EFFECTS OF SMOKE DRYING, BOILING AND DEEP FRYING ON THE MICROBIAL LOAD IN FRESHWATER APPLE SNAIL (*Pila ovata)*

BY

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NOVEMBER, 2025

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SUBMITTED TO THE DEPARTMENT OF AQUACULTURE AND FISHERIES MANAGEMENT, FACULTY OF AGRICULTURE, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE. IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF AGRICULTURE DEGREE B. AGRIC (AQUACULTURE AND FISHERIES MANAGEMENT)

This is to certify that this project was carried out by ALENBALULU, Marvellous, in the Department of Aquaculture and Fisheries Management, Faculty of Agriculture, University of Benin, Benin-city, Edo state

DEDICATION

I dedicate this project to God almighty, the one who found me worthy to be called his own, to him be all glory and honour.To my wonderful parents, Mr and Mrs S.E. Alenbalulu and my dearest Auntie and Uncle Miss Queen Erahbor and Mr. Efosa Oduwa will never be enough to express my gratitude

ACKNOWLEDGMENTS

My utmost appreciation goes to my heavenly Father for keeping and preserving my life throughout this project.

To my admirable and intelligent supervisor, Prof (Mrs). F.A.R. Ehigiator, for constantly pushing and motivating me to reach for the very best during the course of this project. I truly and deeply appreciate your efforts towards making this project a success. My gratitude also goes to the Head of Department, Dr. M. Egwenomhe, for his leadership and relentless efforts towards the betterment of students and the department at large. I also extend my sincere thanks to my lecturers: Prof. V.A. Okonji, Prof. O.J. Abolagba, Dr. (Mrs.) A.E. Odiko, Dr. O.M. Wangboje, Dr. K. Omoruyi, Dr. A. Orowe, Dr. J.N. Nuntah and Dr. S. Osayi.

To my wonderful parents, Mr. S. Alenbalulu and Mrs. E.E. Alenbalulu, thank you for your sacrifices of love, guidance and support throughout my stay in this school, words cannot quantify my gratitude. May God bless and reward your labour of love over me. To my dearest Aunty and Uncle Miss Queen Erhabor and Mr. Efosa Oduwa thank you for being the most wonderful resource person an undergraduate could ask for.

To my friends turned family, Joshua, Godfrey, James, Alex, Jessica, Gift, Queen, Christabel, Itohan, Winner, David, Osazuwa and Osagie thanks for being the best. To my amazing course mates, thanks for the jolly good ride.

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ABSTRACT

The study evaluated the effects of smoke-drying, boiling, and deep-frying on the microbial load and shelf life of *Pila ovata*. *P. ovata* samples were purchased from a local market in Benin city, Edo state. It was washed and processed using the three methods before being stored in clean, airtight containers at ambient temperature (34°C-37°C). Microbial analyses, including total bacterial and fungi counts, were conducted immediately after processing and during storage. Results showed that smoke-dried samples had the lowest bacterial and fungal counts throughout the storage period due to the combined effects of dehydration and antimicrobial compounds in smoke. Fried samples exhibited high initial microbial reduction but showed increased counts during storage, while boiled samples had moderate counts that gradually increased, indicating susceptibility to recontamination. The study concluded that smoke drying is the most effective method for enhancing microbial safety and prolonging the shelf life of *P. ovata*. Proper processing, hygienic handling, and storage in moisture-proof containers are recommended to maintain product quality and consumer safety.

CHAPTER ONE

INTRODUCTION

Freshwater molluscs play vital ecological and socioeconomic roles across aquatic ecosystems. Among these, *Pila ovata* (*Ampullariidae*) stands out as a widely distributed gastropod in West African freshwater systems, particularly in the Niger Delta region, where it serves as an essential protein source for local communities (Adebayo-Tayo *et al.,* 2011; Ehigiator *et al.,* 2015). This species thrives in diverse freshwater habitats, including streams, lakes, and rivers across Southern rainforests, where it is commonly found attached to submerged surfaces like rocks and aquatic vegetation (Brown, 1994). With a shell width of 55–59mm and height of 43–47mm, *P. ovata* is characterized by a globose morphology, a rounded aperture, and a small but deep umbilicus, adaptations that enhance its survival in dynamic freshwater environments (Ehigiator *et al.,* 2015).

It feeds on algae and decaying vegetation and is often associated with macrophytes and is believed by aquarist to act as natural cleaner in aquaria by grazing on algae and detritus (Deeka *et al.,* 2019; Osuala, 2009). However, microbiological assessments reveal that *P. ovata* from polluted habitats like the Nembe Creek in the Niger Delta harbors high microbial loads, including *Proteus, Escherichia coli, Klebsiella* and *Aspergillus spp.,* exceeding Food and Agriculture Organization (FAO)/ World Health Organization (WHO) safety standards for human consumption (Ehigiator *et al.,* 2015). This contamination is linked to anthropogenic activities such as untreated sewage disposal, raising critical public health concerns for communities reliant on this protein source.

Nutritionally, *P. ovata* is valued for its high protein content, comparable to other freshwater molluscs like *Pomacea canaliculata*, which provide 12–14% protein by wet weight (Lv *et al.,*

2013). The FAO (2020) highlights freshwater molluscs as critical dietary components in protein-deficient regions, though species-specific data for *P. ovata* remain understudied. Economically, artisanal harvesting of *P. ovata* supports livelihoods in the Niger Delta, where it is traded locally for food and aquarium maintenance (Adebayo-Tayo *et al.,* 2011; Ofoezie, 2019). However, this specie is subjected to microbes.

Microbial load refers to the quantity of microorganisms present in a given sample, which can include bacteria, viruses, fungi, and parasites (Baker *et al.,* 2021). The effects of elevated microbial loads are multifaceted, potentially leading to spoilage, reduced shelf life, and health hazards for consumers (Almeida *et al.,* 2021). One processing method that has been explored to mitigate microbial load is smoke drying, which involves exposing the fish to smoke from burning wood or other organic materials (Santos *et al.,* 2021). This aids in fish preservation by combining dehydration with antimicrobial effects from smoke constituents, thereby extending shelf life while retaining acceptable quality (Olokor and Ibiama, 2010). Deep frying is also another method used which involves immersing food in hot oil, usually at temperatures between 160 °C and 190 °C. The intense heat exposure rapidly denatures microbial proteins, disrupts cell membranes, and inactivates essential enzymes, leading to a significant reduction in viable microbial populations (Crosa *et al.,* 2021). Compared with lower-temperature cooking methods, deep frying has been shown to more effectively reduce total viable counts in seafood products due to the combined effect of high heat and oil penetration (Zhang *et al.,* 2022). Boiling is another widely applied method in aquatic food processing, typically conducted at ~100 °C for a defined duration. Immersion in boiling water leads to effective thermal inactivation of microorganisms, including common foodborne pathogens such as *Salmonella and Listeria* (Chen *et al.,* 2013). Studies demonstrate that boiling can substantially reduce or eliminate bacterial

loads, improving product safety and contributing to extended storage life (Niamnuy *et al.,* 2008; Martínez-Álvarez *et al.,* 2009).

1.1 JUSTIFICATION OF STUDY

The assessment of how smoke drying, boiling, and deep frying affect the microbial load in *P. ovata* is necessary for improving food safety and processing practices in regions where this species is commonly consumed. *P. ovata* is an important source of animal protein in many African communities, yet it is highly susceptible to microbial contamination that can pose health risks if not properly handled (FAO, 2020). Smoke-drying, a traditional preservation technique, helps to lower moisture content, reduce microbial activity, and extend shelf life, particularly in rural and low-resource settings (Clucas and Ward, 1996). Despite these advantages, microbial regrowth has been reported during storage, compromising product safety (Omoruyi and Ebhodaghe, 2017). Boiling and deep frying are also effective heat-based methods that reduce microbial load, but they do not extend shelf life and often leave the product highly perishable (Obanu, 1988; Akpomie *et al*., 2019).

