PHYSICOCHEMICAL CHARACTERISTICS, MICROBIAL QUALITY AND SENSORY ACCEPTABILITY OF CRICKET (*GRYLLUS BIMACULATUS*) FLOUR PRESERVED WITH GINGER, GARLIC AND TURMERIC EXTRACTS

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DOCTOR OF PHILOSOPHY IN

(Food Science and Nutrition)

JOMO KENYATTA UNIVERSITY

OF

AGRICULTURE AND TECHNOLOGY

2024

Physicochemical Characteristics, Microbial Quality and Sensory Acceptability of Cricket (G*ryllus bimaculatus*) Flour Preserved With Ginger, Garlic and Turmeric Extracts

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A Thesis Submitted in Partial Fulfilment of the Requirements for the Degree of Doctor of Philosophy in Food Science and Nutrition of the Jomo Kenyatta University of Agriculture and Technology

2024

DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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DEDICATION

I dedicate this thesis to my beloved husband, Joshua Oder, and children, Joselyn and Jotham, to my parents, Mr. James Odongo and Mrs. Mary Odongo, and to my siblings: Moses, Denis, Solomon, Collin, Christine, Lucky, and Judith, for their constant support, encouragement, and prayers.

ACKNOWLEDGEMENT

My first and most profound gratitude is to the Almighty God, who has given me life, good health, and the ability to undertake this Ph.D. research successfully. My supervisors, Dr. John Kinyuru, Dr. Beatrice M. Kiage, and Prof. Dorothy Nakimbugwe are well appreciated for their inspiration, guidance, and general contribution to shaping this research.

I am grateful to the management of Busitema University, for allowing me to pursue further training, as well as to all of my colleagues in the faculty of agriculture and animal sciences for their unwavering support during my studies. Similarly, I appreciate the Jomo Kenyatta University of Agriculture and Technology (JKUAT), the host of this training. Thank you to Mr. David Abuga, Ms. Jessica Oruka, and Ms. Jennifer Wambugu from the School of Food and Nutrition Sciences, who all assisted with the laboratory work.

I acknowledge and appreciate the funding provided by the German Academic Exchange Service (DAAD) through the Regional Universities Forum for Capacity Building in Agriculture (RUFORUM)—In-Country/In-Region Doctoral Scholarship Program.

I appreciate the love, support, and encouragement from my beloved husband, Mr. Oder Joshua, and children, Joselyn and Jotham. Thank you for walking with me throughout this journey. My parents, Mr. James Odongo and Mrs. Mary Odongo, have done so much for me in the form of education, upbringing, and daily prayers. I am deeply thankful to them. Similarly, the moral support from my siblings: Moses, Denis, Solomon, Collin, Christine, Lucky, and Judith, is well appreciated.

Many people have contributed to the success of this work, and it is not possible to mention all the friends, family, and members of the lab group. Please receive my gratitude.

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ABSTRACT

Insects are a vital and preferred food among many cultures scattered throughout the world, where they are consumed as a source of protein, fat, minerals, and vitamins. However, with majority of insects still collected from wild environments, their utilization is hampered by regional and seasonal availability, perishability, and high postharvest losses. Therefore, this study aimed to preserve the quality of crickets (*Gryllus bimaculatus*) commonly consumed in East Africa, using spice extracts and determine the physico chemical characteristics, microbial quality and sensory acceptability of cricket flour. Garlic cloves, ginger, and turmeric rhizomes (2.5 kg each) were acquired from a local food market in Northern Uganda (2.2581° N, 32.8874° E), packed in airtight bags, and transported to the Jomo Kenyatta University of Agriculture and Technology (JKUAT) food biochemistry laboratory in Kenya. Samples were carefully cleaned under tap water, and rinsed with distilled water, drained to eliminate excess water, grated and extracted using acetone, ethanol, methanol, and water (2 g in 30 ml). The solution was shaken for 1 hr and kept in the dark for 72 hrs. Standard techniques were used to determine phytochemical composition and antioxidant and antimicrobial activity. Based on the results, ginger and garlic, extracted with ethanol, was chosen for the treatment of blanched crickets and subsequent processing into flour. Blanched crickets were divided into 5 batches of 1000 g each; three batches were chosen randomly and mixed with extracts of ginger, garlic, or ginger+garlic at a ratio of 1:4 (v/w). The other two batches received 0.1% sodium benzoate (positive control) and distilled water (C) (negative control). Following a 30-minute soak in the appropriate solution; any excess was drained off prior to oven drying for two hours at 105 °C (until crisp dry). Samples were milled to flour and packed in packed in zip-top low-density polyethylene bags with a 10 µm thickness. The samples were kept on shelf at ambient conditions; room temperature (23±2 °C) and relative humidity (60±2 %) and subjected to physicochemical, microbiological, and sensory evaluation at days 0, 30, and 60 of storage to evaluate the changes in an interval on 1 month. Phytochemicals and antioxidant activity were determined using standard methods; the antimicrobial activity of extracts was investigated using the agar-well diffusion method against *Staphylococcus aureus, Escherichia coli,* and *Candida albicans;* representing the microrganisms that are of public health significance as they affect food quality and safety (cause food borne illness). The fatty acid composition was assayed using gas chromatography, while the sensory evaluation *f*or color, aroma, texture/fineness, and overall acceptability was conducted using the hedonic test on a 5-point scale. Ginger extracts exhibited significantly higher total phenolic and flavonoid content in organic solvents compared to water extracts ($p<0.05$). The highest total phenolic and flavonoid content was in ethanol and methanol extracts of local ginger; 1968.49 and 2172.65 mg GAE/100 g; 254.24 and 184.62 mg QE/100 g, respectively. Organic solvent extracts of turmeric exhibited significantly higher total phenolic and flavonoid content compared to aqueous extract; 1379.94, 515.60, 561.16, 307.45 mg /100g Gallic acid equivalence and 382.66, 411.88, 339.01, 158.11mg /100g quercetin equivalent in acetone, ethanol, methanol and water aqueous respectively (*$p<0.05$*). Garlic extract followed the same trend as ginger and turmeric but with lower phenolic and flavonoid content.Vitamin C was

significantly higher in aqueous compared to organic solvent extracts of ginger, garlic and turmeric ($p<0.05$); This is of interest because vitamin C has antioxidant properties. Higher phenolic and flavonoids content in organic extracts resulted in a significantly higher free radical scavenging activity of the spice extracts. The strongest antioxidant activity was recorded in the turmeric-acetone extracts and in the acetone and ethanol extracts of the local ginger. Antimicrobial activity at 25 mg/ml varied significantly against the microorganisms, being highest on *C. albicans and* in the range of 18.00 to 30.67; 19.67 to 30.33; and 17.67 to 29.33 mm in ginger, garlic, and turmeric extracts, respectively. Raw garlic extracts exhibited higher antimicrobial activities against *S. aureus, E. coli,* and *C. albicans.* Activity of garlic ethanolic extracts compared favorably with raw extracts against the respective organisms (hybrid; 26.33, 24.33, 25.00; and local: 20.33, 20.33, 27.67 mm). Minimum inhibitory concentrations ranged from 2.5–10 mg/ml in garlic extracts. The major fatty acids in the cricket flour were linoleic, oleic, palmitic and stearic acid, respectively. During storage, slight increase in the proportion of palmitic acid and stearic acid and decrease in oleic and linoleic acid was recorded; changes in the proportions were more pronounced in the untreated samples.The acid value (AV), peroxide value (PV), and thiobarbituric acid reactive substances (TBARS) of flour all stayed within safe levels. Flour preserved with a ginger-garlic mixture showed minimal changes, compared favorably with sodium benzoate treatment. The pH, moisture content and total color change increased during storage but remained within acceptable limits. Fecal coliforms and *Escherichia coli* were not detected in any of the samples. On a five-point hedonic scale, (1. dislike extremely and 5. like extremely), color (3.84 to 2.55), aroma (3.59 to 2.40), texture (4.11 to 3.11) and overall acceptability (3.77 to 2.83) sensory scores were all significantly high on day 0 and low on day 60 of storage, respectively. According to the findings, ginger, garlic, and turmeric have strong antioxidant and antimicrobial activity, which makes them useful as natural antioxidants and antimicrobials. The study concluded that treating crickets with a combination of ginger and garlic minimizes lipid oxidation and that preserving crickets with ginger and garlic extracts produced flour that was safe, shelf-stable, and acceptable to consumers.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Entomophagy, the practice of eating insects, appears to be culturally universal, only varying with location, insect species, and ethnic group (Raheem et al., 2019; Raubenheimer & Rothman, 2013). Worldwide, over 2,000 insect species are reported to be used as human food, with an estimated 524 insect species consumed in Africa, 349 in Asia, 679 in the Americas, 152 in Australia and 41 in Europe (Jongema, 2017). This is due to the fact that insects make up the most diverse group of organisms, with one million different species accounting for 80% of all species in the world (Jongema, 2017). Food and Agricultural Organisation (2013), reported that beetles (Coleoptera) account for 31% of all consumed insects worldwide, followed by caterpillars (Lepidoptera) (18%); bees, wasps, and ants (Hymenoptera) (14%); grasshoppers, locusts, and crickets (Orthoptera) (13%); cicadas, leafhoppers, planthoppers, scale insects, and true bugs (Hemiptera) (10%); termites (Isoptera) (3%); dragonflies (Odonata) (3%); flies (Diptera) (2%); and other orders (5%). In Africa, edible insects are consumed as part of the traditional diet, and of the over 500 species, 100 species are consumed in eastern Africa (Kelemu et al., 2015).

In East Africa, termites (*Macrotermes spp*.), cone-headed grasshoppers (*Ruspolia spp*), blak ants, house crickets (*Acheta domesticus*), and field crickets (*Gryllus bimaculatus*) are the most widely consumed insects (Agea et al., 2008; Akullo et al., 2017; Kelemu et al., 2015; Kinyuru & Kipkoech, 2018). Due to the ease of domestication, the field cricket (*G. bimaculatus*) and the house cricket (*A. domesticus*) have gained more attention among the insects (Mitchaothai et al., 2022; Ng'ang'a et al., 2020; Sorjonen et al., 2019). According to Rumpold & Schlüter (2013), the majority of edible insects, including crickets provide adequate energy and protein in the human diet and also meet the amino acid requirements

of the human body. Insects such as crickets are also rich in essential fatty acids that are required by the human body (Guil-Guerrero et al., 2018; Otero et al., 2020); and trace elements, including copper, iron, phosphorus, selenium, and zinc, and they also contain vitamins like riboflavin, pantothenic acid, biotin, and folic acid (Akullo et al., 2018a; Gere et al., 2019; Weru et al., 2021). Proper processing and preservation of insects is a prerequisite for delivering safe and high-quality raw materials, ingredients, and products for large-scale insect food and feed manufacturing applications (Ojha et al., 2021). This is particularly important because insects are already being utilized to produce a range of novel new products that come in a number of forms, such as powders, pastes, liquids, and oils (Dossey et al., 2016).

On the other hand, various food preservation techniques have been developed in response to the requirement to extend the shelf life of food products while maintaining their quality and safety for human consumption (Bouarab Chibane et al., 2019; Mutungi et al., 2019). Additionally, the increasing demand for natural and organic foods in the last decade has promoted investigations into the application of natural food preservatives for preserving perishable foods. Several studies have shown that most of these natural food preservatives are derived from spices, in the form of powders, extracts, essential oils, or resins (El-Saber Batiha et al., 2021; Negi, 2012). Traditionally, spices such as ginger, garlic and turmeric have been used to improve the flavor, color, and aroma of food (Embuscado, 2015; Shan et al., 2009). However, it has now been demonstrated that spices are useful for food preservation since they have antioxidant and antibacterial properties, primarily because they contain phenolic compounds (El-Saber Batiha et al., 2021; Gottardi et al., 2016; Martínez-Graciá et al., 2015). Spices exhibit antioxidant properties by scavenging free radicals, chelating transition metals, quenching singlet oxygen, and boosting the activities of antioxidant enzymes (Rubió et al., 2013).

Rehman et al. (2003) observed that ginger extract has antioxidant activity equal to that of synthetic antioxidants butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), while turmeric oil was shown to have antioxidant properties similar to those of vitamin E and BHT (Yu et al., 2008). In a different study, analysis of 46 spices and herbs

showed that several of them have antibacterial activity against food pathogens (Shan et al., 2007). Additional research has focused on the ability of spices like ginger, garlic, and turmeric to preserve food (Demirhan, 2020; Martínez-Graciá et al., 2015; Ranjan et al., 2012; Verheyen et al., 2019). However, no studies on the use of spices for insect food preservation have been reported; thus, the goal of this study was to improve the processing and preservation of edible cricket (*G. bimaculatus*) by using spice extracts (ginger, garlic and turmeric).

