA, used in lower concentrations in most foods was at a much higher concentration in this study and because synthetic antioxidants are now considered unsafe, it is advantageous to replace them with natural antioxidant sources like water hyacinth, turmeric and seaweed plant phenolics.

8.10.4 Rheological and thermodynamic properties of dried fish

- These natural antioxidants minimized lipid oxidation in solar dried fish during storage and thereby reduced protein oxidation and cross-linking, leading to lower G' values and reduced toughening compared to dried fish without antioxidants.
- The natural antioxidants lowered heat enthalpy ΔH meaning they had an effect on protein cross-linking.

8.10.5 Carbon Footprint and quality tool – HACCP

- The carbon dioxide emissions were low in this study due to hand labour, and low energy use (direct solar energy). The main emissions were caused by fisheries activities. However, due to the low fuel use in the fisheries practice (use of dhows) the total energy use is very low.
- The drying system used in the study led to shelf stable low water activity products.

- The Hazards identified either as critical points or critical control points in the study are not effective because the final product is cooked eliminating all hazards.

8.11 FURTHER WORK

Solar/windmill drying technology

Drying in the fisheries sector has been a key preservation method for centuries and can be used to control post-harvest losses especially with further improvements. The hybrid windmill tunnel dryer was introduced to help with fish drying during the wet and damp weather conditions and at night. The dryer has shown that drying is possible in the rainy season; however the challenge now is to increase the drying capacity of the dryer to over 1 ton.

This increase in drying capacity will require higher power output which can be achieved with the aid of extra solar panels. Further, it would be advantageous to connect this hybrid windmill dryer to an ice maker so that the fish processing can benefit from readily available ice for hygienically processing fish.

The dryer should not to be limited to drying of fish only but its application should be extended to dry other farm produce. High value microalgae like Spirulina are candidates for uniform drying and with their high price in the market, Return On Investment on the dryer will be faster. It is possible to achieve all the above benefits whilst using renewable energy, and helping to protect the environment.

Quality of fish

The quality of dried fish has to be as high as that for fresh fish products to provide safe nutritious food and enable fisher-folk to earn a higher income.

Further work is required to ascertain the quality characteristics of dried fish products. As undertaken for fresh fish production, where the Quality Index Method has been introduced for ascertaining quality, a method is needed to help characterize the quality of dried fish. Both the levels of biochemical and physico-chemical indicators of spoilage in dried fish are required to be assessed. Levels of peroxides, TBARS and TVB-N are acceptable indicators to monitor because during drying a lot of changes take place, some surpassing the traditional levels.

Vacuum packaging for dried fish was found to be very useful for maintaining quality during drying and storage of fish. However, further work needs to be undertaken to assess the risks from anaerobic bacteria in vacuum packaged fish that will lead to adoption of appropriate packaging by the Government of Kenya.

Training and awareness of quality maintenance of the product is crucial, so that safety is guaranteed at all levels in the value chain. It should be emphasized that quality assessment should be based on losses that may be incurred resulting from a poor quality starting product.

Control of lipid oxidation

Postharvest losses in fish particularly deterioration of proteins and lipids are responsible for low value of fish and economic losses that need to be ameliorated.

The present studies on aquatic plants as a source of natural antioxidants were successful for limiting lipid oxidation. However, more work needs to be done to optimize the levels of natural antioxidants and to consider using synergistic combinations with cinnamon extracts to better control lipid oxidation during fish storage. Abundant raw material sources of natural antioxidants like water hyacinth and seaweeds (some being farmed) can be used to produce higher value products like antioxidants or bioactive peptides to give better economic returns and to support industrialization, health and job creation.

Carbon footprint

The environmentally cleaner technologies in the fisheries industry can help to reduce the carbon footprint, but adopting these technologies requires further work.