Food borne diseases continue to be a major global health concern, with millions of cases linked to contaminated food each year (WHO, 2015). The need for safer processing methods that can reduce microbial risks in widely consumed aquatic foods such as *P. ovata* is therefore critical. Existing study has identified microbial contamination in fresh *P. ovata* (Ehigiator *et al*., 2015) and the potential of smoke drying to initially reduce microbial load (Omoruyi and Ebhodaghe, 2017), but comparative information on how smoke drying, boiling and deep frying differ in effectiveness and storage stability remains limited. This study seeks to bridge that gap by evaluating and comparing these three processing methods. The findings will provide evidence-based guidance for processors and consumers on effective ways to ensure microbial safety and

shelf-life stability, while also supporting food security and public health in communities where *P. ovata* is a common dietary resource.

1.2 Aim and Objectives

The study was aimed at evaluating the effects of smoke drying, boiling and deep frying on the Microbial load in freshwater apple snail (*P.ovata*).

The specific objectives of the study were to;

1. assess the effects of smoke-drying, boiling and deep-frying on the Total Heterotrophic Bacterial Count (THBC) and Total Fungi Count (TFC) of *P. ovata* after processing
2. assess the effects of different storage periods (0, 2, and 4 weeks) on the THBC and TFC of processed *P. ovata.*
3. evaluate the interaction between processing methods and storage periods on THBC and TFC of *P. ovata.*

CHAPTER TWO

2.0 LITERATURE REVIEW

History of the Freshwater snail (*pila ovata*)

Pila ovata was first described in 1804 by the French naturalist Guillaume-Antoine Olivier under the name *Ampullaria ovata* in his publication Voyage dans l'Empire Ottoman l'Égypte et la Perse (Olivier 1804). Throughout the 19th century, the species appeared under various synonyms, including *Ampullaria bridouxi*, *Ampullaria erythrostoma* var. *stuhlmanni*, and *Ampullaria gradata*, as different researchers collected and identified specimens across Africa (Berthold 1991). At one point, the species was placed under the genus Lanistes as *Lanistes ovatus,* reflecting the evolving understanding of its taxonomy during that period (Berthold 1991). In 1882, Smith described a subspecies, *P. ovata* gordoni, adding to the taxonomic record of the species (Smith 1882). Over time, comprehensive malacological studies, including the work of Cowie (2015), consolidated these synonyms and clarified the placement of the species within the genus *Pila* under the family Ampullariidae. Today, *P. ovata* (Olivier 1804) is recognized as a valid species with two subspecies, with type specimens preserved in museum collections that continue to support research on the taxonomy and ecological relevance of this freshwater gastropod (Cowie 2015).

2.1 Classification of Freshwater snail (Taxonomy) According to Olivier:

Phylum *Mollusca*
Class *Gastropoda*
Subclass *Caenogastropoda*
Order *Architaenioglossa*
Superfamily *Ampullarioidea*
Family *Ampullariidae*
Genus *Pila*
Species *ovata*

2.2 Description and Distribution of Freshwater snail

P. ovata is a freshwater snail with a globose, thick shell typically greenish to brown in color, often marked with darker bands (Berthold 1991). The shell has a large, oval aperture and rounded whorls with a relatively short and obtuse spire (Brown 1994). The species possesses an operculum that prevents desiccation during dry periods, enabling it to survive in fluctuating aquatic environments (Cowie 2015). The soft body includes a large muscular foot for crawling on submerged surfaces and has both gills and a lung, allowing it to respire in water and air (Kristensen 1987). The shell of *P. ovata* measures approximately 55–59mm in width and 45–47mm in height, and it is relatively high and spherical with a small but deep umbilicus and a thickened shell lip, while the round aperture contributes to its distinct shape (Ehigiator *et al.,* 2015).

P. ovata is widely distributed across streams, lakes, and rivers in the southern rainforest regions of Nigeria, where it inhabits the bottom of ponds, lakes, and streams, and is often found attached to pebbles and submerged surfaces (Ehigiator *et al.,* 2015). It is also present across West and Central Africa, including Ghana, Cameroon, and the Democratic Republic of Congo, inhabiting rivers, lakes, swamps, and floodplains with abundant vegetation (Brown 1994, Cowie 2015). The species can burrow into mud to aestivate during dry periods, emerging when water levels rise (Kristensen 1987). In Nigeria, it is harvested for food due to its nutritional and economic importance (Ofoezie 1999). It also serves as a cleaning agent in aquaria, feeding on algae and decomposing vegetation, making it valuable for maintaining cleanliness in fish tanks (*Ehigiator et al.,* 2015). Its distribution is influenced by water quality, vegetation cover, and substrate type,

with a preference for habitats rich in decaying plant material, which provides both food and shelter (Berthold 1991).

2.3 Nutritional Value of Mollusc

Molluscs, comprising bivalves and gastropods such as oysters, clams, abalones, and snails, are widely recognized as nutrient-dense aquatic foods that contribute significantly to human nutrition. They are rich in high-quality protein, essential amino acids, polyunsaturated fatty acids (PUFAs), and vital minerals required for metabolic and physiological functions (Pogoda *et al*., 2013; FAO, 2021). Generally, molluscs contain 60–80% moisture, 10–20% protein, 1–3% lipids, and 1.5–3% ash on a fresh-weight basis, although variations may occur depending on species, habitat, and season (Orban *et al*., 2002; Lutaaya *et al*., 2020). Their proteins are rich in lysine, leucine, and methionine, making them valuable dietary sources, while the low lipid content is balanced by a high proportion of beneficial PUFAs such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which promote cardiovascular and neurological health (Gao *et al*., 2011). Molluscs are also notable for their mineral richness, including calcium, iron, zinc, magnesium, and selenium, as well as vitamins such as vitamin B_{12}, niacin, and trace amounts of fat-soluble vitamins (FAO, 2021; Beninger and Stephan, 2016).

Although limited information is available on the nutritional composition of *P. ovata* related species within the Pila genus have been reported to exhibit significant nutritional potential. For instance, *Pila ampullacea* from River Benue, Nigeria, was found to contain 76.32% moisture, 10.67% protein, 0.06% lipid, 5.54% ash, and 7.40% nitrogen-free extract, with mineral contents of calcium (129.18 mg/100 g), potassium (71.13 mg/100 g), phosphorus (60.52 mg/100 g), magnesium (31.19 mg/100 g), iron (10.19 mg/100 g), and zinc (1.31 mg/100 g) (Obande *et al*.,

2013). These values demonstrate that Pila species are rich in high-quality protein and essential minerals but low in fat, aligning with earlier findings on freshwater molluscs, which are regarded as nutritious, low-cholesterol food sources (FAO, 2001; Eneji *et al*., 2008). Considering the close taxonomic relationship between *P. ovata* and *P. ampullacea*, it is plausible that *P. ovata* exhibits a comparable nutrient profile, making it a valuable, affordable, and health-promoting food source capable of enhancing dietary protein intake and supporting food security in aquatic-dependent communities.

2.4 Post-Harvest Mollusc Technology

Post-harvest mollusc technology encompasses the steps taken immediately after harvesting to maintain quality, reduce spoilage, and ensure safety before processing or consumption. It involves practices such as proper handling during harvesting, cleaning, depuration, grading, and storage. Careful handling during and after harvest is critical to prevent shell damage and stress that can accelerate spoilage and mortality (FAO, 2004). Molluscs should be collected using appropriate gear that minimizes physical damage, and harvested individuals should be protected from direct sunlight and high temperatures to maintain viability (Lucas and Southgate, 2012).

For live molluscs, transportation in clean, moist conditions with adequate aeration helps maintain survival rates during transit (Goulletquer, 1997). Temperature control during transport is essential, with most bivalves requiring temperatures between 4–10°C to reduce metabolic rates and maintain quality (FAO, 2021). Post-harvest cleaning involves washing molluscs to remove mud, sand, and epibionts that may degrade quality and reduce consumer acceptability (Lucas and Southgate, 2012). Grading by size and weight ensures uniformity in batches, which facilitates further handling and market pricing (Morse, 1990).

Depuration, or the purging of sand and potential microbial contaminants, is a critical post-harvest step for molluscs such as clams and oysters that are often consumed raw or lightly boiled (Baker and Mann, 1992). It involves placing molluscs in clean, filtered, and often UV-treated seawater under controlled conditions to allow the expulsion of sand and reduction of bacterial loads over 24–72 hours (FAO, 2021; Lee *et al.,* 2008).