1.2 Problem Statement

The lack of storage and preservation facilities in rural areas limits the utilization of farmed insects, resulting in substantial postharvest losses (Kinyuru et al., 2018). The perishability of insects is a result of high levels of moisture, free amino acids, polyunsaturated fatty acids, and nutrient content (Guil-Guerrero et al., 2018; Otero et al., 2020; Rumpold & Schlüter, 2013); in addition to the high amounts of naturally present autolytic enzymes and high pH. The spoilage of these insects results from changes brought about by biological reactions such as the oxidation of lipids, the activity of the intrinsic enzymes, and their metabolic processes. Oxidation induces modifications of lipids and proteins and adversely affects the physical, organoleptic, and nutritional properties of food products (Mokhtar et al., 2014).

The food industry has frequently used synthetic antioxidants such as butylated hydroxyanisole [BHA], butylated hydroxytoluene [BHT], and propyl gallate] ethylene diamine tetraacetic acid (EDTA) to preserve highly perishable and nutrient-dense foods (Brewer, 2011). Ascorbic acid [AA] and -tocopherol are two vitamins that have been used in food preservation. However, as consumers gain more knowledge and awareness about the benefits of eating healthy and organic foods, they prefer minimally processed foods with low levels of chemical preservation. This has led to a renewed interest in natural sources of antioxidants and specifically in food sources like spices that can maintain food quality. In earlier studies, spices were employed to preserve meat and fish since they contain antimicrobial properties (Brewer, 2011; Yerlikaya & Gökoğlu, 2010). On the

other hand, little is known about using spices as natural antioxidants and antimicrobials to preserve insect and insect-derived foods. Therefore there is scare information on the preservation of crickets with spices extracts and the associated effects on the physicochemical characteristics, microbial quality and sensory acceptability of the resultant cricket flour; this gap warranted this study.

1.3 Justification of the Study

The fact that one-third of the world's population eats insects is creating industrial interest in insects as food in most parts of the world (Dossey et al., 2016; Raheem et al., 2019). In Africa, the demand for edible insects is growing mainly because animal protein is becoming expensive and scarce; people are also demanding nutritious, safe, and healthier alternatives such as insects (Payne et al., 2016; Weru et al., 2022). With this trend, more and more people are likely to be involved in insect rearing, both at small-scale and medium-scale levels, to supplement the harvesting of insects from the wild. This development is likely to result in the production of huge volumes of insects as raw materials for complete foods and food ingredients (El-Shanshoury, 2014; Imathiu, 2020; Kinyuru et al., 2018). Improved preservation techniques are therefore necessary to lower post-harvest losses, increase shelf life, boost supply, and maintain the availability of the insect food products.

The addition of antioxidants is the most effective way to prevent oxidation and preserve food, and currently, using natural plant extracts as food preservatives is a popular trend as consumers perceive it as being healthy (Embuscado, 2015). In this regard, a number of studies have recommended the use of spices in food preservation (Beristain-Bauza et al., 2019; Panpatil et al., 2013). Consumers are willing to accept such products because of their perceived health benefits. The spices used in this study (garlic, ginger, and turmeric) are common food ingredients found in most communities (Kumari et al., 2018; Martínez-Graciá et al., 2015). It is anticipated that their use in the region and beyond will be economical and sustainable for the preservation of insect food products in Uganda.

1.4 Main Objective

The main objective of this study was to determine the physicochemical characteristics, microbial quality and sensory acceptability of cricket flour preserved with spice extracts.

1.5 Specific Objectives

1. To determine the phytochemical composition and antioxidant activity of spices (garlic, ginger, and turmeric) extracted using organic solvents and water.
2. To determine the *in vitro* antimicrobial activity of selected spices (garlic, ginger, and turmeric) extracted using organic solvents and water.
3. To evaluate the lipid characteristics and stability of cricket (*G. bimaculatus*) flour preserved with ginger and garlic extracts.
4. To determine the physiochemical properties, microbiological quality, and shelf-life of cricket (*G. bimaculatus*) flour preserved with ginger and garlic extracts.
5. To determine the sensory quality and acceptability of cricket (*G. bimaculatus*) flour preserved with ginger and garlic extracts.

1.6 Research Hypothesis

1. **Ho_1**: Extraction of spices using different solvents does not affect the phytochemical profile or antioxidant activity.
2. **Ho_2**: Extraction of spices using different solvents does not affect the antimicrobial activity.
3. **Ho_3**: Preserving cricket (*G. bimaculatus*) with ginger and garlic extracts does not affect the lipid characteristics and stability of the cricket flour.
4. **Ho_4**: Preserving cricket (*G. bimaculatus*) with ginger and garlic extracts does not increase the shelf-life of the cricket flour.
5. **Ho_5**: Preserving cricket (*G. bimaculatus*) with ginger and garlic extracts does not affect the sensory quality or acceptability of the cricket flour.

CHAPTER TWO

LITERATURE REVIEW

2.1 Consumption of Edible Insects

Insects have been consumed by humans for thousands of years as delicacies, staple foods, and emergency sustenance (Durst & Shono, 2010). However, there is little evidence from Europe and it is often limited to its southern and eastern parts (Bodenheimer, 1951). Generally, entomophagy appears to stretch 45° north and south of the Equator, with the Amazon Basin in Ibero-America, the American Southwest and neighboring Mexico, Central and southern Africa, Southeast Asia, and aboriginal Australia being some of the epicenters of entomophagy in the world (Paoletti et al., 2020; Ramos-Elorduy, 2009; Schrader et al., 2016). Insect consumption has a long history in all tropical countries. In the northernmost and southernmost regions of the world, long periods of dormancy and low average temperatures suppress the development and overall activity of cold-blooded species like insects. The biodiversity, activity, developmental rates, and size of many insect groups tend to increase toward the equator, thereby increasing the opportunities for entomophagy (Meyer-Rochow et al., 2010). Despite the fact that only a small number of species are consumed by humans, insects have enormous potential as a food resource due to their high biodiversity on the planet (Sollai & Solari, 2022). It is estimated that there are 10 to 30 million species of insects on planet Earth (Paoletti et al., 2020). It is believed that the systematic screening of more insect species would likely reveal many more candidates for entomophagy (Meyer-Rochow et al., 2010).

2.2 Insect Consumption in Africa

Edible insects are among the natural, renewable food resources that have the potential to alleviate food insecurity in Africa (Niassy et al., 2016). Fortunately, eating insects in Africa is more than just a survival tactic during food shortages (Illgner & Nel, 2010). Instead, people eat insects as a delicacy or as a vital part of their diet. However, there is a

bias against edible insects driven by the notion that the majority of people in western civilization assume that consuming insects as food in the tropics is a response to starvation; therefore, they reason that eating insects is just a survival strategy (Durst et al., 2010; Durst & Shono, 2010). Currently, both the scientific community and the industry are showing an increasing interest in edible insects as food and feed.

2.2.1 Commonly Consumed Insects in Africa

The number of edible insect species consumed in Africa is constantly being revised as more research is conducted; 246 species were previously reported (Van Huis, 2003); followed by 524 species (Ramos-Elorduy, 1997); and 470 species (Kelemu et al., 2015). The highest biodiversity hot spot was the Central African region with 256 edible species, followed by southern Africa (164 species), eastern Africa (100 species), Western Africa (91 species) and North Africa (8 species) (Kelemu et al., 2015).

The orders Lepidoptera (moths and butterflies), Orthoptera (grasshoppers, locusts and crickets), and Coleoptera (beetles) have the greatest diversity of edible insect species, followed by Isoptera (termites, queens, and reproductives), Hymenoptera (bees), and Hemiptera (true bugs) (Illgner & Nel, 2010; Kelemu et al., 2015; Ramos-Elorduy, 2009; Van Huis, 2003). Among the lepidoptera, *Cirina forda* and *Gonimbrasia belina* represent the most widely consumed species in the southern, central and west Africa, particularly South Africa, Nigeria, Botswana, Malawi and the Democratic Republic of Congo (DRC) (Illgner & Nel, 2010).

The orthopteran, *Acanthacris ruficornis* (Fabricius) and *Ruspolia differens* (Serville) are widely consumed grasshopper species in Sub Sharan Africa (SSA), with the latter reported in southern, central, and eastern Africa (Alamu et al., 2013). The Rhinoceros beetles (Oryctes spp.) and Rhynchophorus spp. constitute the majority of the edible coleopterans in western, central, and southern Africa. In western and Central Africa *Rhynchophorus phoenicis* (Fabricius) has high economic value, regarded as a delicacy in Benin, DRC Cameroon and Cote d'Ivoire (Kelemu et al., 2015). Among the edible termites,

Macrotermes bellicosus, Macrotermes subhyalinus Macrotermes falciger and Macrotermes natalensis are commonly consumed across the SSA countries (Fombong & Kinyuru, 2018). The *Apis mellifera mellifera* Linnaeus and *A. mellifera adansoni* Latreille are the main species of bees (Hymenoptera) consumed all over, both for honey as larvae (Kelemu et al., 2015; Van Huis, 2003). Depending on the species of interest, the insects are eaten at various stages of their life cycles (Illgner & Nel, 2010). The majority of the insect species consumed in western, eastern, and southern Africa are similar, which is an opportunity for complementary research and development activities aimed at species that are frequently consumed in those locations (Kelemu et al., 2015).

2.3 Insect Consumption in East Africa

Insects have long been used in East Africa for food, medicine, and other cultural practices (Kinyuru et al., 2018). However, there have been reports of increased consumption of edible insects in this region due to changes in eating habits, tastes, and food and nutrition insecurity (Ayieko & Oriaro, 2008). In Kenya, traditionally consumed insects included different species of winged termites (alates), long-horned grasshoppers, locusts, black ants, lake flies, may flies and crickets (Ayieko & Oriaro, 2008; Christensen et al., 2009; Fombong & Kinyuru, 2018; Kinyuru et al., 2009). These insects are mostly consumed in the westernand Lake victoria region of Kenya, among the Luo communities (Christensen et al., 2009; Pambo et al., 2016). Termites (*Macrotermes subhyalinus*) were largely accepted as food by the respondents in a study conducted in five different counties in Kenya, with the majority in western Kenya. However, the majority of respondents had a negative opinion about eating meal worms (*Tenebrio molitor*) (Alemu et al., 2017). In another study, Pambo et al. (2016) reported that more than 75% of respondents were open to accepting insects as a substitute for traditional beef in Western Kenya. This demonstrated that the populace was receptive to insects that were customary in their societies.

In Uganda, there are 20 species of edible insects that are consumed as food, nine of which are termite species (Okia et al., 2017). The most commonly reported were *M. subhyalinus*

(62%), *Pseudacanthotermes militaris* (59 %), *Ruspolia differens* (56%), and *M. bellicosus* (54%). In terms of consumption preference, the most preferred species was *R. defferens*, locally known as ‘nsenene’ a Luganda word derived from "Musenene", the Luganda name for the month of November, one of the two annual seasons during which the grasshoppers appear in large numbers. According to other studies, termites (Macrotermes spp.) are a delicacy and considered to be the most popular in the northern regions of Uganda (Akullo et al., 2017); while grasshoppers (*R. differens*) are a delicacy in the central region of Uganda, with a huge potential for commercialization (Agea et al., 2008; Odongo et al., 2018). Due to their tasty nature and relative availability, these insects are preferred and included in the diet when they are in season. However, efforts to conserve the insects were lacking because they were gathered from the wild. This indicates that the supply of edible insect products is bound to be unreliable (Okia et al., 2017).

In Tanzania, the most popular edible insect species is the longhorned grasshopper (*R. differens*), also known as "senene" in Swahili (Mmari et al., 2017). Senene has been widely harvested and consumed as a traditional snack in communities around the Lake Victoria crescent, including Tanzania (Matojo & Yarro, 2013). Originally eaten by the Haya tribe, Senene has since spread to the entire nation and is now commercialized and eaten by various tribes. Currently, harvesting is from the wild based on seasonal availability (Ng’ang’a et al., 2019).

The inventory in the Democratic Republic of the Congo (DRC) revealed that 148 species of insects are consumed there, with the orders of lepidoptera (60.1%), orthoptera (10.1%), coleoptera (8.1%), and hymenoptera (8.1%) dominating (Nsevolo et al., 2022). A study by Ishara et al. (2022) in South Kivu in Eastern DRC, recorded twenty-three (23) edible insects belonging to nine families and five orders, being consumed either in the larval, adult, egg or pupa stages. Among the insects, grasshoppers (*R. differens*), caterpillars (*Imbrasia oyemensis*), honey bee (*Apis mellifera*) larvae, mole crickets (*Gryllotalpa africana*), and Nsike were preferred for their taste. The house crickets (*Acheta domesticus*) were among the insects that were reported to be abundant throughout the year in the area, unlike other insect species which were seasonally available. In Haut-Katanga Province,

DRC, eleven edible insect species belonging to four families were recorded (Bomolo et al., 2017). The most prevalent species of edible insects preferred and consumed by the various communities were various species of caterpillars and the termites (*Macrotermes falciger*).