Using cleaner technology in food production is important as it affects climate change positively. Eco-labelling is now considered important and details on packaging showing how much carbon is emitted in the Life Cycle of the product will be introduced in a matter of time. Therefore extensive and in depth knowledge of new technologies and processes of production that are environmentally friendly is highly necessary to acquire. Further information on the carbon footprint and other greenhouse gas emitting processes cannot be over emphasized

Product safety-HACCP/Risk Analysis

Product safety through well documented procedures boosts consumer confidence and should be focused on in the dry fisheries chain. Proper labelling with scientifically based information on expiry dates of dried fish products will help move products faster in the market because consumers have become very aware of quality of products they purchase. Therefore risk analysis and HACCP to be used for each product is necessary in order to guarantee product safety.

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10.0 PUBLICATIONS AND PATENT REGISTRATION

1. Odote, P. M. O.; G. M. Kituu.; M. Obiero.; R.Ruwa and N.K.Howell **(2015).** "Performance evaluation of hybrid thin layer solar tunnel-windmill dryer in the drying of brined and non-brined Tafi (Siganus sutor) Fish. AgreInt. CGIR Vol. 17 No. 1. Pp 273-283

2. Oduor-Odote Peter; Shamasundar A.; Booman AC.; Howell NK.; Nunes M and Zagarramurdi A (2016): Effect of Raw Material Quality on Quality and Yield of Dried Fish Products. International Journal of Food Processing Technology 3: 54-61

3. "Hybrid windmill-solar-tunnel dryer" by **Oduor-Odote Peter Michael**, Douglas Shitanda & Gareth Kituu. Category: Utility Model.NUMBER 152. Ref: KE/U/2016/660

11.0 ANNEXES

Annex 1: Reference for Carbon footprint was collected from literature used in Tables 6.1 and 6.2 (from SKAO 2014, CO_2-prestation ladder, Generic Handbook, version 2.2)

Activity	Activity specification	Activity details	Value	Unit
Energy carriers	Other than transport class A	Gasoline / Petrol	2.78	kg CO_2 Eq / liter
Energy carriers	Other than transport class A	Diesel	3.135	kg CO_2 Eq / liter
Energy carriers	Other than transport class A	LPG	1.86	kg CO_2 Eq / liter
Energy carriers	Other than transport class A	Fuel oil	3.185	kg CO_2 Eq / liter
Energy carriers	Other than transport class A	Bio-ethanol	1.6	kg CO_2 Eq / liter
Energy carriers	Other than transport class B	Crude oil	3.735	kg CO_2 Eq / kg
Energy carriers	Other than transport class B	Orimulsion	2.61	kg CO_2 Eq / kg
Energy carriers	Other than transport class B	Earth gas condensate	3.4	kg CO_2 Eq / kg
Energy carriers	Other than transport class B	Petroleum	3.71	kg CO_2 Eq / kg
Energy carriers	Other than transport class B	Ethane	3.425	kg CO_2 Eq / kg
Energy carriers	Other than transport class B	Nafta's	3.85	kg CO_2 Eq / kg
Energy carriers	Other than transport class B	Bitumen	3.975	kg CO_2 Eq / kg
Energy carriers	Other than transport class B	Lubrication oils	3.62	kg CO_2 Eq / kg
Energy carriers	Other than transport class B	Petroleum cokes	4.05	kg CO_2 Eq / kg
Energy carriers	Other than transport class B	Refinary raw material	3.92	kg CO_2 Eq / kg
Energy carriers	Other than transport class B	Refinary gas	3.655	kg CO_2 Eq / kg
Energy carriers	Other than transport class B	Chemical residual gas	3.655	kg CO_2 Eq / kg
Energy carriers	Other than transport class B	Other oils	3.515	kg CO_2 Eq / kg
Energy carriers	Other than transport class B	Peet	1.19	kg CO_2 Eq / kg
Energy carriers	Other than transport class B	Charcoal brikets	2.315	kg CO_2 Eq / kg
Energy carriers	Other than transport class C	Earth gas condensate	1.825	kg CO_2 Eq / kg
Energy carriers	Other than transport class C	Biogas	0.4	kg CO_2 / Nm3 unit
Energy carriers	Other than transport class C	Biogas (co-fermentation-mais-fertilizer)	1.3	kg CO_2 / Nm3 unit
Energy carriers	Other than transport class C	Methane	2	kg CO_2 / Nm3 unit