Storage methods for post-harvest molluscs aim to maintain viability and quality. Live molluscs are typically stored in refrigerated conditions or wet storage systems with circulating clean seawater (Goulletquer, 1997). For short-term storage, refrigeration at 4–7°C can maintain quality, while prolonged storage may lead to increased mortality if conditions are not optimal (Lucas and Southgate, 2012). Post-harvest handling and technology directly affect the safety and market value of molluscs. Good practices help minimize microbial contamination and preserve organoleptic qualities, thereby ensuring compliance with health standards for molluscs in domestic and international markets (FAO, 2004). Regular monitoring of water quality during depuration and storage is recommended to ensure the effectiveness of post-harvest safety measures (Lee *et al.,* 2008).

2.5 Spoilage and Deterioration of Freshwater Mollusc

Freshwater molluscs are highly perishable due to their high moisture content, neutral pH, and the presence of endogenous enzymes, which collectively predispose them to rapid spoilage after harvest. Spoilage and deterioration reduce the sensory quality, nutritional value, and safety of molluscs, thereby limiting their marketability and shelf life if not properly managed (Huss, 1995; Ghaly *et al.,* 2010). The primary factors responsible for spoilage in molluscs include microbial activity, enzymatic autolytic action, and lipid oxidation, each contributing distinctly to quality deterioration during post-harvest handling and storage (Gram and Dalgaard, 2002).

2.5.1 Spoilage Mechanism and Shelf-Life Implications

Spoilage mechanisms in freshwater molluscs involve the combined effects of microbial growth, enzymatic degradation, and chemical changes, leading to off-odours, texture breakdown, and colour changes (Ghaly *et al.,* 2010). Micro-organisms, particularly specific spoilage organisms (SSOs), utilise nitrogenous compounds released during autolysis to produce volatile compounds such as ammonia and trimethylamine, resulting in the characteristic fishy odour associated with spoilage (Gram and Dalgaard, 2002).

Enzymatic autolysis breaks down muscle proteins, lipids, and carbohydrates, contributing to softening and water loss in the tissue (Huss, 1995). Lipid oxidation, though limited in many freshwater molluscs due to low-fat content, can lead to rancidity and undesirable flavours when present (Venugopal, 2006). These spoilage processes reduce the acceptability and edibility of molluscs, significantly shortening their shelf life, which may range from 24 to 48 hours at ambient temperature but can be extended under proper refrigeration or processing conditions (Chakraborty *et al.,* 2014).

2.5.2 Enzymatic Autolytic Action

Enzymatic autolytic action refers to the degradation of mollusc tissues by endogenous enzymes following harvest. Proteolytic enzymes degrade muscle proteins and connective tissues, resulting in softening and breakdown of texture (Venugopal, 2006). Glycolytic enzymes contribute to pH decline by converting glycogen to lactic acid, which initially slows microbial activity but may later facilitate microbial growth as pH rises due to ammonia production from protein breakdown (Olafsdottir *et al.,* 1997).

Lipolytic enzymes may hydrolyse lipids, producing free fatty acids that can contribute to rancidity if oxidised (Hultin, 1992). The autolytic activity also releases peptides, amino acids,

and other nitrogenous compounds that serve as substrates for microbial spoilage, thereby indirectly accelerating spoilage processes in molluscs (Gram and Dalgaard, 2002). Controlling enzymatic activity through prompt cooling post-harvest is critical in extending the shelf life of freshwater molluscs (Huss, 1995).

2.5.3 Microbial Load and Spoilage

Microbial load plays a critical role in the spoilage of freshwater molluscs due to their nutrient-rich and moist environment, which supports the proliferation of spoilage organisms (Chakraborty *et al.,* 2014). Bacteria such as *Pseudomonas spp*., *Shewanella putrefaciens*, and *Aeromonas spp.* are commonly implicated in mollusc spoilage, producing slime and off-odours during their metabolic activities (Gram and Dalgaard, 2002). These bacteria utilise the nitrogenous compounds produced during enzymatic autolysis, generating volatile basic nitrogen compounds indicative of spoilage (Huss, 1995). The rate of spoilage is temperature-dependent, with rapid microbial proliferation occurring at ambient temperatures, leading to significant deterioration within 24–48 hours post-harvest (Ghaly *et al.,* 2010). Employing cold storage and hygienic handling can significantly reduce microbial load, thus extending the shelf life and maintaining the quality of molluscs (Chakraborty *et al.,* 2014).

2.5.4 Lipid Oxidation

Although freshwater molluscs generally have lower lipid content compared to marine species, lipid oxidation can still contribute to spoilage, especially under improper storage conditions (Venugopal, 2006). Lipid oxidation involves the reaction of unsaturated fatty acids with oxygen, leading to the formation of peroxides and secondary oxidation products such as aldehydes and ketones that impart rancid and off-flavours (Hultin, 1992).

Lipid oxidation can also lead to the deterioration of nutritional quality, as essential fatty acids are degraded during the process (Hultin, 1992). Factors such as exposure to air, light, high temperatures, and the presence of pro-oxidants can accelerate lipid oxidation in molluscs (Ghaly *et al.,* 2010). Effective methods to minimise lipid oxidation include vacuum packaging, the use of antioxidants, and cold storage to limit oxygen exposure and reduce oxidation rates (Venugopal, 2006).

2.6 Preservation and Processing Methods

Preservation refers to the techniques or activities aimed at protecting food from damage, decay, or spoilage, ensuring its usability for future consumption (Merriam Webster dictionary). It encompasses a range of methods designed to maintain food quality after harvest or catch (Desroseir *et al.,* 2023). Common preservation techniques include drying, refrigeration, and fermentation.

Food processing involves the series of steps taken from the point of harvest or catch until the product reaches the consumer. These methods often transform raw food into different forms to enhance shelf life, safety, or convenience (Omoruyi *et al.,* 2015). In fish processing, various stages can alter the protein structure of muscle tissues, influencing the final product's quality. Effective preservation begins immediately after harvest or catch to minimize deterioration and maintain nutritional value, flavour, colour, and texture. The choice of method depends on factors such as the type of seafood, intended use, and desired shelf life. The various preserving and processing methods include:

2.6.1 Curing/Brining

Curing involves adding salt to fresh meat or seafood to aid moisture reduction and control the growth of undesirable bacteria. This method can also enhance the colour and flavour of the

product (Alvila-Alvarez *et al.,* 2022). Curing reduces water activity by using salt, sugar, spices, vinegar, or sodium nitrite, which helps draw out moisture from the product, thereby minimising microbial growth. The two primary methods of curing are dry-salting and pickle-curing.

In pickle-curing, also referred to as wet-salting, the product is either submerged in a brine solution (a mixture of salt and water) or dry salt is applied without draining the resulting moisture (Catsberg, 2013). This technique enhances preservation while also improving flavour, often with the addition of spices and herbs.

2.6.2 Drying

Drying is a preservation technique that involves removing moisture from food products by maintaining controlled temperatures, typically within the range of 45–85°C, until the moisture content is sufficiently reduced. During the initial drying phase, known as the constant rate period, surface moisture evaporates while the product's temperature remains stable. In the final stage, or falling rate period, the temperature rises as moisture removal continues (Calin-Sánchez *et al.,* 2020). Drying effectively reduces water activity, thereby inhibiting microbial activity and enzymatic reactions that can lead to spoilage.

2.6.3 Smoke Drying

Smoke drying is a preservation method that combines heat and smoke to extend the shelf life of fish and fishery products. The quality of smoke-dried products is evaluated based on characteristics such as colour, texture, aroma, flavour, overall acceptability, and microbial load. application of heat in smoke-drying helps maintain food safety and quality, although it may lead to protein denaturation, which can reduce the nutritional and functional quality of the product (Sutikno *et al.,* 2019).

2.6.4 Smoking

Smoking is used as a preservation technique to control the physicochemical properties of food, including pH, volatile basic nitrogen (VBN), and thiobarbituric acid reactive substances (TBARS). It also influences fatty acid profiles, texture, sensory attributes, and shelf-life extension (Huang *et al.,* 2019). Smoking with wood generates smoke compounds that inhibit microbial growth and slow oxidative changes in the product (Fuentes *et al.,* 2010). There are two major smoking techniques: hot smoking and cold smoking.