A total of 13 edible insects were reportedly consumed in Burundi and 6 in Rwanda. Termite alates, *M. facinger* and *M. subhyalinus* were reportedly the most collected and consumed in the majority of Burundi and Rwandan households, respectively (Okia et al., 2017). According to Odongo et al. (2018), the most commercially valuable edible insect species in the Lake Victoria area, including Burundi, is the grasshopper (*R. deferens*). The trade in the grasshoppers and their products are mainly in metropolitan areas, where they are prized as delicacies (Agea et al., 2008).

2.3.1 Status of Edible Insect Rearing in East Africa

The East African region has potential for rearing a number of edible insect species (Tanga et al., 2021). Due to the possibility of raising insects cheaply and using commonly accessible organic waste, insect farming has significantly increased in East Africa in less than ten years (Chia et al., 2020; Onsongo et al., 2018). *A. domesticus, Scapsipedus icipe, G. bimaculatus, Schistocerca gregaria, R. differens, Hermetia illucens, T. molitor, and Rhynchophorus phoenicis* are just a few of the edible insects that are being farmed in East Africa (Egonyu et al., 2021; Magara et al., 2021).

In Kenya, Tanzania, and Uganda, several small scale businesses have emerged in insect rearing, the market for edible insects is expanding in the region, and over 95% of these farms have the potential to be changed into more automated systems in the future (Onguko et al., 2022; Tanga et al., 2021). In Kenya, a number of initiatives focusing on different aspects of rearing edible insects for food are reported (Harriet et al., 2019; Kamau et al., 2021; Kinyuru & Kipkoech, 2018; Onguko et al., 2022). Crickets are the most common insect currently farmed for food in East Africa (Halloran et al., 2021; Kamau et al., 2021;

Oloo et al., 2021; Tanga et al., 2021). *S. icipe*, *A. domesticus*, and *G. bimaculatus* are three species of crickets that are commonly farmed (Tanga et al., 2018, 2021).

According to Tanga et al. (2021), small- and medium-scale production of crickets is growing quickly in Kenya (378 farmers) and Uganda (140 farmers). On the other hand, a large-scale insect farm in Kenya, InsectiPro Ltd., uses automated technology to generate about a ton of cricket powder each month. The adoption of cricket rearing in East Africa has been facilitated by the presence of the international insect science research center, (ICIPE) in Kenya, working in collaboration with other research, academic institution and partners to promote research in edible insect rearing since 2012. The center is currently researching on the rearing of 20 different edible species, including several species of crickets, grasshoppers, locusts, African fruit beetle, darkling beetles (mealworm), silkworm, saturniid caterpillar, African palm weevil, black soldier flies and tephritid fruit fly species that are available for promotion and upscaling trials (Egonyu et al., 2021; Tanga et al., 2018).

In addition to research, the adoption of cricket farming is attributed to the awareness created on the ease of rearing, the current market demand for cricket products and the risk aversion potential of the enterprise. However, among the challenges facing cricket farmers is the high cost of feeds (Ng'ang'a et al., 2020); hence, research is required to find alternate diets for rearing that are more cost-effective (Ayieko et al., 2016; Halloran et al., 2021; Kusia et al., 2021).

2.4 Role of Insects in Food Security and Sustainability

The current world population of 7.3 billion is expected to reach 8.5 billion by 2030, 9.7 billion in 2050 and 11.2 billion in 2100 (Department of Economic and Social Affairs (DESA)-United Nations, 2015). The population increase will necessitate a 100% increase in food production (Belluco et al., 2013). The demand for food is driven by the growing human population, but there is a corresponding reduction in the amount of land that can be used to cultivate that food, which is anticipated to be exacerbated by climate change.

It is predicted that low-income countries might experience the harsh effects of climate change and food insecurity, including widespread malnutrition and poverty. Therefore, global improvements in socioeconomic conditions and the availability of food are required (Lange & Nakamura, 2021).

Insects for feed and food can play a significant role in both ensuring food security and living more sustainably. They can aid in achieving the Sustainable Development Goals (SDG) by 2030 (DESA-UN, 2019). Specifically, SDG 2, to end hunger; SDG 12, to ensure sustainable consumption and production patterns; SDG 13, to take urgent action to combat climate change and its impacts; SDG 15, to protect, restore, and promote sustainable use of terrestrial ecosystems; sustainably manage forests; combat desertification; and halt and reverse land degradation and halt biodiversity loss (Raheem et al., 2019).

The World Food Summit of 1996 defined food security as a "situation that 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 preference for an active and healthy life" (Food and Agriculture Organization of the United Nations, 1996). Among the alternative sources of animal proteins, edible insects represent the option that most closely meets the necessary requirements for food security (Verneau et al., 2021). However, when insects are promoted for food security, the amount that can be harvested from the wild is insufficient, let alone the unreliable supply limitation (Durst & Hanboonsong, 2015; van Huis, 2015). Fortunately, insects can be produced more sustainably and with a much smaller ecological footprint than most vertebrate livestock, such as cattle and swine. The effectiveness is a result of the diversity of insects, their high fecundity, feed conversion efficiency, and their ability to bio transform a variety of organic materials into edible insect biomass while emitting fewer greenhouse gases (Nakagaki & Defoliart, 1991; Oonincx et al., 2010, 2015; van Huis & Oonincx, 2017).

2.4.1 Feed Conversion Efficiency and Fecundity of Edible Insects

In comparison to mammals, insects are far more efficient at converting plant proteins into insect proteins (Oonincx et al., 2015). For instance, it has been reported that crickets need less than 2 kg of feed for every kg of weight gain. Contrarily, the normal feed requirement (feed-to-meat conversion rate) for an increase in bodyweight of 1 kilogram is 2.5 kg for chicken, 5 kg for pork, and up to 10 kg for beef (Smil, 2002). After adjusting these figures for edible weight, the benefits of rearing and eating insects become even evident (van Huis, 2013).

According to estimates, 80% of whole cricket may be consumed and digested, compared to 55% for chickens, pigs, and cattle, and only 40% for pigs (Nakagaki & Defoliart, 1991; Smil, 2002; van Huis, 2013). Insects convert food into energy very effectively, and they also have shorter life cycles and faster growth rates than vertebrate livestock. Insects tend to reproduce quickly, with high fecundity and large biomass (Belluco et al., 2013). A female house cricket, for instance, can lay over 1500 eggs in her lifetime. Since insects produce hundreds of offspring, they can scale up and recover from losses much more quickly than other types of livestock farms (Gahukar, 2016).

In terms of ensuring food security, insects can be produced in large quantities with little adverse effect on global warming. For instance, it has been established that compared to pigs or cattle, insects produce less ammonia and greenhouse emissions. When compared to cattle and beef, the greenhouse gas emissions from raising edible insects like crickets, locusts, and mealworm larvae have been found to be reduced by a factor of almost 100 (Oonincx et al., 2010). Ammonia from animal waste, such as manure and urine, causes nitrification and soil acidity, and it also pollutes the environment (Aarnink et al., 1995). Commonly farmed insect species such as; crickets, locusts, and mealworm larvae emit less ammonia when compared to pigs, with a tenfold difference (Oonincx et al., 2010).

2.5 Nutritional Value of Edible Insects

Insects offer an important nutritional resource for humans; they are rich in protein, amino acids, fat, carbohydrates, various vitamins, and trace elements (Christensen et al., 2009; Kinyuru et al., 2013; Weru et al., 2021). The amounts of these nutrients vary greatly with species, as shown in Table 2.1.

Table 2.1: Proximate Composition and Energy Value of Edible Insect per 100g of Dry Matter

Order	Proteins (g)	Crude fat (g)	Ash (g)	Carbohyd-rates (g)	Energy (Kcal)
Orthoptera (Grasshoppers and locust)	56-77	4-32	2-17	16-40	362-427
Hemiptera (bugs)	33-65	9-54	1-18	7-19	329-622
Homoptera (tree hoppers)	29-72	4-33	3-11	19-26	394-470
Coleoptera (beetles)	21-54	18-52	1-7	12-34	410-574
Lepidoptera (butterflies)	15-60	7-77	2-8	3-32	293-762
Hymenoptera (bees, wasps, ants)	4.9-81	6-62	2-6	5-80	416-473
Isoptera (termites)	49-67	2- 40	4-5	13-34	535

Adapted from; (Weru et al., 2021)

Differences in species, environmental factors, food preferences, and insect developmental phases are thought to be the causes of variations in nutrient across species (Fontaneto et al., 2011; Kulma et al., 2020; Raksakantong et al., 2010).

2.5.1 Protein Content of Edible Insects

The protein content of insect orders; Orthoptera was high among the insect orders, ranging from 56 to 77 g/100 g dry weight, which is comparable to the protein content of meat (Bukkens, 1997; Ramos-Elorduy et al., 1997). In another study, Rumpold & Schlüter, (2013) reported that the average protein contents of the insect orders ranged from 35.34% for Isoptera (termites) to 61.32% for Orthoptera (crickets, grasshoppers and locusts). Most insect species convert plant protein to insect protein efficiently; insect are compared

favorably with beef, eggs, cow milk and soybean seeds as a source of dietary protein (Churchward-Venne et al., 2017). According to Weru et al. (2021), in a review of publications involving 91 edible insect species, the highest protein content was 81.11g/100 g and the lowest was 1.11g/100 g, additionally, 65 (60.19%) insects would meet the Recommended Daily Allowance (RDA) for females (46 g/day) whereas 42 (38.88%) insects could meet the RDA for men (56 g/day).

2.5.2 Dietary Protein Quality and Digestibility of Edible Insects

It is important to evaluate the quality of dietary proteins to ascertain how well they can satisfy protein and essential amino acid needs (Churchward-Venne et al., 2017). The quality of a protein and subsequently its nutritional value is dependent on the amino acid composition and the protein digestibility. Insect proteins are reported to contain 10-30% of essential amino acids and 35-50% of all of amino acids, which is close to the amino acid model proposed by the World Health Organization and Food and Agricultural Organization (WHO, FAO, 2007).

According to Köhler et al. (2019), four insects showed a high protein content, ranging from 27 g to 54 g/100 g edible portion in fresh weight basis; with tryptophan being the limiting amino acid in locusts and crickets, lysine in the scarab beetle and leucine in the silkworm. Only Silkworm met the FAO/WHO requirements for 40% essential amino acids and 0.6 ratio of essential to non-essential amino acids. Ramos-Elorduy et al. (1997) observed that protein digestibility ranged from 77% to 98% in different edible insect species. Ajayi, (2012) reported protein digestibility of 83.41% in winged termites and 81.10% in soldier termites. This is higher than the digestibility of legumes such as; chick peas (69.0 %), lentils (72.7 %), red kidney beans (64.1%) cooked by boiling (Rehman & Shah, 2005); while 49.86% and 41.13% were reported for boiled and fried tree locusts respectively (El Hassan et al., 2008). Akullo et al, (2018) reported protein digestibility of 31.38, 44.39 and 50.22% for *Syntermes soldiers, M. bellicosus* and Brachytrupes spp respectively.

The presence of chitin, an insoluble fiber and element of the insect exoskeleton, in the sample is one of the elements that may alter the digestibility of insect protein. In an *in vivo* investigation, it was observed that removing the chitin from insect products enhanced the protein quality in terms of digestibility, amino-acid availability, net protein utilization, and protein efficiency ratio (Churchward-Venne et al., 2017).

2.5.3 Fat Content and Energy Value of Edible Insects

The fat content of edible insects varies according to development stage and insect order; larvae and pupae stages have higher fat content than adult stages; Isoptera (termites) and Lepidoptera (caterpillars) rank among the highest in fat content (Raksakantong et al., 2010). Lepidoptera contain 7-77%, Hymenoptera 6-62%, Hemiptera 9-54%, Cloeoptera 8-54%, Homoptera 4-33% and Orthoptera 4-32% (Weru et al., 2021). The energy value of edible insects varies with species and order. The highest (762 Kcal/100 g) was in *phasus triangularis* (Lepidoptera). The lowest range (377-400 Kcal/100 g) was in Orthoptera. The energy value of edible insects is influenced by the species, lipid content, and stage of development (Magara et al., 2021).

2.5.4 Fatty Acids Composition of Edible Insects

The fatty acid composition of insects is varied, as shown in Table 2.2. The kind of fatty acid present is significantly influenced by the type of feeds consumed by the insects. For instance, winged termites are detritivores, while crickets are herbivores and phytophagous, hence they are able to accumulate linoleic acid from their feeds. According to Raksakantong et al. (2010), polyunsaturated fatty acid (PUFA) is the predominant fatty acid in edible insects, followed by saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA). However, other studies report that MUFA is the major fatty acid in other edible insects such as; termites and crickets (Akullo et al., 2018). One distinctive feature of saturated fatty acids is their suppression of Low Density Lipoproteins (LDL) receptor expression, which raises blood LDL cholesterol levels and may contribute to atherosclerosis and other chronic diseases (Medeiros, 2007).