Energy carriers	Other than transport class C	Propane	1.53	kg CO_2 / Nm3 unit
Energy carriers	Electric energy	Coal – Power central	0.82	kg CO_2Eq / kWh
Energy carriers	Electric energy	Biomass – cofiring with coal	0.74	kg CO_2Eq / kWh
Energy carriers	Electric energy	Gas – combined cycle	0.49	kg CO_2Eq / kWh
Energy carriers	Electric energy	Biomass – dedicated	0.23	kg CO_2Eq / kWh
Energy carriers	Electric energy	Solar PV – utility scale	0.048	kg CO_2Eq / kWh
Energy carriers	Electric energy	Solar PV – rooftop	0.041	kg CO_2Eq / kWh
Energy carriers	Electric energy	Geothermal	0.038	kg CO_2Eq / kWh
Energy carriers	Electric energy	Solar power concentrated	0.027	kg CO_2Eq / kWh
Energy carriers	Electric energy	Hydropower	0.024	kg CO_2Eq / kWh
Energy carriers	Electric energy	Wind offshore	0.012	kg CO_2Eq / kWh
Energy carriers	Electric energy	Nuclear	0.012	kg CO_2Eq / kWh
Energy carriers	Electric energy	Wind onshore	0.011	kg CO2Eq / kWh
Energy carriers	Electric energy	Average supply	0.455	kg CO_2Eq / kWh
Energy carriers	Heat delivery	Steam and gas central	11.3	kg CO_2/GJ
Energy carriers	Heat delivery	Coal central	18.5	kg CO_2/GJ
Energy carriers	Heat delivery	AV / others	20	kg CO_2/GJ
Energy carriers	Heat delivery	Gas engine WKK	70.3	kg CO_2/GJ
Energy carriers	Heat delivery	Geothermic	3	kg CO_2/GJ
Transport	Transport class A	Gasoline / Petrol	2.78	kg CO_2 Eq / liter
Transport	Transport class A	Diesel	3.135	kg CO_2 Eq / liter
Transport	Transport class A	LPG	1.86	kg CO_2 Eq / liter
Transport	Transport class A	Fuel oil	3.185	kg CO_2 Eq / liter
Transport	Transport class A	Bio-ethanol	1.6	kg CO_2 Eq / liter
Transport	Transport Class B	Delivery truck	0.63	kg CO_2Eq/tonkm
Transport	Transport Class B	Truck 3.5 - 10 ton	0.48	kg CO_2Eq/tonkm
Transport	Transport Class B	Truck 10 - 20 ton	0.3	kg CO_2Eq/tonkm
Transport	Transport Class B	Truck >20 ton	0.13	kg CO_2Eq/tonkm
Transport	Transport Class B	Tractor and trailer	0.095	kg CO_2Eq/tonkm
Transport	Transport Class B	Train elektric	0.02	kg CO_2Eq/tonkm
Transport	Transport Class B	Train diesel	0.025	kg CO_2Eq/tonkm
Transport	Transport Class B	Train combination	0.022	kg CO_2Eq/tonkm
Transport	Transport Class B	River ship 32 TEU	0.065	kg CO_2Eq/tonkm

Transport	Transport Class B	River ship 96 TEU	0.075	kg CO_2Eq/tonkm
Transport	Transport Class B	River ship 200 TEU	0.06	kg CO_2Eq/tonkm
Transport	Transport Class B	River ship 470 TEU	0.05	kg CO_2Eq/tonkm
Transport	Transport Class B	Seaship 150 TEU	0.085	kg CO_2Eq/tonkm
Transport	Transport Class B	Seaship 580 TEU	0.045	kg CO_2Eq/tonkm
Transport	Transport Class B	Seaship 4000 TEU	0.023	kg CO_2Eq/tonkm
Coulant		R22	1.81	kg CO_2Eq / kg
Coulant		R404a	3.92	kg CO_2Eq / kg
Coulant		R507	3.985	kg CO_2Eq / kg
Coulant		R407c	1.775	kg CO_2Eq / kg
Coulant		R410a	2.09	kg CO_2Eq / kg
Coulant		R134a	1.43	kg CO_2Eq / kg

Annex 2: Environmental CO_2 footprint records for solar drying of Siganids in Kipini