Hot smoking: Hot smoking is commonly practised in tropical regions, involving the gradual smoking of fish or meat at temperatures ranging from 40°C to 100°C, but never below 40°C (Fecicilar and Genccelep, 2017). Hot smoking integrates cooking, smoking, and drying processes, which reduces moisture content and can enhance the nutritional composition of the product. The quality of hot-smoked products depends on optimal time and temperature control as well as the type of wood used during smoking.

Cold smoking: This method includes salting, drying, and smoking while maintaining temperatures at or below 30°C, ensuring that proteins are not denatured during the process. Cold smoking can be carried out by exposing the product to smoke without heat or by using liquid smoke containing the same chemical components as traditional smoke (Arvanitoyannis and Kotsanopoulous, 2012). Following initial salting, the product undergoes smoking to develop desirable sensory characteristics and to incorporate antimicrobial and antioxidant compounds (such as aldehydes, ketones, alcohols, and phenols), significantly extending its shelf life compared to fresh products (Sampels, 2015). Smoke-drying and smoking techniques are beneficial to consumers, producers, and contribute to the economic value within the food processing sector.

2.6.5 Frying

Frying is a thermal processing method in which food is boiled in hot oil or fat at high temperatures, typically between 150°C and 190°C (Bognár, 2002). It involves both heat and mass transfer, leading to moisture evaporation from the food surface while oil is absorbed, resulting in a crispy texture and characteristic flavour (Saguy and Dana, 2003).

Frying reduces the moisture content of food significantly, which can contribute to short-term preservation by lowering water activity, thereby inhibiting microbial growth (Fellows, 2009). However, because frying often involves high fat content, fried products can still be prone to quality deterioration during storage due to lipid oxidation and rancidity (Shyu and Hwang, 2001). In addition to enhancing flavour and texture, frying can also affect the nutritional composition of foods. It may lead to losses of heat-sensitive nutrients such as some vitamins, while increasing the energy density of the food due to oil absorption (Bognár, 2002). It can also be applied to various food types, including fish, meat, vegetables, and molluscs, for immediate consumption or short-term storage, though it is not a long-term preservation method like drying or smoking (Fellows, 2009). One of the common frying methods include deep frying.

Deep frying

Deep-fried foods are highly popular due to their distinctive sensory and organoleptic qualities. The deep-fat frying process causes macro- and micro-level changes in the structure of foods (Abdulla *et al.,* 2022). It also contributes to the preservation of foods by inactivating enzymes and reducing microbial activity through the application of heat, thereby enhancing food safety (Dehghannya and Ngadi, 2021). This high-temperature method decreases the moisture content on the surface of food, which helps extend its shelf life. Various physical and chemical changes occur during frying, including protein denaturation, starch gelatinization, browning, and crust formation, which are essential to the interaction between the hot oil and the food. The extent of

oil absorption during frying depends on several factors, including temperature, frying time, pre-treatment procedures, and the physicochemical properties of both the food and the oil used (Frakolaki *et al.,* 2023).

However, a significant issue associated with deep-fried foods is their high fat content, which can contribute to metabolic health problems such as hypertension, obesity, and elevated cholesterol levels. The intake of large quantities of cooking oil and saturated fats is linked to an increased risk of developing chronic conditions like type 2 diabetes, cardiovascular diseases, cancer, obesity, and high blood pressure (Oke *et al.,* 2018). Moreover, fried foods with oil content exceeding 50% by weight are at a higher risk of acrylamide formation. Higher frying temperatures further increase the likelihood of acrylamide development, oil oxidation, and nutrient degradation during frying (Mesias *et al.,* 2021).

2.6.6 Boiling

Boiling is one of the most common thermal processing methods used in aquatic foods, including fish and snails. It involves cooking at 100°C in water, which induces protein denaturation, alters nutrient profiles, and inactivates a wide range of microorganisms. In traditional processing, boiling is also applied as a preparatory step before smoking, drying, or freezing, particularly in snail handling where it helps remove slime and detach meat from shells (Marimuthu *et al.,* 2011; Pratama *et al.,* 2023; Ligaszewski *et al.,* 2024).

From a microbiological perspective, boiling substantially reduces bacterial and parasitic loads, thereby improving food safety. For example, it has been shown to lower risks from pathogens such as *Salmonella* and *E. coli* in snails, and spoilage bacteria in fish tissues (Kougiagka *et al.,* 2022; Pratama *et al.,* 2023). However, boiling alone does not guarantee long-term preservation,

as enzymatic activity and oxidative spoilage can still occur if products are not combined with other preservation methods such as smoking or refrigeration (Manz Koule *et al.,* 2024).

Nutritionally, boiling causes variable changes in proximate composition. Protein and fat levels typically decrease due to heat denaturation and leaching into cooking water. In *Pomacea canaliculata*, protein dropped from 15.6% in raw meat to 13.7% after boiling, while fat content decreased by about half (Pratama *et al.,* 2023). Similar patterns are observed in fish, where boiling reduces lipids but often leads to relative increases in ash content due to concentration effects (Marimuthu *et al.,* 2011; Manz Koule *et al.,* 2024). Minerals such as phosphorus and potassium are also reduced through leaching, though this can have beneficial implications, such as lowering the phosphorus-to-protein ratio in fish consumed by patients with kidney disease (Boaz *et al.,* 2021).

Boiling also affects lipid quality and vitamins. While total lipid content is reduced, boiling generally preserves polyunsaturated fatty acids better than frying, making it a relatively protective method for maintaining omega-3 fatty acids (Sampels, 2015). On the other hand, water-soluble vitamins, particularly the B-complex and vitamin C, are highly vulnerable to heat degradation and loss into the cooking water (Sampels, 2015). Retaining the cooking liquid, such as in soups or broths, helps conserve these nutrients (Boaz *et al.,* 2021). Though it is an accessible and effective method for cooking fish and snails, it is not sufficient for long term storage unless combined with other preservation strategy

2.7 Storage method

Storage is a critical factor in maintaining the quality and safety of aquatic foods such as fish and snails. Freshly harvested products are highly perishable due to their high moisture content, enzymatic activity, and susceptibility to microbial growth, making effective storage essential to

extend shelf life (Okpala *et al.,* 2014). Plastic containers are widely used for storage because they are affordable, lightweight, and provide a barrier against external contamination. They are particularly suitable for short-term preservation of fish and snails, especially when used in combination with chilling or freezing (Akintola and Brown, 2020). Studies have shown that sealed plastic packaging can minimize moisture loss and reduce microbial contamination, thereby maintaining sensory and nutritional quality for longer periods compared to unpackaged products (Abolagba and Igbinevbo, 2010).

Beyond plastic, alternative storage methods such as modified atmosphere packaging (MAP) and vacuum packaging have been increasingly studied. These methods slow down microbial proliferation and lipid oxidation, extending the shelf life of fish fillets and other seafoods (Sivertsvik *et al.,* 2002). Recent innovations also include the use of biodegradable and active packaging materials designed to improve sustainability and enhance preservation (García-Soto *et al.,* 2021).

Overall, storage method plays a decisive role in the safety and acceptability of aquatic foods. While plastic containers remain a common and practical option, integrating them with other preservation strategies such as refrigeration, MAP or smoking can significantly enhance the effectiveness of storage.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of Study Area

The study was conducted at the Experimental Fish Farm of the Department of Aquaculture and Fisheries Management, Faculty of Agriculture, University of Benin, Ugbowo Campus, Edo State, Nigeria (latitude 6.3949°N, longitude 5.6°E). All processing operations, including smoke drying, boiling, and deep frying were carried out at the facility. Microbial analyses of the processed samples were later performed at Global Point Analytical Laboratory Ltd, Iduowina, Benin City, Edo State.

3.2 Experimental Design

The experiment was carried out using a 3 × 3 factorial experiment in a Completely Randomized Design (CRD) with three replicates to evaluate the effects of smoke drying, boiling, and deep frying on the microbial load of *P. ovata*.

3.3 Sample Collection

Samples of *P. ovata* were obtained from a local market in Benin City, Edo State, Nigeria. The samples were placed in clean plastic containers and transported immediately to the processing area under hygienic conditions to prevent contamination before processing.

3.4 Preparation of Samples and Equipment

3.4.1 Preparation of the Smoking Kiln

The smoke-drying process was carried out using a Magbon-Alade smoking kiln. The smoking racks were thoroughly washed with water and an iron sponge to remove debris from previous use. They were rinsed properly with clean water and allowed to air dry. The racks were lightly oiled to prevent the samples from sticking during drying. The coal chamber of the smoking kiln was also cleaned and filled with fresh charcoal before use.