Consuming PUFAs, particularly n-3 PUFAs, is becoming more popular because they have positive effects on lowering the risk of diabetes by preventing insulin resistance, reducing glucose intolerance, lowering blood pressure, lowering LDL cholesterol, and helping to prevent a number of other diseases like thrombosis, hypertension, inflammatory disease, arrhythmia, and coronary heart disease (Medeiros, 2007). PUFAs are therefore crucial for preserving good health; in the nerve tissue and retina, n-3 fatty acids are crucial as a structural membrane lipid (Gil & Gil, 2015; Kapoor et al., 2021).

Table 2.2: Fatty Acid Composition of Edible Insects

Fatty acid	June beetle	Queen caste	Weaver ant	Termites	Cicada	Short tail cricket
C14:0	Nd	0.44 ± 0.04	0.27 ± 0.02	0.13 ± 0.01	1.99 ± 0.05	nd
C15:0	Nd	0.06 ± 0.03	0.09 ± 0.02	0.02 ± 0.01	nd	nd
C16:	0.77 ± 0.01	1.68 ± 0.01	1.19 ± 0.01	0.78 ± 0.01	2.47 ± 0.04	1.61 ± 0.05
C18:0	27.92 ± 0.06	32.41 ± 0.04	27.67 ± 0.03	31.90 ± 0.02	52.53 ± 0.70	35.79 ± 0.02
SFA	**29.42 ± 0.10**	**34.59 ± 0.11**	**29.23 ± 0.07**	**32.83 ± 0.06**	**56.98 ± 0.79**	**37.54 ± 0.08**
C16:1	0.51 ± 0.02	nd	0.55 ± 0.04	0.19 ± 0.02	0.28 ± 0.05	0.71 ± 0.03
C18:1	5.59 ± 0.05	1.96 ± 0.02	1.80 ± 0.01	1.86 ± 0.01	0.92 ± 0.02	3.4 ± 0.03
MUFA	**6.11 ± 0.07**	**1.96 ± 0.02**	**2.36 ± 0.05**	**2.06 ± 0.03**	**1.20 ± 0.07**	**4.11 ± 0.06**
C18:3n-3	Nd	nd	0.36 ± 0.02	0.34 ± 0.03	nd	nd
C20:3n_6	13.86 ± 0.08	5.82 ± 0.03	9.88 ± 0.10	8.90 ± 0.03	10.77 ± 0.33	7.94 ± 0.04
C20:4n_6	47.26 ± 0.35	57.73 ± 0.28	57.82 ± 0.07	56.01 ± 0.07	33.03 ± 0.71	50.43 ± 0.55
C22:6n_3	3.39 ± 0.12	nd	0.74 ± 0.03	nd	nd	nd
PUFA	**64.50 ± 0.55**	**63.55 ± 0.31**	**68.80 ± 0.22**	**65.25 ± 0.13**	**43.80 ± 1.03**	**58.37 ± 0.59**

Adapted from: Raksakantong et al. (2010)

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; nd, not detected

Additionally, n-3 fatty acids compete with n-6 fatty acids for the enzymes needed to produce long-chain n-3 fatty acids. Hence, it influences the ratio of n-3 to n-6 fatty acid-derived eicosanoids (Finley & Shahidi, 2001; Siriwardhana et al., 2012). The presence of essential fatty acids like linoleic and linolenic acid points to the nutritional value of oils from the insects. Linoleic acid acts as a precursor for arachidonic acid, which in turn serves as the precursor for eicosanoids (prostaglandins, thromboxane, and leukotrienes), which are essential in body defense mechanisms (Saini et al., 2021). However, high amounts of polyunsaturated fatty acids such as linoleic and linolenic acid in foodstuffs gives them low oxidative stability (Saini et al., 2021), due to lipid peroxidation which is one of the reasons for the high perishability of insects. The fatty acid composition has a much higher influence on the stability of its oils (Adam Mariod, 2013).

2.5.5 Ash and Mineral Composition of Edible Insects

Edible crickets possess a significant amount of ash that varies among the insect orders, being high in orthopteran (2-17 mg/100 g) and hemipteran (1-18 mg/100 g) (Ramos-Elorduy, 1997). The higher the ash content, the higher the value of the mineral elements for human health. For instance, crickets have been reported to have a higher content of ash when compared to goat, broiler, and pork meat (Magara et al., 2021). On the mineral profile, edible insects are rich sources of minerals such as potassium (K), sodium (Na), calcium (Ca), copper (Cu), iron (Fe), zinc (Zn), manganese (Mn) and phosphorus (P) (Christensen et al., 2009; Köhler et al., 2019; Weru et al., 2021). According to Magara et al. (2021), the most prevalent macro mineral elements in edible crickets are phosphorus, which ranges from 0.80 to 1169.60 mg/100 g; potassium, ranging from 28.28 to 1079.90 mg/100 g; and sodium, which ranges from 0.99 to 452.99 mg/100 g. In previous studies, 9.66 and 22.43 mg/100g of iron and zinc were reported in *G. bimaculatus* (Ghosh et al., 2017); while 8.75 and 20.22 mg/100g were recorded in *A. domesticus* (Finke, 2002). Mineral levels and availability are also impacted by species difference and how insects are processed. The potential bioavailability of Ca, K, Fe, Zn, and Co was higher in fried Sudanese tree locusts, than in the boiled samples, while Na and Mg potential bioavailability were higher in the boiled samples (El Hassan et al., 2008).

2.5.6 Vitamin Content of Edible Insects

Vitamins are a group of organic compounds that are necessary for metabolism in human bodies. As vitamins cannot be synthesized in the human body, they must be supplied constantly by food. Thiamine, riboflavin, niacin, and vitamin B_{12} are a few water-soluble and lipophilic vitamins in edible crickets (Finke, 2002; Oibiokpa et al., 2017). Thiamine content was reported at 0.4mg/100 g (dry weight) of house crickets (*A. domesticus*), while a range of 0.1 to 4 mg/100 g of dry matter thiamine content reported in other edible insects (Bukkens, 1997). The amount of vitamins present in edible insects varies depending on their diet, such as food waste and commercial feed (Baiano, 2020). Most vitamins are sensitive to light and heat treatment during processing; Solar drying decreased the quantity of these vitamins (riboflavin, niacin, pyridoxine, retinol, ascorbic acid, folic acid, and a-tocopherol in winged termites and grasshoppers (Kinyuru et al., 2010).

2.6 Traditional Methods of Processing and Preservation of Edible Insects

Depending on the type of insect, post-harvest treatment of insects entails blanching in hot water or steaming to kill the insects, which is followed by removing foreign objects accidentally collected with the insects, wings, legs, and other inedible parts, followed by washing with cold water (Van Huis, 2003). Traditionally, the methods used for processing and serving edible insects are shown in Table 2.3. This includes roasting, smoking, toasting or pan-frying, deep frying, mincing, and boiling followed by sun drying, depending on the insect (Mutungi et al., 2019). However, for some insects, such as termites, a small portion may be eaten raw as relish during harvesting (Ayieko & Oriaro, 2008; Srivastava et al., 2009). Whole termites, grasshoppers, and crickets are pan-fried in their own fat under low heat and eaten whole; this product has a short shelf life and must be consumed within 1-2 days.

2.6.1 Dry Pan Frying/Roasting

In different parts of the world, dry pan frying or roasting is a common method of processing edible insects (Melgar-Lalanne et al., 2019). In Kenya, termites are de-winged, toasted, and then sun-dried (Kinyuru et al., 2009); whereas in Uganda they are steamed in banana leaves or fried and pounded into a cake (Van Huis, 2003). Frying of termites, crickets, and grasshoppers are also methods of cooking insects in Thailand (Siriamornpun & Thammapat, 2008). According to Nonaka (1996), the San women in the central Kalahari roast grasshoppers and crickets in hot ash and sand

Table 2.3: Traditional Methods of Processing and Serving Edible Insects

Insect species	Processing	Consumption
Macrotermes spp. (Winged termites)	1.Pan frying, boiling and sun drying, dried insects are made in to paste or stew	Snack/main/side dish
	2. Fresh de winged insects are grounded with spices, made in to meat balls and boiled or steamed into a popular dish known as 'Alakena'	Main dish
Syntermes soldiers (Soldier termites)	Blanching & grinding with spices to make meat balls which is boiled or steamed into a popular dish known as 'Alakena'	Main/side dish
Brachytrypes spp. (Crickets)	Deep-frying, grilling roasting, steaming and currying with spices such as onions and pepper	Snacks/side dish
Ruspolia differens (Longhorn grasshoppers)	Deep frying and air drying, pan frying with salt without oil; boiling followed by sun drying	Snacks
Apis mellifera (Honey bees)	Boiling with porridge or rice, pan frying without oil	Snacks
Cytacanthacris naeruginosus unicolor (Shorthorn grasshoppers)	Roasting, frying with spices like chili	Snacks
Zonocerus variegates (Grasshoppers)	Roasting and panfrying	Snacks/side dish

Source: (Akullo et al., 2017)

In Nigeria**,** dried winged termites are ground into a paste and eaten as a side dish with tapioca or combined with other ingredients like honey (Ekpo et al., 2007; Igwe et al., 2011). In the southern regions of Africa, mopane caterpillars and ground crickets are prepared by degutting, washing, boiling in salty water, roasting, and then sun-drying or smoking. The product is subsequently put into large tins or plastic containers for sale to traders and customers, or into sacks for storage (Illgner & Nel, 2010; Musundire et al., 2014).

2.6.2 Boiling and Sun Drying

Boiling is a method that is used for preserving large quantities of edible insects for consumption at a later date after harvest. Boiling is usually followed by sun drying or the addition of salt (Illgner & Nel, 2010). Sun drying is the most popular technique for preserving edible insects at the household level, given its low energy input and affordability. Termites are prepared by boiling for a few minutes the morning after the swarm, and then they are spread out on mats, iron sheets, or sacks to dry in the sun for 3–4 days depending on the weather (Ayieko et al., 2010). While drying reduces the moisture content of fresh products by gradually transforming them into more stable products, boiling serves the purpose of deactivating the insect's enzymes. Drying preserves food by lowering its water content to between 10 and 15 percent, which makes it less susceptible to microbial deterioration and enzymatic degradation (Melgar-Lalanne et al., 2019). Water activity (*aw*) is directly correlated with microbial growth (*aw*). At $aw<0.65$, most microorganisms stop growing, but when water conditions are favorable, they can resume growth (Grabowski & Klein, 2017a, 2017b). A reduction of free water increases the dry matter concentration significantly without damaging the tissues or changing the physical appearance of foods and is an important step for food ingredient extraction (Lamidi et al., 2019).

2.6.3 Smoking

Food is preserved through smoking by being exposed to smoke from burning or smoldering plant materials, most frequently wood. This is due to the presence of several chemicals in smoke that have anti-microbial and anti-oxidant properties and since heat is used, it also helps to dry the food material out (Adeyeye & Oyewole, 2016). In Uganda, sun dried winged termites are put in a pot and kept above the cooking area. This method keeps the insects fresh in addition to maintaining their original flavor. Fresh insects that have been fried or boiled and then sun-dried can be smoked (Van Huis, 2003). During the process, the combined action of enzymes and heat promotes protein and lipid changes (Tiencheu et al., 2013).

2.7 Processing of Edible Insects as Ingredients in Food Production

Edible insects are abundant in Africa and provide a great mass of material possessing genuine food value for both humans and livestock (Ayieko et al., 2010; Kelemu et al., 2015; Niassy et al., 2016). The creation and application of suitable food processing and preservation techniques are necessary for the full realization of the nutritional benefits of insects as a range of commodities. Processing is an important factor for consideration in order to incorporate insects into more conventional meals at all scales, including large-scale industrial, cottage industry, restaurant, and professional cuisine, as well as at the household level (Dossey et al., 2016).

Good processing eliminates or destroys possible safety hazards like pathogens while maintaining the longest shelf life possible and retaining or enhancing the nutritional, organoleptic (taste, aroma, texture, color), and functional properties of raw materials processed into food ingredients (Mutungi et al., 2019). The two processing factors that are most crucial are functionality and format. Functionality describes how a substance functions in a food and interacts with other components during the process of converting raw materials into finished products like foods and beverages (Lucas-González et al., 2019). Functionality examples include the capacity to bind oil and moisture, produce gels,

and maintain their integrity (Gravel & Doyen, 2020; Kim, Lee, et al., 2020). Format is the form in which the ingredient comes; this includes powders, pastes, and liquids (Dossey et al., 2016).