Type of data	Parameter	Choice option or pre-set value	Value	Data type and unit
CO_2Eq energy used for fishing (IF KNOWN)	If KNOWN from literature otherwise input Diesel use	0 IF not known	0	CO_2eq/kg or another unit
	Is literature available?		~~N~~	Y/N
Total energy used for fishing (IF KNOWN)	If KNOWN otherwise fossil energy use	0 IF not known	0	MJ/kg or another unit
	Is literature available?		N	Y/N
	Type of fossil energy used	Diesel	3.135	CO_2 Eq/l combusted
	Type of fossil energy used	Petrol	2.78	
Fuel use for the fishing trip	Amount of fuel used for one fishing trip		12	liters
Type of fisheries	Short description type of fisheries	The snapper fishery from the north bank. The handlines used for fish. This fishery replaced the *Siganid* fishery during the fishing season		Landings in Kg
Vessel type	Indication of vessel type (to be used in general database)	Motorized Dhows are used for this fishery		Number of motorized vessels
Type of fuel used	crude oil, diesel, gasoline/petrol	Petrol is used		
Total volume of fish landed	What is the total volume of landed mixed fish	Snapper/Siganid	175	kg or tonnes per landing
Potential reference document	What reference document can be provided for verification?	BMU records	None	BMU records
	Fuel use for fishing to landing (total catch)		0.07	litre /kg landed mixed fish

Duration of average fishing trip	Total duration of the fishing trip		7hrs	number of hrs or days
Stripping on board	Is the product stripped on board?	The products lands gutted	Y	Yes
	Is stripping waste discarded on board? (Y/N)		Y	Discarded
	Is the product cooled on board?		Y	
	How is the product cooled?		Icing	
Registration capacity	How are catches registered (or what is possible)	Registered by BMU	per catch/per vessel	Per catch or per vessel
	Is the product filleted on board?		N	N
	Are by products (frames, head, tails) discarded?		N	N/A
	What is the mass of discarded by-products (exl. Stripping)		N/A	kg or tonnes per landing
	What is the Percentage of discarded by-products (exl. Stripping)		N/A	% Discarded product (frames)
Landed target Product species	Name product and mass	Siganid	100	Mass or % (per catch or total fleet)
Landed By-products whole fish	Name product and mass		0	Mass (per catch or total fleet)
Landed By-product other	Name product and mass		0	Mass (per catch or total fleet)
Landed Waste (stripping)	Name product and mass		0	Mass (per catch or total fleet)
Total landed catch	Total landed catch (Sum of the above)			
Landed product	How is economic value available?			Indication or documented
Landed target Product species	Name product (0 if not used)		100	Economic value (Currency/kg or total fleet)
Landed By-products whole fish	Name product (0 if not used)		0	Economic value (Currency/kg or total fleet)
Landed By-product other	Name product (0 if not used)		0	Economic value (Currency/kg or total fleet)
Landed Waste (stripping)	Name product (0 if not used)		0	Economic value (Currency/kg or total fleet)

Annex 3: Environmental CO_2 footprint records for Landing Solar Dried Siganids in Kipini

Type of data	Parameter	Choice option or pre-set value	Value	Data type and unit
Landing procedure	What is the landing procedure	Manual transport		Short
	Landing procedure (landing, sorting, storing) prior to transport	Landing no sorting		Description
Fuel use landing to processing, excl. transport	Calculated based on total fuel / processed product	If no data 0.	0	l/kg fish
Volume Landed mass	Volume Landed mass		700	Kg or tonnes
Used fuel for landing mass	Used fuel for landing mass	If 0 type 0.0001	0.001	liters
Fuel use landing	Used fuel for landing mass		1.4E-06	l /kg fish
	Type of fuel used	Diesel	3.135	CO_2 Eq /l
Electricity use landing	Total energy use for landing		0.0000	kWh
	Total energy use for landing		0.0E+00	kWh / kg fish
	Type of energy used for landing	Average supply	0.455	CO_2 Eq / kWh
Loss at landing stage	What is the amount of spoilage/losses at landing		0	%
	What type of losses are there		None	Short description
Loss at landing stage	Is the waste product used in another production chain?		No	Y/N
	Are losses processed, sold or used?		No	Processed, sold or used
Loss at landing stage	Economic value target products whole fish entering the facility	Snappers/Siganid gutted	100	Economic value (/kg)
Loss at landing stage	Economic value losses product if sold		0	Economic value (/kg)