3.4.2 Deep Fryer

A manually operated deep fryer was used for the deep-frying process. The setup consisted of a metal frying pot and a perforated scoop for handling the snail meat. The fryer was heated over a controlled flame using a gas burner. Fresh cooking oil was used for each batch, and care was taken to prevent overheating of the oil.

3.4.3 Boiling

The boiling process was carried out using a stainless-steel cooking pot with a fitted lid. The pot was thoroughly washed and rinsed with clean water before use. Potable water was measured into the pot, and the snail samples were fully immersed. The pot was placed over a controlled flame using a gas burner, and the samples were boiled at 100°C for the required duration. After boiling, the samples were removed using a perforated ladle, drained, and allowed to cool.

3.4.4 Preparation of Samples

In the laboratory, the snail samples were washed with clean potable water to remove mud, mucus, and other impurities. The shells were cracked using a sanitized mallet, and the edible portion (meat) was extracted. The extracted snail meat was rinsed again, drained using a clean stainless-steel sieve, weighed with a digital balance, and then assigned to their respective treatment groups.

3.5 DETERMINATION OF MICROBIAL LOAD

3.5.1 Materials

Petri dishes, aluminum foil, autoclave, masking tape, test tubes, measuring cylinder, Gram stain reagents, weighing balance, beakers, marker pens, cotton wool, ethanol, lab coat, face mask, syringe (5 ml), spatula, disposable gloves, pressure cooker, knife, and distilled water were used during the study.

3.5.2 Sterilization of Materials

All glassware used during the experiment were thoroughly washed and rinsed with distilled water to remove contaminants. The glassware were placed upside down on canisters to drip dry and then sterilized in an autoclave at 121°C for 15 minutes.

3.5.3 Preparation of Samples and Serial Dilution

Microbial analyses were conducted for both bacteria and fungi. One gram of each Pila ovata sample was aseptically cut into small portions using a sterile blade and placed in a test tube containing 9 ml of sterile distilled water (stock solution). From this, 1 ml was transferred serially into new test tubes containing 9 ml of sterile distilled water to obtain dilutions of 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}. From each dilution, 1 ml was inoculated into sterile Petri dishes in triplicate. The plates were incubated at 34–37°C for 24 hours before colony counting and isolation.

3.5.4 Preparation and Sterilization of Culture Media

All culture media used were commercially obtained and prepared according to the manufacturer's instructions. They were sterilized in an autoclave at 121°C for 15 minutes. The media used included Nutrient Agar (NA) for bacteria and Potato Dextrose Agar (PDA) for fungi.

3.5.5 Nutrient Agar

Twenty-eight grams (28 g) of nutrient agar powder was dissolved in one litre (1 L) of distilled water in a conical flask covered with cotton wool and aluminium foil. The medium was mixed thoroughly and sterilized by autoclaving at 121°C for 15 minutes. It was then allowed to cool to 45–50°C before being dispensed aseptically into sterile Petri dishes.

- **Isolation of Bacteria**

One gram (1 g) of homogenized Pila ovata sample was weighed into a sterile test tube, and 9 mL of sterile distilled water was added. The mixture was serially diluted using a ten-fold dilution technique up to 10^4. An aliquot of 1 mL from the appropriate dilution was plated onto nutrient agar for bacterial isolation. The culture plates were incubated at 37°C for 24 hours. Thereafter, the number of discrete colonies was counted and expressed as colony forming units per gram (cfu/g). The viable counts were calculated by multiplying the number of colonies by the dilution factor.

- **Enumeration of Bacteria**

The bacterial counts obtained were used to determine the total viable counts of the isolates. Discrete colonies on the nutrient agar plates were counted, and the mean colony count was calculated. The total viable counts were expressed as colony forming units per gram (cfu/g) (Holt *et al.,* 2000).

3.5.6 Potato Dextrose Agar (PDA)

Potato Dextrose Agar (PDA) was used for the cultivation of fungi. Thirty-nine grams (39 g) of PDA powder was dissolved in one litre (1 L) of distilled water in a sterile conical flask covered with cotton wool and aluminium foil. The mixture was stirred thoroughly and sterilized by

autoclaving at 121°C for 15 minutes. The medium was cooled to 45–50°C and dispensed aseptically into sterile Petri dishes.

- **Isolation of Fungi**

One gram (1 g) of each Pila ovata sample was weighed into a sterile test tube, and 9 mL of sterile distilled water was added. The solution was serially diluted using a ten-fold serial dilution method up to 10^3. One millilitre (1 mL) of the appropriate dilution was plated onto Potato Dextrose Agar (PDA) for fungi isolation. The plates were incubated at room temperature (28°C) for 72 hours. After incubation, discrete colonies were counted as colony forming units per gram (cfu/g), and the viable counts were calculated based on the dilution factor used.

- **Enumeration of Fungi**

The fungi counts were used to determine the total viable counts of the isolates. Discrete colonies on the PDA plates were counted, and the mean colony count was calculated. The total viable counts were expressed as colony forming units per gram (cfu/g) (Holt *et al.,* 2000).

3.5.7 Identification and Characterization of Isolates

Isolates were identified using gram staining, physiological reactions fermentation of sugar according to taxonomic schemes (Fawole and Oso 2001)

3.5.8 Morphological and Cultural Characteristics

Distinct bacterial and fungi colonies were examined based on their morphological and cultural characteristics, including colour, shape, size, elevation, margin, and texture. Pure cultures were obtained by sub-culturing isolated colonies onto fresh media plates using the streak plate method.

3.5.9 Gram Staining

Gram staining was performed to differentiate bacterial isolates. A thin smear of each isolate was made on a clean slide, heat-fixed, and stained with crystal violet for 1 minute. The slides were treated with Gram's iodine for 1 minute, decolorized with 95% ethanol for 20 seconds, and counterstained with safranin for 45 seconds. After air-drying, the slides were examined under an oil immersion microscope (×1000). Bacteria that appeared purple were recorded as Gram-positive, while those that appeared pink were recorded as Gram-negative (Cheesbrough, 2006).

3.5.10 Catalase Test

A small portion of each bacterial colony was placed on a clean glass slide, and a drop of 3% hydrogen peroxide (H_2O_2) was added. The immediate production of gas bubbles indicated a positive catalase reaction, while the absence of bubbles indicated a negative result (Fawole and Oso, 2007).

3.5.11 Oxidase Test

Oxidase activity was determined using oxidase reagent (tetramethyl-p-phenylenediamine dihydrochloride) on filter paper. A portion of a fresh bacterial colony (18–24 hours old) was smeared on the reagent-impregnated area using a sterile wooden applicator. The development of a blue to dark purple colour within 10 seconds indicated a positive oxidase reaction, while no colour change within that time indicated a negative result. Appropriate control organisms (Pseudomonas aeruginosa as positive and Escherichia coli as negative) were used (Cheesbrough, 2006).

3.5.12 Indole Production Test

Each bacterial isolate was inoculated into sterile test tubes containing 5 ml of tryptone broth and incubated at 37°C for 48 hours. After incubation, 0.5 ml of Kovac's reagent was added carefully.

The appearance of a red ring at the surface of the broth indicated a positive indole reaction, while the absence of colour change (yellow layer) indicated a negative result (Fawole and Oso, 2007).

3.6 Shelf- Life Evaluation

All samples for the different processing methods were packed into different sealed containers with tight lids and labelled accordingly. These containers were stored under ambient (room) temperature (27°C - 30°C) and periodic microbial analysis was carried out for a period of four weeks

3.7 Statistical Analysis

Data will be analyzed using two-way ANOVA to test for significant differences ($p < 0.05$) among treatments. Duncan's Multiple Range Test (DMRT) will be use to separate means. Analysis will be done using SPSS version 25.0

Plate 1 : Freshwater Apple Snail Samples

CHAPTER FOUR

4.0 Results

Table 1 represent the mean comparison of the microbial load expressed as Total Heterotrophic Bacterial Count (THBC) and Total Heterotrophic Fungi Count (THFC) in boiled, smoke-dried and deep-fried *P. ovata* samples stored and sampled across a period of four weeks (0, after two weeks and after four weeks).