2.7.1 Production of Edible Insect Flour as Ingredients in Food Processing

Edible insects are processed into flour for various uses. The procedure involves spreading adequately cleaned insects out on a lightly greased cookie sheet and drying them in the oven until they are fairly brittle and easy to crush. For instance, drying of crickets at 110°C for 3 hr was reported to be sufficient to reduce the moisture content to < 5% as well as ensure microbial safety (Fröhling et al., 2020). Dried insects are ground or milled until they are about the consistency of wheat germ. The flour can almost be used in any recipe, including those for bread, porridge, salad dressings, soups, and other baked products (Osimani et al., 2018; Tao & Li, 2018). According to Dossey et al. (2016), the most optimum insect-based ingredient format for the majority of products will be a dry powder with fine particle size. This is accurate for several reasons: Powders can be blended successfully with a wide range of other ingredients without affecting the texture or structural integrity of the final product. Powders can have a mild flavor and aroma as well as a light color, depending on the processing method (Akullo et al., 2017). Powders have the longest shelf life compared to other product formats based on packaging and storage conditions.

Generally, a powder is the best way to incorporate an insect into a product without changing it or causing the consumer to "notice the insect," which is ideal for market acceptability (Kinyuru et al., 2009). Powders are also appropriate because they can be poured into or flowed through and used in the largest range of food equipment (extruders, etc.). Powders are perfect for any product because of their versatility and long shelf life, even though the finished product might not be dry (Kamau et al., 2018). This is because the ingredient can be transported, kept for a long time, and then used as needed; making it easy for an entrepreneur to buy and store larger amounts of insect-based ingredients for a long time for future use.

2.7.2 Edible Insect Flour as an Ingredient in Food Production

The focus of the most recent study has been on using edible insects to fortify more conventional food types like bread, cookies, pasta, burgers, and sausages, boosting their nutritional content. In addition to utilizing all the environmental, technological, and nutritional benefits connected with insect cultivation, this movement has made it possible for the marketing of insect-based food products and ingredients that go beyond conventionally flavored snacks (Melgar-Lalanne et al., 2019). Kinyuru et al. (2021) developed a nutrient-dense cereal-cricket porridge suitable for school feeding programs in Kenya. Evaluation of the porridge among the children showed considerable acceptability, compared to the conventional porridge consumed in the area (Kinyuru et al., 2021).

Homann et al. (2017) reported developing a cricket-based biscuit suited for school feeding programs and testing the biscuits' acceptance among Kenyan schoolchildren. In comparison to a similar biscuit with milk, the results showed that the cricket biscuits had a high level of acceptability and that their organoleptic qualities were above average, though generally lower than those of milk biscuits. The nutritional and sensory properties of wheat buns enhanced with termite flour as a source of protein and micronutrients were reported by Kinyuru et al. (2009) in Kenya. Consumers found the product with a 5% termite meal substitution in place of wheat flour to be acceptable, and it had significantly increased quantities of protein, retinol, riboflavin, iron, and zinc. A different study reported the development of a pre-cooked complementary food (Winfood Classic) based on the extrusion cooking of flour composites containing amaranth grain (71%), maize (10.4%), edible termites (10%), dagaa fish (*Rastrineobola argentea*) (3%), soybean oil (0.6%), and sugar (5%), as a complementary food to combat child malnutrition (Kinyuru et al., 2015). The foods were shelf-stable for 6 months of storage, with neither pathogenic microorganisms nor aflatoxins reported. A complementary weaning food made of amaranth and maize grains and fortified with dagaa fish (3%) and edible termites flour (10%) was evaluated among young children and their mothers in western Kenya; the

result was that the product received a similar acceptability rating to one without a termite enrichment (Konyole et al., 2012).

In Uganda, Akullo et al., (2018b) developed crackers enriched with 5, 10, and 15% of different insect flours (crickets, soldiers, and winged termites) in substitution of wheat flour. Winged termite-enriched crackers were most preferred by consumers, compared favorably with the control. The nutrient content per 100 g increased significantly ($p<0.05$) with increasing insect proportion. The study recommended the utilization of termite flour in the bakery industry. Akullo et al.(2017b) formulated honey spreads enriched with different insect flours; honey spread enriched with soldier termite flour processed by pan-frying was the most preferred. Increased honey substitution with soldier termite, up to 24% increased both the sensory acceptability score and nutrient content.

In Nigeria, Adepoju and Daboh (2013) produced nutritious composite flour by enriching maize and sorghum flours with *C. forda* larvae powder as a source of protein and micronutrients in complementary foods for children. On the other hand, the production of maize flour tortillas enhanced with *T. molitor* larval flour was reported in Mexico (Aguilar-Miranda et al., 2002). Mealworm powder combined with 7% resulted in tortillas with higher protein content (by 2%) and essential amino acids. The product was shown to have excellent consumer acceptance, an enhanced mouthfeel sensation, a better taste, and improved functional qualities for rolling tacos. These illustrations demonstrate the potential for novel insect consumption strategies.

2.8 Antioxidants as a Potential Preservation Method of Edible Insect as Food

Aware of the nutritive value of edible insects and their potential as ingredients in the food industry, there is a need to develop better methods of preservation so as to provide raw materials with functional properties that can be used in a variety of food products. Insects are perishable due to their high levels of moisture, free amino acids, polyunsaturated fatty acids, and nutrient content, as well as their high pH and the number of naturally occurring autolytic enzymes (Guil-Guerrero et al., 2018; Otero et al., 2020; Rumpold & Schlüter,

2013). The biological factors that cause these insects to spoil include oxidation of lipids due to ease of reaction of the polyunsaturated fatty acids with oxygen in the environment, the activity of their intrinsic enzymes, and their metabolic activities.

The physical, organoleptic, and nutritional characteristics of food products are impacted by oxidation, which also modifies lipids and proteins hence lowering the quality of food (Mokhtar et al., 2014). To preserve highly perishable and nutrient-dense foods such as fish and meat, the food industry routinely uses synthetic antioxidants such butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propyl gallate, Ethylenediaminetetraacetic acid (EDTA), vitamins ascorbic acid (AA) and tocopherol ((Brewer, 2011; Rathod et al., 2021; Ribeiro et al., 2022). Antioxidants could be utilized to increase the shelf life of raw insects and the products made from them for use in a variety of food applications. Antioxidants are substances that, when present in small amounts compared to an oxidizable substrate, significantly slow down the oxidation of that substrate (Fereidoon Shahidi & Zhong, 2010).

Lipid oxidation is frequently a major contributor to the deterioration of food quality in fatty foods, resulting in the development of off flavors and odors, a decrease in shelf life, changes in texture and color, and a decrease in nutritional value (Brewer, 2011; Mokhtar et al., 2014). Antioxidants are commonly added to high-fat foods like beef and fish in order to inhibit oxidation and preserve the food. Therefore, the best approach to minimizing food spoilage and maintaining its quality is the addition of antioxidants.

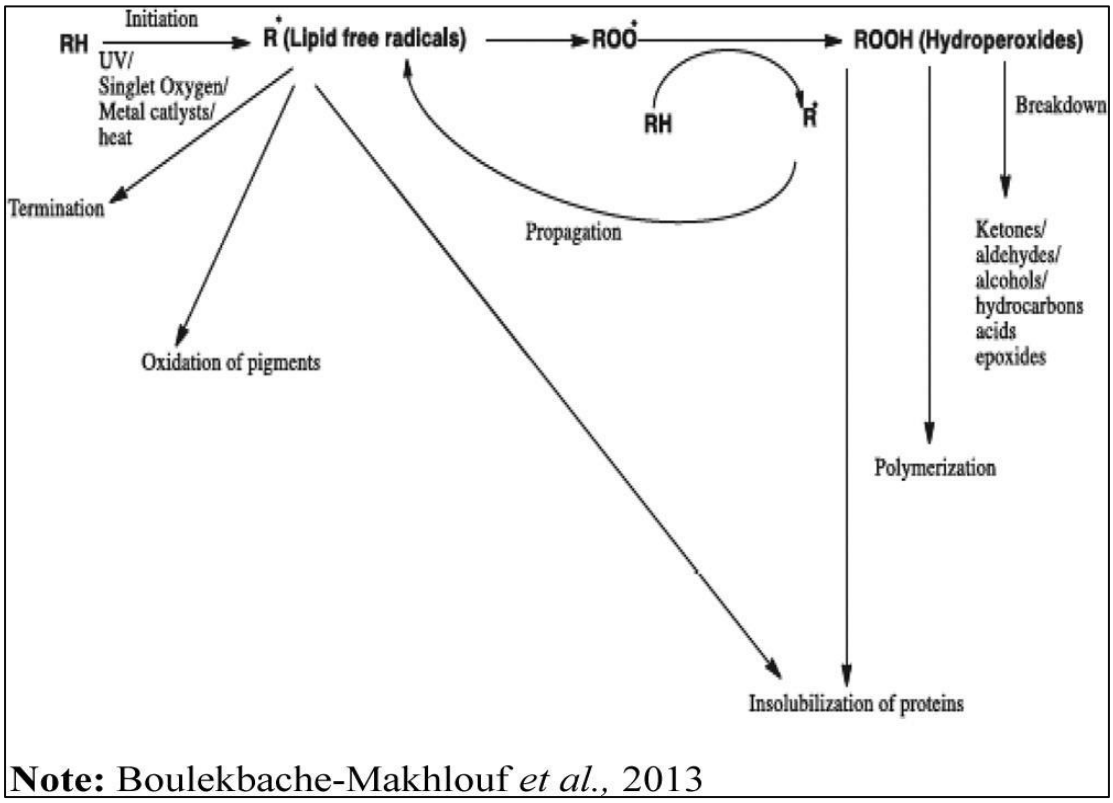
Antioxidants are an essential group of food additives, mainly because of their distinctive properties of extending the shelf-life of food products without any negative effect on the sensory or nutritional qualities. Although synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and tert-butylhydroquinone (TBHQ) are most widely used in foods, some reports have indicated that certain synthetic antioxidants such as BHA and BHT may possess weak carcinogenic effects at high levels (Fereidoon Shahidi & Zhong, 2010). In light of the revelations on the health effects of synthetic antioxidants, current attempts have been to use herbs, spices,

and other plant extracts as natural antioxidants to preserve foods that are highly susceptible to spoilage due to oxidation and microbial degradation (Embuscado, 2015).

Previous studies have already alluded to the use of natural antioxidants in preserving the quality of high-protein and fatty foods such as meat and fish (Cao et al., 2020; Gutiérrez-Del-Río et al., 2021; Hassoun & Emir Çoban, 2017; Medina et al., 2009; Rathod et al., 2021; Ribeiro et al., 2022). However, these investigations have not been conducted on insect-based foods. It is vital to do research to show that the use of natural antioxidants such as spices in insects can result in the production of affordable, convenient, wholesome, and safe food for local communities.

2.8.1 Mechanism of Lipid Peroxidation and Action of Antioxidants

Lipid autoxidation occurs through a free radical chain mechanism involving three separate stages of initiation, propagation, and termination, which results in a number of intricate chemical alterations (Fereidoon & Ying, 2010). A simplified illustration of the autoxidation of lipids is shown in Figure 2.1. The abstraction of a hydrogen atom ($H^{\bullet}$) from an unsaturated fatty acid (RH) forms an alkyl radical ($R^{\bullet}$), which initiates lipid oxidation. Production of the lipid radical is usually initiated by the availability of other radical compounds ($R^{\bullet}$), singlet state oxygen ($^1O_2$), decomposition of hydroperoxides (ROOH), or pigments that act as photosensitizers (Brewer, 2011). In order to stabilize, the alkyl radical ($R^{\bullet}$) usually undergoes a shift in the position of the double bond (cis to trans) and the production of a conjugated diene system. The $R^{\bullet}$ can react with O_2 to form peroxyl radical ($ROO^{\bullet}$). The peroxyl radical can then abstract a hydrogen atom ($H^{\bullet}$) from another unsaturated fatty acid, forming a hydroperoxide (ROOH) and a new, free alkyl radical ($R^{\bullet}$). This reaction may be repeated several thousand times during propagation until no hydrogen source is available or the chain is interrupted (Fereidoon & Ying, 2010).