Annex 4: Environmental footprint records for CO_2 for transport fish to the solar Dryer in Kipini

Type of data	Parameter	Choice option or pre-set value	Value	Data type and unit
Transport landing to processing	Transport distance landing to processing	Processed in Kipini	0	km
	Is this an average distance or specific example?		average	average, most or specific example
	What is the average distance?		0	km
	Fuel used for transport	Truck 3.5 - 10 ton	0.00048	kg CO_2 Eq / kmkg
Transport method	What is the transport method(Lorry/car/foot/motor)		None	Specify
Chilled	Is the product chilled during this stage?		Ice	Specify

Frozen	Is the product frozen during this stage		0	
Average time prior to processing	How long is the product stored prior to transport		1	Days in cooler
Type of cooling system	What type of cooling system is used		Ice box	Specify
Any other transport issues				Describe if expected relevant
	How much more energy is consumed for freezing, cooling		0	% extra fuel consumption

Annex 5: Environmental footprint records for CO_2 during solar drying of Siganid fish in Kipini

Type of data	Parameter	Choice option or pre-set value	Value	Data type and unit
CO2Eq energy use IF KNOWN	Is the CO_2 output of processing known? Eg. earlier studies		No	Yes or No
	What is CO_2 output of processing?		0	CO_2 Eq/kg raw material
				Other unit
Total energy use IF KNOWN	Is the Energy use of processing known? Eg. earlier studies		No	Yes or No
	What is the CO_2 output of processing?		0	kWh/kg raw material
Measurable size	What is the minimum size or batch on which electricity data is available		day	Batch, day production, month production, year production
	Is this measured in finished product or raw material (filet, stripped or whole)?		raw material	Raw material
	What is the production volume in this period?		100	kg
Electricity use	How much electricity is used for this batch, year, or other unit of production		6.48	kWh per unit of production (kg)
Type of electricity is used	What type of fossil energy source is used?	Solar PV – rooftop	0.041	Green, solar, net,
Energy use (if not electrical)	How much energy is used for this batch, year, or other unit of production		0.0648	kWh / kg production
	How much own energy is generated		50	% Solar energy on total energy consumption
	How much own energy is generated		50	% Wind energy on total energy consumption
Type of heating electricity	What type of electricity is used?	Average supply	0.455	Eg. Diesel generator
Electricity use per unit	Use of electricity from solar energy		0.0324	kWh / kg production
Electricity use per unit	Use of electricity from wind energy		0.0324	kWh / kg production
Electricity use per unit	Use of electricity from fossil sources		0	kWh / kg production
Fuel use	How much fuel is used for this batch, year, or other unit of production		0.0	Liters

	How much fuel is used for this batch, year, or other unit of production		0.0	Liters/kg raw material
Type of fuel used	What type of fuel is used?			Diesel, gasoline/petrol
Use of coolant	Amount of coolant total		0.0	Total amount (liters)
	Volume of produced fish using this amount		100	Total mass (kg)
	How much coolant is used (per tonne of production).		0.0	l/kg raw material
Type of coolant	What type of coolant is used?		R22	
Loss at processing	Percentage of loss during processing		0	% of loss due to spoilage
	Type of spoilage?		Deterioration	What type of spoilage is it (spillage, deterioration)
Loss at processing	What is done with the spoilage?		Waste	Waste/processing
Loss at processing	Is the waste product used in another production chain?		No	Y / N
Processed Product	Name product (0 if not used)	Name product	100	Economic value (/kg or total fleet)
Processed By-products	Name product (0 if not used)	Name product	10	Economic value (/kg or total fleet)
Processed By-product other	Name product (0 if not used)	Name product	10	Economic value (/kg or total fleet)
Processed Waste (stripping)	Name product (0 if not used)	Name product	0	Economic value (/kg or total fleet)