The THBC was highest in the smoked samples throughout storage, beginning with an initial value of ($9.83 \pm 2.12 \times 10^5$ cfu/g) and increasing steadily to ($2.08 \pm 0.11 \times 10^6$ cfu/g) by week 4. The boiled samples recorded an initial THBC of ($5.10 \pm 0.70 \times 10^5$ cfu/g), which peaked at week 2 with ($1.95 \pm 0.10 \times 10^6$ cfu/g) before slightly declining to ($1.47 \pm 0.04 \times 10^6$ cfu/g) at week 4. The fried samples, however, had the lowest bacterial count at the start ($2.30 \pm 0.62 \times 10^5$ cfu/g), showing a gradual rise to ($8.97 \pm 0.60 \times 10^5$ cfu/g) by week 4. Duncan Multiple Range Test (DMRT) indicated that the bacterial loads differed significantly ($P < 0.001$) among the processing methods.

Similarly, the total heterotrophic fungi count (THFC) was lowest in the smoked samples ($2.00 \pm 1.00 \times 10^4$ cfu/g) and highest in the boiled samples ($4.67 \pm 0.58 \times 10^4$ cfu/g) at week 0. Fungi counts generally increased across all processing methods, reaching their peak at week 2, with boiled samples showing the highest fungal load of ($8.67 \pm 0.58 \times 10^4$ cfu/g). According to the DMRT, there was a significant difference ($P < 0.001$) among the processing methods in terms of fungi load.

Table 1: Table showing the mean comparison of the microbial load in processed *P. ovata* during storage across a period of four weeks

Processing Method	Storage Time (weeks)	THBC (cfu/g)	THFC (cfu/g)
Boiled	0	$5.10 \pm 0.70 \times 10^{5b}$	$4.67 \pm 0.58 \times 10^{4b}$
Boiled	2	$1.95 \pm 0.10 \times 10^{6b}$	$8.67 \pm 0.58 \times 10^{4b}$
Boiled	4	$1.47 \pm 0.004 \times 10^{6b}$	$5.33 \pm 1.15 \times 10^{4b}$
Fried	0	$2.30 \pm 0.62 \times 10^{5c}$	$3.67 \pm 0.58 \times 10^{4c}$
Fried	2	$6.70 \pm 0.46 \times 10^{5c}$	$5.33 \pm 0.58 \times 10^{4c}$
Fried	4	$8.97 \pm 0.60 \times 10^{5c}$	$5.67 \pm 0.58 \times 10^{4c}$
Smoked	0	$9.83 \pm 2.12 \times 10^{5a}$	$2.00 \pm 1.00 \times 10^{4a}$
Smoked	2	$1.63 \pm 0.38 \times 10^{6a}$	$5.67 \pm 1.53 \times 10^{4a}$
Smoked	4	$2.08 \pm 0.11 \times 10^{6a}$	$3.00 \pm 1.73 \times 10^{4a}$

Values are expressed as Mean ± Standard Deviation (SD). For both THBC and THFC.Means in the same column with different superscript are significantly different (THBC: $P < 0.001$; THFC: $P < 0.001$) according to Duncan's multiple range test.

ANOVA Results: THBC: $F=89.7$, $P<0.001$ | THFC: $F=15.43$, $P<0.001$

Table 2 represents the log reduction and microbial growth rates for the Total Heterotrophic Bacterial Count (THBC) and Total Heterotrophic Fungi Count (THFC) in boiled, smoke-dried, and deep-fried *P ovata* samples during storage.

For the THBC, the highest log reduction was recorded in the deep-fried samples (0.631), followed by the boiled samples (0.285) while no reduction was observed in the smoke-dried samples (0.000). However, during storage bacterial growth rate was highest in the fried samples (0.148 log cfu/g per week), followed by boiled samples (0.115 log cfu/g per week) and lowest in the smoked samples (0.081 log cfu/g per week). The THBC showed that all the processing methods were significantly different ($P<0.001$) from each other.

For the TFC, no reduction was observed in the smoked samples (0.000), while negative log reduction values were obtained for the boiled (–0.368) and fried (–0.263) samples. The growth rate of fungi was highest in the fried samples (0.047 log cfu/g per week), followed by the smoked samples (0.044 log cfu/g per week) and lowest in the boiled samples (0.014 log cfu/g per week).The THFC were also significantly different ($P<0.001$) among all processing methods.

Table 2: Table two showing the log reduction and microbial growth rates of the processing methods

Processing	Log_Reduction_THBC	Log_Reduction_THFC	Growth_Rate_THBC	Growth_Rate_THFC
Boiled	0.285	-0.368	0.115	0.014
Fried	0.631	-0.263	0.148	0.047
Smoked	0.000	0.000	0.081	0.044

Growth rates expressed as log cfu/g per week

Table 3 shows the percentage frequency and diversity of bacterial and fungi isolates obtained from processed *P ovata* immediately after processing (0 weeks of storage). The bacterial isolates included *Bacillus spp., Pseudomonas spp.,* and *Staphylococcus spp*., while the fungi isolates comprised *Aspergillus sp*., *Penicillium sp*., and *Fusarium spp*. The most frequently occurring bacteria were *Bacillus spp*., *Pseudomonas spp*., and *Staphylococcus spp*., each accounting for 14.3% of the total bacterial isolates. Similarly, the most prevalent fungi were *Aspergillus sp*., *Penicillium sp*., and *Fusarium spp*., each representing 28.6% of the total fungi isolates. All three fungi species were detected in the boiled samples.

Table 4 shows the percentage frequency of occurrence and diversity of bacterial and fungi isolates from processed *P ovata* after two weeks of storage. The bacteria detected were *Bacillus spp*. and *Staphylococcus spp*., while the fungi isolates included *Aspergillus sp*., *Penicillium sp., Fusarium spp., and Cladosporium sp*. The most frequently occurring bacterium was *Bacillus spp*. (30.0%), whereas *Aspergillus sp*. was the most dominant fungi species (20.0%). The least occurring fungus was *Cladosporium sp*. (10.0%), which was found exclusively in the smoke-dried samples.

Table 5 presents the percentage frequency and diversity of bacterial and fungi isolates from processed *P ovata* after four weeks of storage. The bacterial isolates identified were *Bacillus spp*. and *Staphylococcus spp*., while the fungi included *Aspergillus sp*., *Penicillium sp*., and *Fusarium spp*. The most frequently occurring bacterium was *Staphylococcus spp*. (33.3%), while *Aspergillus sp*. was the dominant fungus (33.3%).

Table 3: Percentage frequency of occurrence and diversity of bacteria and fungi in the different processing methods at 0 weeks (immediately after processing)

Organisms	Frequency	%Frequency	Smoke-dried	Boiled	Deep-Fried
BACTERIA					
Bacillus sp	1	14.3	✓	×	×
Staphylococcus sp	1	14.3	×	×	✓
Pseudomonas sp	1	14.3	×	✓	×
Bacteria frequency	3		1	1	1
Bacteria% frequency		42.9	33.3	33.3	33.3
Bacteria diversity	3		1	1	1
Bacteria% diversity		100	33.3	33.3	33.3
FUNGI					
Aspergillus sp	2	28.6	×	✓	✓
Penicillium sp	2	28.6	✓	✓	×
Fusarium sp	2	28.6	×	✓	✓
Fungi frequency	6		1	3	2
Fungi% frequency		85.7	16.7	50	33.3
Fungi diversity	3		1	3	2
Fungi% diversity		100	33.3	100	66.7
Microbial frequency	9		2	4	3
Microbial% frequency		100	22.2	44.4	33.3
Microbial diversity	6		2	4	3
Microbial% diversity		100	33.3	66.7	50

Table 4: Percentage frequency of occurrence and diversity of bacteria and fungi in the different processing methods after two weeks of storage

Organisms	Frequency	%Frequency	Smoke-Dried	Boiled	Deep-Fried
BACTERIA					
Bacillus sp	3	30	✓	✓	✓
Staphylococcus sp	2	20	✓	✓	x
Bacteria frequency	5	50	2	2	1
Bacteria% frequency			40	40	20
Bacteria diversity	2	100	2	2	1
Bacteria% diversity			100	100	50
FUNGI					
Aspergillus sp	2	20	x	✓	✓
Penicillium sp	1	10	x	✓	x
Fusarium sp	1	10	x	x	✓
Cladosporium sp	1	10	✓	x	x
Fungi frequency	5		1	2	2
Fungi% frequency		50	20	40	40
Fungi diversity	4		1	2	2
Fungi% diversity		100	25	50	50
Microbial frequency	10		3	4	3
Microbial% frequency		100	30	40	30
Microbial diversity	6		3	4	3
Microbial% diversity		100	50	66.7	50