Note: Boulekbache-Makhlouf *et al.,* 2013

Figure 2.1: A General Pathway for the Autoxidation of Polyunsaturated Lipids

Source: Adapted from (Sardarodiyan & Mohamadi Sani, 2016)

Lipid hydroperoxides (ROOH) are the primary products of lipid oxidation; they are tasteless, odorless, and unstable. When exposed to heat, metal ions, and/or light, they decompose into volatile secondary compounds such as aldehydes, ketones, organic acids, and epoxide polymers, which have a strong and pungent taste. These volatile compounds are responsible for the rancid off-odors and off-tastes in food (Sun et al., 2011)

2.8.2 Mechanism of Action of Antioxidants

Antioxidants are substances or systems that inhibit the formation of free radicals or interrupt their propagation by one or a variety of mechanisms. They may decrease oxygen concentration, intercept singlet oxygen ($^1O_2$), prevent first-chain initiation by scavenging initial radicals such as hydroxyl radicals, bind metal ion catalysts, decompose primary

products of oxidation into non-radical species, and prevent continued hydrogen abstraction from substrates (Huang et al., 2005; Niki, 2010; Hong Wang et al., 1996). Antioxidants act at different levels in the oxidative sequence involving lipid molecules.

Additionally, antioxidant molecules are not all equally powerful in reacting according to these varied mechanisms. For instance, phenolic acids are effective in trapping free radicals but not as good at chelating metals, while flavonoids can scavenge free radicals and chelate metals (Badarinath et al., 2010). As a result of several mechanisms employed by antioxidants, a single assay can use different modes of action (Brewer, 2011). Antioxidants capable of interrupting the free radical chain reaction are usually the most effective (Saeed et al., 2012). They are characterized by aromatic or phenolic rings and act by donating a hydrogen atom to free radicals formed during oxidation; in the process, they transition into a radical form themselves. However, these radical intermediates are stable due to resonance delocalization of the extra electron within the aromatic ring and the subsequent formation of stable quinones (Brewer, 2011).

2.8.3 Plant as Sources of Natural Antioxidants

Most plants contain antioxidants, varying only in quantity; plants use antioxidants to protect themselves from solar radiation and pests and also regulate the production of chemical energy (Shah & Mir, 2021). Fruits, vegetables, herbs, and spices have a high level of phenolic antioxidants, which can be exploited by the food industry since they have been used since ancient times as healthy foods (Santos-Sánchez et al., 2018). Phenolic compounds are the most important group of natural antioxidant compounds, they have strong antioxidant activity and are beneficial effects on human health (Fereidoon Shahidi & Zhong, 2010). Table 2.4 shows the major plant antioxidants in common food sources, which can be extracted for food applications.

Table 2.4: Common Plant Food Antioxidant Sources

Antioxidants	Example	Source

Tocopherols	α, β-, γ- and δ-tocopherols	Seeds, cereal and legume grains, nuts, vegetable oils
Tocotrienols	α-, β-, γ- and δ-tocotrienols.	Palm oil, rice bran oil
Ascorbic acid	Ascorbic acid, ascorbates	Fruits, vegetables
Carotenoids	b-carotene, lycopene, astaxanthin, fucoxanthin	Carrots, tomato, fish/shellfish, marine algae
Phenolics/ polyphenolics	Ferulic acid, quercetin, catechin, resveratrol, cyanidin	Fruits, vegetables, nuts, cereals

Source: Fereidoon Shahidi & Zhong, (2010)

2.8.4 Extraction of Antioxidants

Antioxidants are extracted from foods and medicinal plants using a variety of solvents. The chemical makeup and polarity of the antioxidant chemicals to be extracted determine the suitability of solvents for extraction (Wong & Kitts, 2006). The majority of the antioxidants in plants, including phenolics, flavonoids, and anthocyanins, are hydrosoluble. The polar and medium polar solvents such as; water, ethanol, methanol, propanol, acetone, and their aqueous mixtures are often used for its extraction (Belwal et al., 2016;Nguyen et al., 2016). On the other hand, carotenoids are lipid-soluble antioxidants, and common organic solvents, such as mixtures of hexane with acetone, ethanol or methanol, or mixtures of ethyl acetate with acetone, ethanol, or methanol, have been used for extraction (Strati & Oreopoulou, 2011).

As illustrated in Figure 2.2, a variety of extraction techniques, which may be generally divided into conventional and non-traditional extraction methods, can be employed to extract antioxidants from foods and medicinal plants. The most common conventional extraction techniques include hydrodistilition, maceration, and Soxhlet extraction. These procedures are reasonably straightforward to carry out, but they have the drawback of taking a long time and using a lot of organic solvents, resulting in low extraction yields (Azmir et al., 2013). In addition, long heating process may degrade some of the thermolabile compounds.

Ultrasound, microwave, pressurized liquid, enzyme hydrolysis, supercritical fluids, high hydrostatic pressure, pulsed electric fields, and high voltage electrical discharges have all

been investigated as non-traditional methods to obtain antioxidants from plants in an energy-efficient and economically sustainable way. Xu et al. (2017) provide a summary of the description of the non-conventional techniques including the cost involved, energy efficiency, merits, and drawbacks of using each technique.

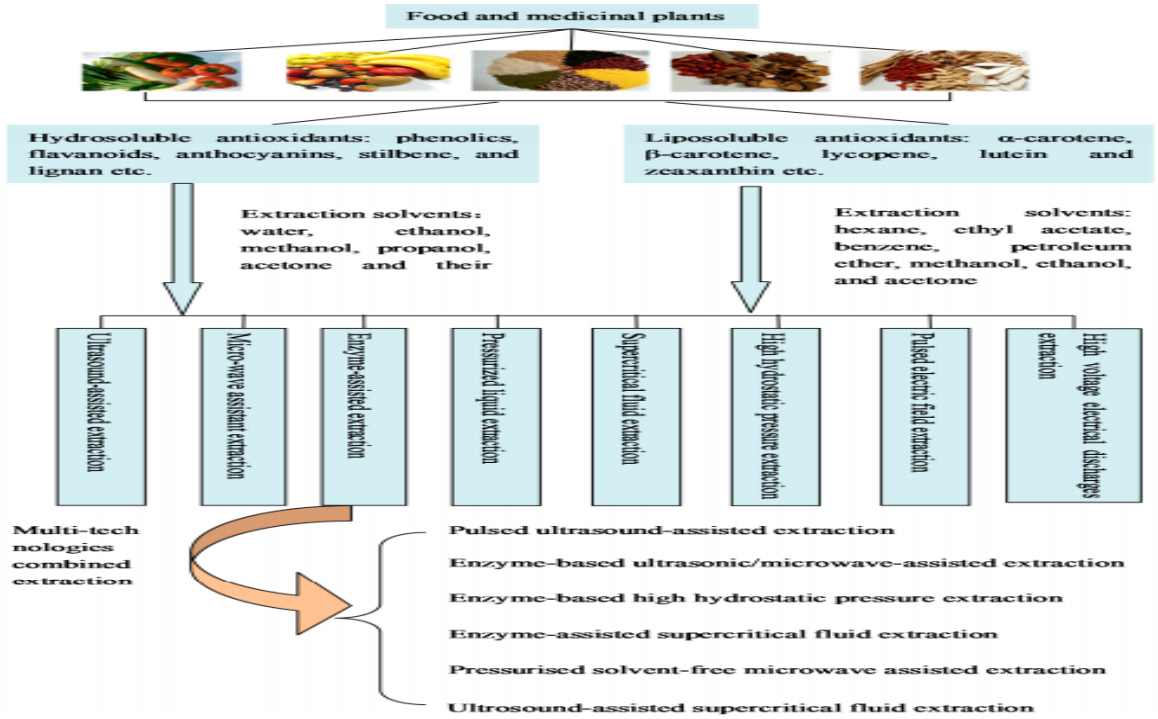


Figure 2.2: Methods of Extraction of Antioxidants from Food and Medicinal Plants

Source: (Xu et al., 2017)

2.8.5 Methods of Evaluating the Antioxidant Capacity of Plant Extracts

Based on the mechanism of action, methods of evaluating the antioxidant activity of plant extracts at a chemical level can be broadly divided into two types: single electron transfer (SET) and hydrogen atom transfer (HAT). SET-based methods measure the ability of an antioxidant to transfer one electron to reduce target charged compounds, such as radicals and metal ions (Antolovich et al., 2002; Huang et al., 2005; Prior et al., 2005). Some of these SET-based assays are based on the capacity to neutralize stable free radicals, such as the Folin-Ciocalteu reagent (FCR) assay, the diphenylpicrylhydrazyl (DPPH) assay, and the Trolox equivalence antioxidant capacity (TEAC), or are based on the capacity to neutralize metal ions, such as the ferric ion reducing antioxidant power (FRAP) and the cupric reducing antioxidant (CUPRAC).

HAT-based assays determine the ability of an antioxidant to quench free radicals by hydrogen donation, which is more relevant to the radical chain-breaking antioxidant capacity. Oxygen radical absorbance capacity (ORAC), total radical trapping antioxidant parameter (TRAP), and preventing the oxidation of low-density lipoprotein (LDL) are examples of HAT-based assays (Prior et al., 2005). To study different aspects of antioxidants, the combination of TEAC, FRA, and FCR methods is used; the total phenol assay by FCR examines the phenolic contents of samples, whereas the TEAC assay assesses the capacity to scavenge free radicals, and the FRAP assay evaluates the reducing capacity of antioxidants (Pulido et al., 2000; Xu et al., 2017).

2.8.6 Herbs and Spices as Natural Antioxidants

It is well known that spices and herbs contain bioactive substances that can inhibit the oxidation of lipids and oils. One of the ways that spices and herbs work to counteract free radicals is by scavenging them and inhibiting them by donating hydrogen atoms (Table 2.5). The antioxidant free radicals that are produced are stabilized by the delocalization of their electrons due to the low activation energy of these antioxidant chemicals, and they

do not easily react to make new free radicals. The structures of antioxidant compounds derived from spices and herbs clearly support the mechanism of action, where the presence of aromatic rings and substituent groups permits the structure to remain stable even after hydrogen atom donation (Embuscado, 2015).

Table 2.5: Antioxidant Compounds in Selected Herbs and Spices and Their Mode of Action

Spice/herb	Scientific name	Antioxidant compounds	Mode of action
Rosemary	*Rosemarinus officinalis*	Carnosol, carnosic acid, rosmanol, rosmadial, diterpenes rosmariquinone, rosmarinic acid	Scavenge superoxide radicals, metal chelator
Sage	*Salvia officinalis L.*	Carnosol, carnosic acid, rosmanol, rosmadial, methyl and ethyl esters of carnosol, rosmarinic acid	Free radical scavenger
Oregano	*Origanum vulgaris*	Rosmarinic acid, caffeic acid, protocatechuic acid, 2-caffeoyloxy- 3-[2-(4-hydroxybenzyl)-4,5- dihydroxy] phenylpropionic acid; flavonoids	Free radical scavenger
Ginger	*Zingiber officinale*	Gingerol, shogaol, zingerone	Free radical scavenger
Turmeric	*Curcuma longa L.*	Curcumins, 4-hydroxycinnamoyl methane	Free radical scavenger
Black pepper	*Piper nigrum L.*	Kaempherol, rhamnetin, quercetin	Free radical scavenger
Chili pepper	*Capsicum frutescence L.*	Capsaicin, capsaicinol	Free radical scavenger
Clove	*Eugenia caryophyllata*	Phenolic acids (gallic acid), flavonol glucosides, phenolic volatile oils (eugenol, acetyl eugenol, isoeugenol), tannins	Free radical scavenger, metal chelator
Cumin	*Cumimum Cyminum*	Cuminal, γ-terpinene, pinocarveol, linalool, 1-methyl-2-(1-methylethyl) benzene, carotol	Free radical scavenger, metal chelator
*Garlic	*Aliuum sativum L*	allicin, diallyl thiosulfonate; alliin, S-allyl-cysteine sulfoxide; ; SAC, S-allyl-cysteine; Z-ajoene	Free radical scavenger

*Source: (Embuscado, 2015) *(Shang et al., 2019)*

Ginger, Garlic and Turmeric as Sources of Natural Antioxidants

Ginger (*Zingiber officinale*) is a tropical flowering plant that was originally cultivated in South Asia but is now widely cultivated the world over (Mustafa et al., 2019). It is classified as a member of the *Zingiberaceae* family that produces a cluster of greenish-purple flowers about three feet tall. Rhizomes of ginger contains 60–70% carbohydrates, 9% protein, 3%–8% crude fiber, 8% ash, 3%–6% fatty acids, and 2%–3% volatile oils, phenolic compounds, and terpenes (Ajanaku et al., 2022). The major phenolic compounds of ginger are gingerol and shogaol, while the terpenes components are zingiberene, beta-bisabolene, alpha-farnesene, beta-sesquiphellandrene, and alpha-curcumene and these compounds are responsible for the bioactivity of ginger (Mao et al., 2019)

Similar to ginger, turmeric (*Curcuma longa*) is a member of the *Zingiberaceae* family and is grown and consumed primarily in tropical and subtropical areas of the world (Ramkumar et al., 2020). Turmeric contains 69.4% carbohydrates, 6.3% protein, 5.1% fat, and 3.5% minerals (Gan et al., 2017). It is a rich source of essential oils, with sesquiterpenes (53%), zingiberene (25%), and other minor components (Ali Ghasemzadeh, 2012). The main phenolic compound in turmeric is curcumin, which makes up 2.5 to 6% of the rhizomes and gives turmeric its characteristic yellow color and therapeutic properties. There is evidence that curcuminoids (curcumin, demethoxycurcumin, and bisdemethoxycurcumin) have antioxidant properties (Guimarães et al., 2020).