Annex 6: Environmental footprint records for CO2 for fossil fuel during solar drying of Siganid fish in Kipini

Type of data	Parameter	Choice option or pre-set value	Value	Data type and unit
CO2Eq energy use IF KNOWN	Is the CO_2 output of processing known? Eg. earlier studies		No	Yes or No
	What is CO_2 output of processing?		0	CO_2 Eq/kg raw material
				Other unit
Total energy use IF KNOWN	Is the Energy use of processing known? Eg. earlier studies		No	Yes or No
	What is the CO_2 output of processing?		0	kWh/kg raw material

Measurable size	What is the minimum size or batch on which electricity data is available		day	Batch, day production, month production, year production
	Is this measured in finished product or raw material (filet, stripped or whole)?		raw material	Raw material
	What is the production volume in this period?		100	kg
Electricity use	How much electricity is used for this batch, year, or other unit of production		6.48	kWh per unit of production (kg)
Type of electricity is used	What type of fossil energy source is used?	Solar PV – rooftop	0.041	Green, solar, net,
Energy use (if not electrical)	How much energy is used for this batch, year, or other unit of production		0.0648	kWh / kg production
	How much own energy is generated		0	% Solar energy on total energy consumption
	How much own energy is generated		0	% Wind energy on total energy consumption
Type of heating electricity	What type of electricity is used?	Average supply	0.455	Eg. Diesel generator
Electricity use per unit	Use of electricity from solar energy		0	kWh / kg production
Electricity use per unit	Use of electricity from wind energy		0	kWh / kg production
Electricity use per unit	Use of electricity from fossil sources		0.0648	kWh / kg production
Fuel use	How much fuel is used for this batch, year, or other unit of production		0.0	Liters
	How much fuel is used for this batch, year, or other unit of production		0.0	Liters/kg raw material
Type of fuel used	What type of fuel is used?			Diesel, gasoline/petrol
Use of coolant	Amount of coolant total		0.0	Total amount (liters)
	Volume of produced fish using this amount		100	Total mass (kg)
	How much coolant is used (per tonne of production).		0.0	l/kg raw material
Type of coolant	What type of coolant is used?		R22	
Loss at processing	Percentage of loss during processing		0	% of loss due to spoilage

	Type of spoilage?		Deterioration	What type of spoilage is it (spillage, deterioration)
Loss at processing	What is done with the spoilage?		Waste	Waste/processing
Loss at processing	Is the waste product used in another production chain?		No	Y / N
Processed Product	Name product (0 if not used)	Name product	100	Economic value (/kg or total fleet)
Processed By-products	Name product (0 if not used)	Name product	10	Economic value (/kg or total fleet)
Processed By-product other	Name product (0 if not used)	Name product	10	Economic value (/kg or total fleet)
Processed Waste (stripping)	Name product (0 if not used)	Name product	0	Economic value (/kg or total fleet)

Annex 7: Environmental footprint records for CO2 during filleting of solar dried Siganids in Kipini

Type of data	Parameter	Choice option or pre-set value	Value	Data type and unit
Fillet factor	% fillet / fish		60	%
By Product[1]	What by-product is resulting	Frames		Product name
By Product[1]	Volume product (as a percentage of raw material)		30	Mass % of raw material
By poduct[2]	What by-product is resulting	Skin and bones		Product name
By poduct[2]	Volume product (as a percentage of raw material)		10	Mass % of raw material
Product characteristics	What % of moisture is in the product before critical processing step		80	% Water in product
Dried product characteristics	What % of moisture is in the product after processing step (e.g. Drying)		10	% Waste in frozen or dried product

Annex 8: Environmental footprint records CO_2 calculations for solar assisted drying in Kipini

		Value choices	**Value**	**unit**	**CO_2 Eq /unit**	**unit**	**CO_2 Eq / kg**	
CO2Eq energy use Fishing IF KNOWN	If KNOWN from literature otherwise input Diesel use	Choose in database	0	CO_2eq/kg	0	CO_2 Eq/kg fish	0	