Table 5: Percentage frequency of occurrence and diversity of bacteria and fungi in the different processing methods after four weeks of storage

Organisms	Frequency	%Frequency	Smoke-dried	Boiled	Deep-fried
BACTERIA					
Bacillus sp	1	11.1	✓	x	x
Staphylococcus sp	3	33.3	x	✓	✓
Bacteria frequency	4		1	1	2
Bacteria% frequency		44.4	25	25	50
Bacteria diversity	2		1	1	1
Bacteria% diversity		100	50	50	50
FUNGI					
Aspergillus sp	3	33.3	✓	✓	✓
Penicillium sp	2	22.2	✓	✓	x
Fusarium sp	2	22.2	x	✓	✓
Fungi frequency	7		2	3	2
Fungi% frequency		77.8	28.6	42.9	28.6
Fungi diversity	3		2	3	2
Fungi% diversity		100	66.7	100	66.7
Microbial frequency	11		3	4	4
Microbial% frequency		100	27.3	36.4	36.4
Microbial diversity	5		3	4	3
Microbial% diversity		100	60	80	60

Keys: ✓ represents the presence of the microorganisms and x represents the absence of the micro-organisms

CHAPTER FIVE

DISCUSSION

5.1 Microbial Load

Bacteria and fungi were present in the processed *P. ovata* samples when stored for four weeks. The presence of microorganisms in smoked, boiled, and fried aquatic products has also been reported by Ehigiator *et al*. (2015), Omoruyi and Ebhodaghe (2017), and Huang *et al*. (2019). The microbial counts varied among the processing methods, with smoke-dried samples generally recording the lowest fungi load, while fried and boiled samples showed comparatively higher counts. This variation can be attributed to differences in temperature, processing duration, and the level of moisture retained in each product.

The reduced microbial load in the smoke-dried samples could be linked to the high temperature and prolonged exposure to smoke, which facilitated effective moisture removal and introduced antimicrobial compounds that suppressed microbial growth. According to Fuentes *et al*. (2010), smoke contains phenols, aldehydes, and organic acids that have antimicrobial properties, helping to reduce the growth of bacteria and fungi and extend product stability. In contrast, fried samples exhibited lower microbial counts immediately after processing but showed higher microbial activity during storage. This may be due to oil retention, which can promote oxidative rancidity and provide a nutrient medium for microbial proliferation, as noted by Bognár (2002). Boiled samples recorded moderate microbial counts, which increased gradually during storage. The reappearance of microorganisms in boiled samples can be attributed to recontamination during cooling, handling, or packaging, since boiling alone does not deposit antimicrobial compounds or reduce moisture content significantly (Huss, 1995).

The variations in bacterial and fungi counts observed in this study are consistent with the findings of Ehigiator *et al*. (2015), who reported that the microbial diversity in *P.ovata* is influenced by the handling environment, hygiene conditions, and storage method. Improper handling, exposure to air, and moisture reabsorption during storage could also account for the gradual increase in microbial load over time. Generally, the smoke-dried samples demonstrated better microbial stability, as they recorded the lowest fungi load and more stable microbial counts during storage, making smoking the most effective method for preserving *P. ovata* compared to boiling and frying.

5.2 Bacterial Load

Bacterial load was significantly different ($P<0.001$) among the processing methos with smoke-dried samples showing higher bacterial counts than boiled and fried samples. The gradual increase in bacterial load during storage could be linked to residual moisture and nutrient availability that supported bacterial proliferation. Fried samples consistently maintained the lowest bacterial counts, suggesting that deep frying was the most effective method for bacterial inactivation during processing. This agrees with the findings of Bognár (2002) and Dehghannya and Ngadi (2021), who reported that high frying temperatures effectively destroy vegetative bacterial cells, resulting in immediate microbial reduction.

The bacterial isolates identified included *Bacillus spp., Staphylococcus spp., and Pseudomonas spp*., which has also been reported in *P. ovata* by Ehigiator *et al*. (2015). The dominance of *Bacillus spp*. can be attributed to their spore-forming ability, which enables them to survive heat treatments such as boiling and smoking. *Staphylococcus spp.* are known for their tolerance to mild heat and for being common post-processing contaminants, especially during handling and storage (Huss, 1995). *Pseudomonas spp.* are psychrotrophic spoilage organisms that proliferate

in moist and protein-rich environments, and their occurrence in processed samples has been linked to post-processing contamination (Okpala *et al*., 2014).These results suggest that bacterial diversity in processed *P. ovata* is influenced by the intensity of heat applied during processing and the hygiene of post-handling conditions.

5.3 Fungi Load

Fungi load also differed significantly among the processing methods ($P < 0.001$). The highest fungi counts were generally observed in boiled and fried samples, while smoke-dried samples maintained the lowest fungi counts throughout the storage period. The low fungi growth in smoke-dried samples is attributed to the antimicrobial compounds present in smoke such as phenols, organic acids, and aldehydes which inhibit fungi spore germination and mycelial development. This is consistent with the reports of Fuentes *et al*. (2010) and Huang *et al*. (2019), who stated that smoke contains bioactive compounds that retard fungi proliferation in fish and other aquatic foods.

The fungi species identified included *Aspergillus sp., Penicillium sp., Fusarium spp*., and *Cladosporium sp*. These fungi have been similarly reported by Ehigiator *et al.* (2015) in *P. ovata,* confirming that they are common environmental fungi associated with aquatic and soil habitats. *Aspergillus and Penicillium* are xerophilic fungi that can tolerate low moisture conditions, explaining their occurrence even after drying (Chakraborty *et al*., 2014). *Fusarium spp.* are typically introduced through contaminated water or materials used during processing (Venugopal, 2006), while *Cladosporium sp*. often originates from airborne contamination during storage (Hultin, 1992). The relatively low fungi load in smoke-dried samples demonstrates that smoking is highly effective in limiting fungi contamination and ensuring microbiological stability during

storage. These findings confirm that smoking not only enhances product flavour but also extends shelf life through microbial suppression.

5.4 Shelf Life Implications

The shelf life of *P. ovata* is determined by the effectiveness of the processing method in reducing microbial activity, limiting moisture content, and preventing recontamination during storage. Among the processing methods, smoke drying proved to be the most effective in prolonging shelf stability, while boiling and frying offered comparatively shorter preservation effects. Smoke-dried samples maintained the lowest microbial counts throughout the storage period, indicating that smoking effectively inhibited both bacterial and fungi proliferation. This can be attributed to the combined effect of heat and antimicrobial compounds present in smoke such as phenols, aldehydes, and organic acids which destroy microorganisms and suppress their subsequent growth (Fuentes *et al*., 2010; Huang *et al*., 2019). Furthermore, the dehydration effect of smoke-drying lowers water activity, creating unfavorable conditions for microbial survival. This observation agrees with Omoruyi and Ebhodaghe (2017), who found that smoke-drying *Lanistes libycus* reduced microbial load to acceptable levels and significantly extended its storage life. They noted that although microbial counts slightly increased during long-term storage, the total load remained within safe limits, provided the product was stored in low humidity conditions.