Garlic (*Allium sativum L.*) is one of the most popularly grown spices and medicinal plants in the world, and it is among the most extensively researched medicinal plants (Kopeć et al., 2020). It has been utilized among many cultures as an herbal remedy for the treatment and management of cardiovascular and metabolic diseases such as; atherosclerosis, hypertension, dementia, diabetes, cancer, thrombosis among others (Khashan, 2014). The main biologically active compounds of garlic are its organosulfur compounds, which are composed primarily of allicin, allin, and ajoene (Haiping Wang et al., 2015). Allicin is

responsible for the strong, characteristic smell of garlic (Shang et al., 2019). The biological activity of allicin is due to its ability to act as an antioxidant, and the antioxidant potential is known to increase with plant maturity (Lachowicz et al., 2017). A comparison of commercial garlic supplements made from fresh and different types of garlic reveals that those made from aged garlic had higher antioxidant potential (Ajanaku et al., 2022). This is attributed to the difference in the extraction method, which tends to concentrate the stable and highly accessible water-soluble organosulfur compounds.

2.8.7 Antioxidant Activity of Herbs and Spices in Food Application

Dietary herbs and spices have been used as food additives for ages all throughout the world, not only to enhance the sensory qualities of foods but also to increase their shelf life. These additives are regarded as GRAS (generally recognized as safe). Due to the health benefits, natural preservatives are now preferred over synthetic ones, and customer demands have increased interest in non-chemical preservation methods in the food industry. Herbs and spices serve as natural antioxidants and a source of natural antimicrobials in the food preservation process, thereby extending the shelf life of food and maintaining quality (Shan et al., 2009; Shang et al., 2019).

A study on the effect of natural antioxidant treatment on raw beef patties showed that the inclusion of rosemary extract, chitosan, and carnosine, added individually or in combination, reduced lipid oxidation (Mokhtar et al., 2014). In samples where the antioxidants were used in combination, the results were greater compared to the individual addition of antioxidants in inhibiting lipid oxidation, which is suggestive of the synergistic effect of antioxidants. Lower TBARS readings were also noted by Georgantelis et al. (2007) for fresh pork sausage treated with rosemary extract, chitosan, or these mixtures; indicating minimal level of lipid oxidation. The measurement of thiobarbituric acid reactive substances (TBARS) is a valid method for assessing the degree of lipid oxidation in meat and meat products (Fernández et al., 1997).

During chilled storage of pork patties, Volatile Basic Nitrogen (VBN) values, which are indicators of protein degradation during food storage, decreased significantly with the 0.3% procyanidin treatment and increased significantly during storage. While TBARS values were markedly lower in procyanidin-treated meat than in the untreated control (Jeong et al., 2015). Procyanidin is a natural antioxidant that is abundant in grape pomace and seeds.

In another study, treatments of raw pork with extracts from five spices (cinnamon stick, oregano, clove, pomegranate peel, and grape seed) increased the stability of raw pork against lipid oxidation; clove was the most effective for retarding lipid oxidation and presented the highest antioxidant activity in raw pork (Shan et al., 2009). Rababah et al. (2011) examined the impact of natural green tea extract or commercial grape seed extract, in combination with synthetic tert-methylbutylhydroquinone at various concentrations, on the lipid oxidation of ground fresh goat meat held at 5 °C for 9 days. For the raw and cooked meat samples, the TBARS levels varied from 0.21-1.21 and 0.31-4.57 mg MDA/kg meat, respectively. Tert-methylbutylhydroquinone and plant extracts considerably reduced the level of lipid oxidation in goat meat; the addition of tert-methylbutylhydroquinone at a higher concentration was more efficient in reducing lipid oxidation.

2.8.9 Antimicrobial Activity of Herbs and Spices Used in Food

Herbs and spices have antimicrobial effects; they can extend the shelf life of foods by reducing or eliminating the survival of pathogenic bacteria. Shan et al. (2007a) used the agar-well diffusion method to examine the in vitro antibacterial activity of a total of 46 extracts from dietary spices and medicinal plants against five foodborne pathogens (*Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella anatum*). Additionally, their total phenolic contents were assessed, and the results indicated that numerous extracts of herbs and spices had high phenolic content and exhibited antimicrobial activity against foodborne pathogens. The Gram-positive bacteria were more sensitive to the extracts evaluated than the Gram-negative bacteria. The most

vulnerable microorganism was *S. aureus*, whereas the most resistant was *E. coli*. It was revealed that the studied extracts' antibacterial activity was strongly correlated with their phenolic components.

In a study by Witkowska et al. (2013), the antimicrobial activity of 30 different commercially available herbs and spices that are regularly used in the production of ready meals was compared. Different solvent extracts of spices were tested using the microdilution broth method for their antimicrobial activity against *E. coli*, *L. innocua*, *S. aureus*, and *Pseudomonas fluorescens*. All of the microorganisms tested were susceptible to the ethanol and hexane extracts of oregano, clove, sage, rosemary, and celery. Water extracts, on the other hand, had little to no antimicrobial effect. The study demonstrated the effect of solvent and type of spice on the antimicrobial activity for possible use as food additives.

Shan et al. (2009) reported on the inhibitory effects of cinnamon stick, oregano, clove, pomegranate peel, and grape seed extracts on *L. monocytogenes*, *S. aureus* and *Salmonella enterica* in raw pork at room temperature. The population of *L. monocytogenes* in the control significantly increased after storage; after 9 days, samples treated with extracts of spices and herbs contained 0.80-2.24 log CFU/g lower than the control. *S. aureus* and *S. enterica* were present in lower concentrations in the treated samples. An investigation on the effects of spice extracts on the freshness of chicken meat revealed that the incorporation of spice extracts improved the microbiological quality of fresh meat and effectively decreased lipid peroxidation. The addition of spice extracts extended the shelf life of the fresh chicken meat by 15 days; and combining the spice extracts resulted in synergistic effects in meat preservation (Radha Krishnan et al., 2014).

2.9 Effect of Herbs and Spices on the Sensory Quality and Acceptability of Food

Mokhtar et al. (2014) investigated the effect of the addition of rosemary extract, chitosan, and carnosine either separately or together on the color and sensory qualities of raw beef patties at days 0, 1, 3, 6, 9, 12, and 15 of storage. When compared to the control, the

treated and untreated samples had a significantly higher odour, color, taste, and overall acceptability scores, but no significant difference in texture was observed. The antioxidant combinations were superior to individual additions of antioxidants in improving the sensory attributes.

The addition of 200 mg/kg of the essential oils of marjoram and rosemary significantly ($P>0.05$) increased the sensory scores of beef patties during the frozen storage period. Panelists detected a rancid flavor in beef patties formulated without the addition of essential oils during storage, and the flavor scores of beef patties prepared without the addition of essential oils were significantly lower than those of other formulas (Mohamed & Mansour, 2012).

Pork patties treated with procyanidin showed lower lightness and higher redness values than untreated controls, and procyanidin treatment reduced pH values (Jeong et al., 2015). The colour indices of the raw pork samples treated with extracts of cinnamon stick, oregano, clove, pomegranate peel, and grape seed changed slightly during storage, however, the control samples underwent significant changes (Shan et al., 2009).

CHAPTER THREE

PHYTOCHEMICAL PROFILE AND ANTIOXIDANT ACTIVITY OF VARIOUS SOLVENT EXTRACTS OF GINGER *(ZINGIBER OFFICINALE L)*, GARLIC (*ALLIUM SATIVUM L*) AND TURMERIC (*CURCUMA LONGA L*)

Abstract

This study aimed to determine the phytochemical profile and antioxidant activity of two varieties of ginger, garlic and turmeric commonly consumed in Uganda. Fresh ginger rhizomes and garlic cloves of "local" and "hybrid" varieties were acquired from a local food market, washed, grated, and extracted using acetone, ethanol, methanol, and water. Standard techniques were used to determine phytochemical composition. Total phenolic and flavonoid content were assayed using Folin-Ciocalteu and aluminium chloride assays, respectively. Antioxidant activity was determined using the 2,2-Diphenyl-1-picryl hydrazyl (DPPH) assays. Organic solvent extracts exhibited significantly higher total phenolic and flavonoid content compared to the water extracts of the spices ($p<0.05$). The highest total phenolic content, 1968.49 and 2172.65 mg GAE/100 g, and flavonoid content, 254.24 and 184.62 mg QE/100 g were in ethanol and methanol extracts of the local ginger, respectively. Level of Vitamin C and alkaloid was significantly high in aqueous extracts than the organic solvent extracts of ginger, garlic and turmeric ($p<0.05$). Free radical scavenging activity of extracts varied depending on concentration; The half maximal inhibitory concentration ranged from 0.16 to 8.93 mg/ml in local ginger, 4.43 to 6.44 mg/ml in hybrid ginger, and 3.93 to 5.64; 4.44 to 5.27 mg/ml, in local and hybrid garlic; and 0.10-6.98 mg/ml in turmeric, respectively. The best antioxidant activity was exhibited by acetone ectracts of turmeric and ethanol extracts of the local ginger.

Key words: **Phenolic, flavonoids, inhibitory concentration, natural antioxidants**

3.1 Introduction

Herbs and spices have been used for centuries not only to enhance the flavor of food but also to preserve it from spoiling during storage, as well as to treat or prevent human ailments (Dini, 2018; Leja & Czaczyk, 2016). Herbs and spices are often considered safe for human consumption compared to conventional treatment because of their long history of use in food preparation (Mostafa et al., 2018; Sommano et al., 2016). They are utilized in food either in their natural state (whole or ground materials) or as extracts; the use of extracts is more common (Jessica Elizabeth et al., 2017).

Ginger *(Zingiber officinale*) belongs to the Zingiberaceae family. It is grown and used as a spice and medicinal herb in the tropics of Asia, Africa, America, and Australia (Aludatt et al., 2016). More than 150 zingiberaceous species have been identified in the wild and in cultivation (Ujang et al., 2015). Cultivated ginger has a wide range of rhizomes and vegetative features, according to research, and environmental factors have a significant impact on its important bioactive compounds (Kizhakkayil & Sasikumar, 2011).

Garlic (*Allium sativum*) is a perennial bulbous herb belonging to the Alliaceae family. There are two types of garlic: hardneck and softneck (Kshirsagar et al., 2018). The center stalks of hard neck garlic are hard and woody and extend all the way down to the bulb's basal plate. Soft-necked garlic has a non-woody pseudostem made up of overlapping leaf sheaths, and it rarely produces a flower stalk unless the environment is stressful. It is thought that garlic with a soft neck originated from garlic with a hard neck. Kshirsagar et al. (2018) examine the genetic diversity of garlic classification.

Turmeric (*Curcuma longa*) is a rhizomatous herbaceous perennial plant of the ginger family, Zingiberaceae. It is widely grown in the tropical and subtropical regions of the world (Li, 2011). In traditional food preparation, turmeric is used as a spice to impart a characteristic yellow color to the food along with its flavor and taste (Panpatil et al., 2013). The yellow colour of turmeric is contributed by the polyphenolic compound curcumin.

Turmeric is also reported to improve the shelf life of food (Maizura et al., 2011). Spices such as turmeric are rich sources of polyphenolic compounds, which have strong biological activities.

In Uganda, two species of ginger and garlic together with turmeric are extensively used as medicinal plants and condiments in food preparation to improve flavour, aroma and keeping quality (Muhanji, 2009; Safiriyu et al., 2018). "Local" ginger and "hybrid" ginger, as well as "local" garlic and "hybrid" garlic, are terms used by indigenous people (traders and consumers). The term "local" ginger refers to a ginger cultivar with short rhizomes, thick brown skin, yellow flesh/powder, and a pungent scent. The variety with big rhizomes, light yellow skin, off-white flesh/ powder, and a less pungent aroma is referred to as "hybrid" ginger. "Local" garlic, on the other hand, refers to a locally grown type with small bulbs, purple skin, and woody stalks that is typically grown in the coldest parts of the country. While the "hybrid" garlic, also known as "Chinese garlic," has large bulbs, silver white bulb scales, and a tender peel. According to traders, hybrid garlic is not grown in the country but is imported from China and has a longer shelf life. The hard-neck and soft neck classifications of garlic types correspond to the characteristics of local and hybrid garlic varieties, respectively (Kshirsagar et al., 2018). Soft-neck garlic, as opposed to hard-neck garlic, have a higher number of protective shells and thus a longer shelf life (Akan, 2019).