	If NOT KNOWN use unit->							
Fuel use fishing	Fuel use for fishing to landing	0.38	0.069	litre /kg landed mixed fish				
Fue type used	Type of fuel used		Diesel		3.135	CO_2 Eq/l combusted	0.21	
Stripping on board		Y/N	Y	Yes or No				
					Ratio			
Landed target Product species	Name product		100	Mass or % (per catch or total fleet)	100.00			
Landed By-products whole fish	Name product		0	Mass (per catch or total fleet)	0.00			
Landed By-product other	Name product		0	Mass (per catch or total fleet)	0.00			
Landed Waste (stripping)	Name product		0	Mass (per catch or total fleet)	0.00			
				Ratio Target product	1.00			
							Ratio	
Landed target Product species	Name product		100	Economic value (/kg or total fleet)	10000	Calculated Contribution ratio	1.00	
Landed By-products whole fish	Name product		0	Economic value (/kg or total fleet)	0	Calculated Contribution ratio	0.00	
Landed By-product other	Name product		0	Economic value (/kg or total fleet)	0	Calculated Contribution ratio	0.00	
Landed Waste (stripping)	Name product		0	Economic value (/kg or total fleet)	0	Calculated Contribution ratio	0.00	
				Total economic +mass	10000			
Total CO2 footprint RM fisheries							**0.21**	**CO_2Eq per kg target species**

Landing procedure	Landing procedure (only landed and transported ?)		0					
Diesel use landing to processing, excl. transport	Calculated based on total fuel / landed product		1.42857E-06	l/kg fish	3.135	kg CO_2 Eq / liter	4.47857E-06	
Electricity use landing	Calculated based on total energy / landed product		0	kWh/kg fish	0.455	kg CO_2Eq / kWh	0	
Loss at landingstage	Spoilage		0	%				
Loss at landingstage	What is done with the spoilage?		None	If waste no calculation in J35				
Loss at landingstage	Is the waste product used in another production chain?		No					
Total CO2 footprint landing without correction							0.000	
Loss at landingstage	Economic value of target species at this stage		100	Economic value (/kg)	100			
Loss at landingstage	Economic value of by product at this stage		0	Economic value (/kg)	0			
Processing step	What process step is done?		No	Total economic + mass	100			
Total CO2 footprint at landing							**0.00**	**CO_2 Eq / kg target species**

Transport landing to processing			0	km				
Transport method	Fuel use for transport		Truck 3.5 - 10 ton		0.0005	kg CO_2Eq/kmkg	0.0000	
Energy use transport	Is cooling, freezing applied during transport?		Ice	Y/N				
Energy use transport	Additional fuel consumption does to cooling, freezing		0	% extra fuel consumption			0	
Total transport to processing							**0.0000**	
CO2Eq energy use IF KNOWN	If KNOWN otherwise unit		0	CO_2 Eq/kg	0	CO_2 Eq/kg	0	CO_2/kg processed product
Total energy use IF KNOWN	If KNOWN otherwise unit		0	MJ/kg			0.00	
Electricity use per unit	Use of electricity from solar energy		0.0324	kWh/ kg production			0.00	CO_2 / kg production
Electricity use per unit	Use of electricity from wind energy		0.0324	kWh/ kg production			0.000	CO_2 / kg production
Electricity use per unit	Use of electricity from fossil sources		0	kWh/ kg production			0.000	CO_2 / kg production
Fuel use per unit	Use of fuel per unit		0.0000001	l/kg raw material			0.00	CO_2 / kg production
Coolant use per unit	Use of coolant per unit		0.000001	l/kg raw material			0.00	CO_2 / kg production

Processed target product	Name product		100	Economic value (/kg)	60	Calculated Contribution ratio	0.94	
Processed By-product1	Name product		10	Economic value (/kg)	3	Calculated Contribution ratio	0.05	
Processed By-product 2	Name product		10	Economic value (/kg)	1	Calculated Contribution ratio	0.02	
				Total economic +mass	64			
Total processing							**0.00**	kg CO_2Eq / kg
							0.22	

Annex 9: Environmental footprint records CO_2 calculations fuel based drying Kipini