In contrast, boiled samples exhibited higher microbial counts during storage, suggesting a limited shelf life. Boiling, although effective at inactivating vegetative microorganisms initially, often fails to provide long-term protection because it does not involve dehydration or the deposition of preservative compounds. Once cooled, boiled samples are prone to recontamination, particularly

from handling and environmental exposure, as moisture provides a favorable medium for microbial regrowth (Okpala *et al*., 2014). This aligns with the findings of Huss (1995), who observed that moist heat treatments, such as boiling, reduce microbial load temporarily but do not prevent post-process spoilage if the product is not dried or stored under controlled conditions. Fried samples showed the greatest microbial reduction immediately after processing due to the high temperature of the oil, which destroys both bacterial and fungi spores. However, during storage, fried products may experience faster microbial growth compared to smoke-dried samples. This may be due to the retention of residual moisture in the core of the product and oil oxidation, which creates a surface environment conducive to microbial development (Bognár, 2002). Dehghannya and Ngadi (2021) similarly noted that although frying effectively reduces microbial load, its long-term stability depends on proper packaging and the exclusion of oxygen to prevent microbial reactivation and rancidity. Overall, smoke-drying offers superior shelf-life extension compared to boiling and frying because it combines dehydration with antimicrobial smoke constituents. These factors jointly inhibit microbial growth, maintain product quality, and enhance storage stability. This finding supports the reports of Eyo (2001) and Khan and Khan (2001), who concluded that smoking remains one of the most efficient preservation methods for aquatic foods due to its ability to lower water activity and prevent microbial spoilage. Proper packaging, low-humidity storage, and protection from moisture reabsorption are therefore essential to maintain the microbial safety and quality of processed *P. ovata* during extended storage.

CHAPTER SIX

6.0 Conclusion

This study has provided a clear understanding of how processing techniques affect the microbial quality and shelf stability of Pila ovata. Among the three methods examined, smoke-drying emerged as the most efficient preservation method, effectively suppressing both bacterial and fungi growth throughout the storage period. This can be attributed to the combined effects of heat, dehydration, and the antimicrobial compounds in smoke. Deep-frying also achieved a notable reduction in microbial load immediately after processing but was less stable during storage. Boiling, on the other hand, proved least effective due to its high moisture retention, which promoted microbial recontamination. The findings from this study highlight the importance of proper processing and drying techniques in ensuring food safety, reducing spoilage, and extending the shelf life of *P. ovata.* By adopting appropriate preservation methods such as smoke-drying, processors can improve product quality, minimize post-harvest losses, and enhance consumer safety.

6.1 Recommendations

In view of the findings obtained from this study, the following recommendations are made:

- The processing and packaging of *P. ovata* should be done under strict hygienic conditions to prevent contamination during and after processing.
- Smoke-drying should be adopted as the most reliable method for extending the shelf life of *P. ovata*, as it effectively reduces microbial growth and enhances product stability.
- The smoke-drying process should be conducted at moderate temperatures (around 70–80°C) and for a longer period to ensure proper removal of moisture, which is essential for microbial inhibition.

- Processed *P. ovata* should be stored in clean, airtight, and moisture-proof containers to prevent rehydration and microbial regrowth during storage.
- Future studies should investigate how smoke-drying affects the nutritional and sensory quality of *P. ovata* over time and evaluate possible combinations of drying and preservation methods for optimal results.

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APPENDIX

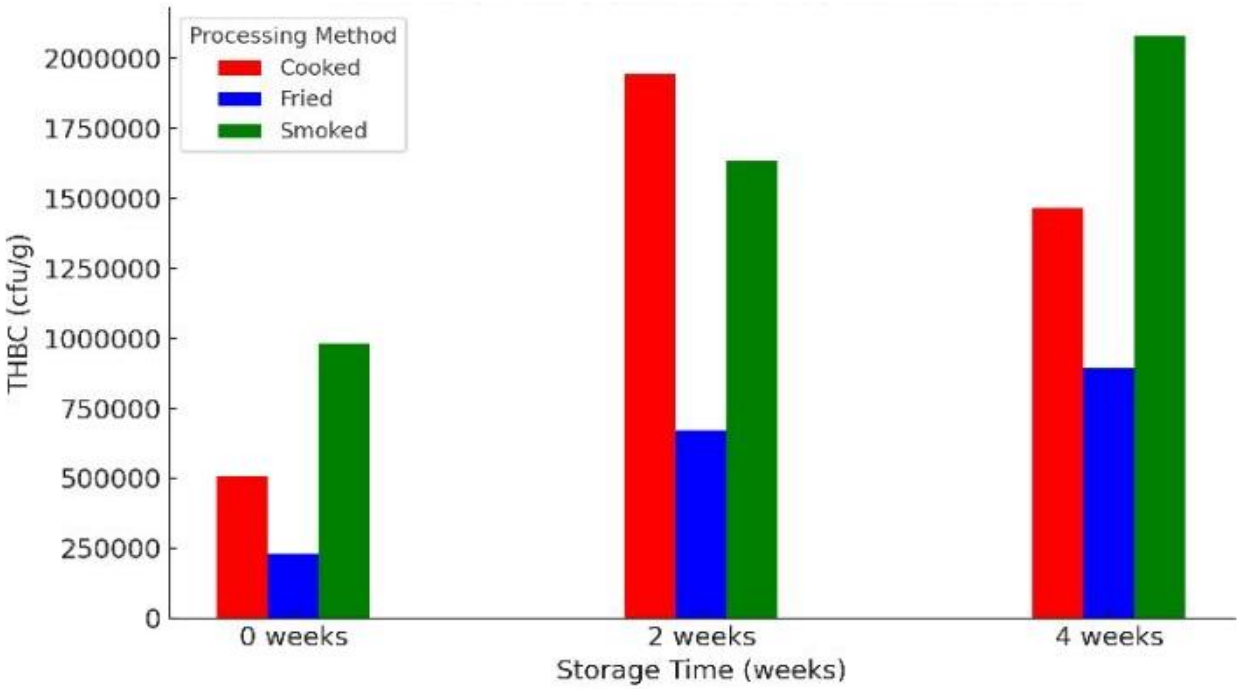

Figure 1: Total Heterotrophic Bacterial Count (THBC)

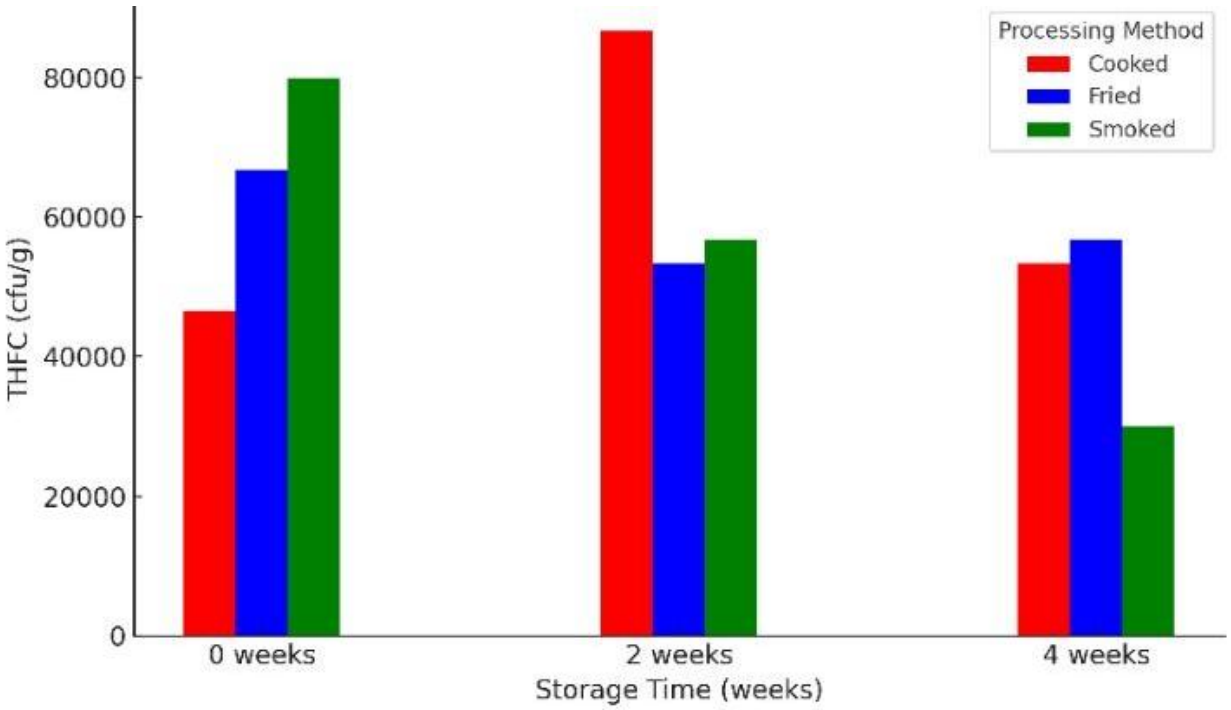

Figure 2: Total Heterotrophic Fungi Count (TFC)