			Value	**unit**	**CO_2 Eq /unit**	**unit**	**CO_2 Eq / kg**
Raw material	CO2Eq energy use Fishing IF KNOWN	If KNOWN from literature otherwise input Diesel use	0	CO_{2e}q/kg	0	CO_2 Eq/kg fish	0
		If NOT KNOWN use unit->					
	Fuel use fishing	Fuel use for fishing to landing	0.069	litre /kg landed mixed fish			
	Fue ltype used	Type of fuel used	Diesel		3.135	CO_2 Eq/l combusted	0.21
	Stripping on board		Y	Yes or No			
					Ratio		
	Landed target Product species	Name product	100	Mass or % (per catch or total fleet)	100.00		
	Landed By-products whole fish	Name product	0	Mass (per catch or total fleet)	0.00		
	Landed By-product other	Name product	0	Mass (per catch or total fleet)	0.00		

	Landed Waste (stripping)	Name product	0	Mass (per catch or total fleet)	0.00		
				Ratio Target product	1.00		
							Ratio
	Landed target Product species	Name product	100	Economic value (/kg or total fleet)	10000	Calculated Contribution ratio	1.00
	Landed By-products whole fish	Name product	0	Economic value (/kg or total fleet)	0	Calculated Contribution ratio	0.00
	Landed By-product other	Name product	0	Economic value (/kg or total fleet)	0	Calculated Contribution ratio	0.00
	Landed Waste (stripping)	Name product	0	Economic value (/kg or total fleet)	0	Calculated Contribution ratio	0.00
				Total economic +mass	10000		
Raw material	**Total CO_2 footprint RM fisheries**						**0.21**
Landing	Landing procedure	Landing procedure (only landed and transported?)	0				
	Diesel use landing to processing, excl. transport	Calculated based on total fuel / landed product	1.42857E-06	l/kg fish	3.135	kg CO_2 Eq / liter	4.47857E-06
	Electricity use landing	Calculated based on total energy / landed product	0	kWh/kg fish	0.455	kg CO_2Eq / kWh	0
	Loss at landingstage	Spoilage	0	%			
	Loss at landingstage	What is done with the spoilage?	None	If waste no calculation in J35			
	Loss at landingstage	Is the waste product used in another production chain?	No				

	Total CO_2 footprint landing without correction						0.000
	Loss at landingstage	Economic value of target species at this stage	100	Economic value (/kg)	100		
	Loss at landingstage	Economic value of by product at this stage	0	Economic value (/kg)	0		
	Processing step	What process step is done?	No	Total economic + mass	100		
Landing total	**Total CO_2 footprint at landing**						**0.00**
Transport	Transport landing to processing		0	km			
	Transport method	Fuel use for transport	Truck 3.5 - 10 ton		0.0005	kg CO_2Eq/kmkg	0.0000
	Energy use transport	Is cooling, freezing applied during transport?	Ice	Y/N			
	Energy use transport	Additional fuel consumption does to cooling, freezing	0	% extra fuel consumption			0
Total Transport Landing to processing	**Total transport to processing**						**0.0000**
Processing	CO_2Eq energy use IF KNOWN	If KNOWN otherwise unit	0	CO_2 Eq/kg	0	CO_2 Eq/kg	0
	Total energy use IF KNOWN	If KNOWN otherwise unit	0	MJ/kg			0.00
	Electricity use per unit	Use of electricity from solar energy	0	kWh/ kg production			0.00

	Electricity use per unit	Use of electricity from wind energy	0	kWh/ kg production			0.000
	Electricity use per unit	Use of electricity from fossil sources	0.0648	kWh/ kg production			0.02764
	Fuel use per unit	Use of fuel per unit	0.0000001	l/kg raw material			0.00
	Coolant use per unit	Use of coolant per unit	0.000001	l/kg raw material			0.00
	Processed target product	Name product	100	Economic value (/kg)	60	Calculated Contribution ratio	0.94
	Processed By-product1	Name product	10	Economic value (/kg)	3	Calculated Contribution ratio	0.05
	Processed By-product 2	Name product	10	Economic value (/kg)	1	Calculated Contribution ratio	0.02
				Total economic +mass	64		
Total Processing	**Total processing**						**0.03**
TOTAL product base							**0.24**