Even though numerous studies have reported on the phytochemicals and antioxidant activities of ginger and garlic and turmeric, there is limited information on the phytochemical profile and antioxidant activity of ginger, and garlic varieties commonly consumed in Uganda, let alone the effect of the extraction solvent on phytochemical composition and antioxidant activity. This could have an impact on its widespread use and industrial applications. As a result, the current study used various solvent extraction systems to investigate the phytochemical composition and antioxidant activities of ginger and garlic cultivars commonly used in Uganda.

3.2 Materials and Methods

3.2.1 Collection and Preparation of Samples

Rhizomes of hybrid and local ginger, cloves of hybrid and local garlic, and turmeric (2.5 kg each) were acquired from Lira City food market in Northern Uganda (2.2581° N, 32.8874° E). In the market, the samples were identified by their morphological and phenotypic characteristics (described in the introduction). The samples were packed in airtight bags, and transported to the Jomo Kenyatta University of Agriculture and Technology (JKUAT, Kenya)-food biochemistry laboratory. Samples were carefully cleaned under tap water, drained and grated for extraction in the laboratory.

3.2.2 Phytochemical Extraction and Sample Preparation for Antioxidant Assays

With minor adjustments, samples were extracted using 99.9% acetone, ethanol, methanol, and distilled water at room temperature (23±2 °C) according to the technique of Kang (2015). Two (2) grams of freshly grated ginger, garlic and turmeric were placed separately in 100 ml amber bottles, containing 30 ml of the solvent; 30 ml of distilled water was also used to make aqueous extracts. Both the organic and aqueous mixtures were shaken for 1 hour at 300 rpm in a mechanical shaker (Labortechnik KS 250b, Germany) and then kept in the dark for 72 hours to avoid the flask contents reacting with light. After that, the extracts were filtered using Whatman filter paper No. 1 and tested for various phytochemical components and antioxidant activity as described below.

3.2.3 Phytochemical Analysis of Ginger, Garlic and Turmeric Extracts

Determination of Total Phenolic Content

The total phenolic content (TPC) of the spice extracts was determined using the Folin-Ciocalteu method, according to previously described procedures (Ezeonu & Ejikeme, 2016). Precisely, 10 mg of gallic acid was dissolved in 100 ml of 50% methanol and diluted to 10, 20, 30, 40, 50, and 60 g/ml. An aliquot (1 ml) from each dilution was

transferred to a test tube and diluted with 10 ml of distilled water. Then 2 ml of Folin Ciocalteu reagent was added, vortexed, and left to incubate for 5 minutes at room temperature. In each test tube, 4 ml of 0.7 M Na_2CO_3 was added, adjusted with distilled water up to the mark of 25 ml, vortexed, and held for 30 minutes at room temperature. The absorbance of the standard was measured against a blank (distilled water) using a UV/VIS spectrophotometer (Shimadzu model UV-1601 PC, Kyoto, Japan). Similarly, 1 ml of the sample extracts were prepared and analyzed using a comparable process. A standard curve was then plotted using garlic acid, and total phenolic contents were calculated using the linear equation on the standard curve. Total phenolic components were calculated as mg of gallic acid equivalent (GAE) per 100 g of ginger, garlic or turmeric extract

Determination of Flavonoid Concentration

Flavonoids were determined using the aluminum chloride colorimetric technique (Chan, 2012). A portion of distilled water (4 ml) and 1 ml of sample extracts were added to a 10 ml volumetric flask; after 3 minutes, 0.3 ml of a 5% sodium nitrite solution was added. Then, 0.3 ml of 10% aluminum chloride was added after 3 minutes. After 5 minutes, 2 ml of 1 M sodium hydroxide was added, and the volume was brought up to 10 ml with distilled water. A UV-Vis spectrophotometer was used to detect absorbance at 415 nm (Shimadzu model UV-1601 PC, Kyoto, Japan). Quantitative determination of total flavonoids was done on the basis of a standard curve of quercetin. The linearity of the calibration curve was achieved between 0 and 1000 µg/ml concentration for quercetin (r^2=0.9965). Total flavonoids were reported in mg quercetin equivalent (QE)/100g of ginger, garlic or turmeric extract.

Determination of Tannin Concentration

Tannins were measured using a modified vanillin-hydrochloric acid method (Broadhurst & Jones, 1978). Catechin hydrate was used to make standards at concentrations of 0, 10, 20, 40, 60, 80, and 100 mg/ml (r^2=.0.9987). Duplicate aliquots of 1 mL of each sample

extract were placed in test tubes, one of which acted as a sample blank; to the blanks, 5 mL of 4% HCl in methanol without the reagent (vanillin) was added. Samples and standards, on the other hand, were treated for 20 minutes with 5 ml of vanillin-HCl reagent (made by mixing equal quantities of 8% HCl in methanol and 1% vanillin in methanol right before use). At 500 nm, the absorbance of the standards, samples, and blanks was measured on A UV-Vis spectrophotometer (Shimadzu model UV-1601 PC, Kyoto, Japan); and tannin content was estimated as percent catechin equivalent (CE) using a standard calibration curve derived from catechin absorbance at various concentrations.

Determination of Ascorbic Acid Concentration

The High performance Liquid Chromatography (HPLC) technique was used to determine the ascorbic acid content of the samples (Stan et al., 2014). The extracts was combined with 10 mL of 0.8% metaphosphoric acid and centrifuged at 10000 rpm for 4 minutes. The supernatant was filtered, and 10 mL of 0.8% metaphosphoric acid was added to it. This was filtered using a 0.45 µl filter and 20 µl injected into the HPLC machine. Shimadzu UV-VIS detector was used for HPLC analysis; at a flow rate of 1.2 ml/min and a wavelength of 266.0 nm, the mobile phase was 0.8 percent metaphosphoric acid. A calibration curve was generated from several concentrations (0, 20, 40, 60, 80, 100 µg/ml) of ascorbic acid standards (r^2=0.9881). Ascorbic acid content was reported in mg of ascorbic acid equivalent (AAE)/100g of ginger or garlic extracts.

Determination of Alkaloid

The alkaloid content was determined using the procedures of Ezeonu & Ejikeme (2016), with some variations. The extract from 2 g of sample was concentrated to one quarter of its original volume in a water bath (50 °C) for 4 hours. Dropwise additions of absolute ammonium hydroxide (10 ml) to the concentration were made until the precipitation was complete. The precipitate was collected, rinsed with dilute (2 M) ammonium hydroxide, and filtered after the solution had settled. After that, the residue was classified as alkaloid

and was dried and weighed. The proportion of alkaloids in the sample was calculated using the formula below.

$$\text{Alkaloid (\%)} = \left(\frac{W3-W2}{W1}\right) * 100$$

Where; *W3*= weight of sample residue before drying; *W2*=weight of residue after drying; *W1*= original sample weight.

Terpenoids and Saponin Screening

The Salkowski test was used to determine terpenoids qualitatively, as per the previously reported technique (Malik et al., 2017). Each extract (5 ml) was carefully combined with 2 ml of 100% chloroform (v/v) and 3 ml of absolute H_2SO_4 to produce a layer. The presence of terpenoids was revealed by the formation of a reddish-brown layer at the point of contact. The basic foam test was used to determine the amount of saponin (Ashour et al., 2019). Each extract (5 ml) was transferred to a test tube and diluted with 5 ml of distilled water; the mixture was firmly agitated for 2 minutes. The presence of foam that lasted at least 15 minutes confirmed its presence.

3.2.4 Antioxidant Assays of Ginger, Garlic and Turmeric Extracts

The antioxidant activity of organic solvent and aqueous extracts of ginger and garlic was determined using free radical scavenging ability; evaluated *in vitro* using the 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical (Sigma-Aldrich, USA) assay, following the procedure of Antolovich et al. (2002), with modifications. DPPH is a stable free radical that generates a deep purple colour in solution. When DPPH accepts an electron from an antioxidant, it becomes colourless or pale yellow. This neutralization process can be measured spectrophotometrically from the changes in absorbance at 517nm. In a study by Chen et al. (2013), the DPPH assays of spice extracts showed a significant correlation to other methods of antioxidant evaluation, thereby demonstrating its reliability. Different concentrations of 0, 1, 2, 4, 6, 8, and 10 mg/ml was made by diluting the extracts in

methanol in methanol (analytical grade), and vitamin C was utilized as an antioxidant standard. In a test tube, 1 ml of the extract was added to 3 ml of methanol, followed by 0.5 ml of 1 m M DPPH in methanol. A control solution was made with the same amount of methanol and DPPH. Methanol was used to zero the spectrophotometer, and the absorbance was read at 517 nm after 5 minutes. The radical scavenging activity was calculated using the following formula:

$$\% \text{ inhibition of DPPH} = \left(\frac{AB-AA}{AB}\right) * 100$$

Where *AB* is the absorption of blank sample and *AA* is the absorption of tested extract solution.

The results were expressed as percentage inhibition of DPPH and minimum inhibitory concentrations (IC50), determined using a linear equation from a plot of percentage inhibition of DPPH versus concentration. The IC50 value is a parameter widely used to measure the antioxidant activity of test samples. It is calculated as the concentration of antioxidants needed to decrease the initial DPPH concentration by 50%. Thus, a lower IC50 value depicts higher antioxidant activity.

3.2.5 Data Analysis

All analysis were done in triplicates, and values were reported as mean ± standard deviation (SD). ANOVA tests were performed using GenStat software to determine significant differences among extracts. Statistical comparisons were done using Bonferroni adjustments at 95% confidence level ($P \leq 0.05$).3.3 Results and Discussion

3.3.1 Total Phenolic, Flavonoid and Tannin Content of Ginger, Garlic and Turmeric Extracts

The phenolic, flavonoid and tannin of content of ginger, garlic and turmeric extracts are shown in Table 3.1. The phenolic content of aqueous and organic solvent extracts of hybrid and local ginger differed significantly ($p<0.05$). The phenolic content of ethanol

extracts of hybrid and local ginger (1595 and 1968 mg GAE/100 g, respectively) were higher than reported for three Malaysian ginger varieties, which had 1053, 1127, and 1276 mg GAE/100 g (Ghasemzadeh et al., 2016; Mustafa et al., 2019). The Total phenolic content (TPC) of organic solvent extracts of ginger in this study was higher than previously reported for acetone and methanol extracts of ginger (1093.4 and 871.5 mg/100 g, respectively) (Aludatt et al., 2016). In the study by Ali et al. (2018), the highest total phenolic content was obtained from the chloroform/methanol extract of fresh ginger rhizomes (60.34 mg GAE/g), when compared to pure chloroform or methanol extracts.

On the other hand, organic solvent extracts of garlic had a higher phenolic content than aqueous extracts; ethanol extracts of hybrid garlic had a much higher phenolic content than local garlic. The phenolic content of garlic extracts in this study (40.87 to 207.2 mg GAE/100 g) was higher than the 42.6 mg GAE/100 g reported in an acidified methanol extract of garlic from Poland (Lachowicz et al., 2017); but lower than a range of 17.17 to 42.53 mg GAE/g reported for 43 garlic cultivars in China (Chen et al., 2013). According to Beato et al. (2011), total phenolic content in garlic varieties ranged from 3.4 to 10.8 mg GAE/ g, with white garlic cultivars and Chinese garlic cultivars having higher total phenolic content than purple garlic cultivars. The findings show how different varieties affect total phenolic content. The presence of OH groups in the chemical structure of phenolic compounds confers antioxidant capabilities. Therefore, the total phenolic content has been used to screen for antioxidant activity in plants (Rachkeeree et al., 2020; Shirin Adel & Prakash, 2010).

Similarly, the phenolic content of the organic solvents (acetone, ethanol, and methanol) of turmeric extracts was significantly higher than the corresponding aqueous extracts ($P<0.05$). The TPC of turmeric-acetone extract (1379.94 GAE/100 g) was the highest among the organic solvent extracts, followed by methanol and ethanol.

Table 3.1: Phenolic, Flavonoid and Tannin Content of Ginger, Garlic and Turmeric Extracts

Spice (Type/variety)	Solvent	Total Phenolic (mg GAE /100g)	Flavonoids (mg QE/100g)	Tannins (mg CE/100g)
Hybrid ginger	Acetone	1217.85 ± 76.31^{b}	168.52 ± 6.02^{bc}	35.08 ± 5.45^{bc}
	Ethanol	1595.98 ± 67.80^{c}	205.86 ± 24.30^{d}	33.13 ± 0.94^{bc}
	Methanol	1322.05 ± 17.74^{b}	148.33 ± 6.36^{b}	55.47 ± 5.84^{d}
	Water	385.13 ± 36.94^{a}	104.50 ± 4.00^{a}	3.69 ± 1.82^{a}
Local ginger	Acetone	1278.85 ± 40.78^{b}	168.52 ± 16.70^{bc}	28.12 ± 1.45^{b}
	Ethanol	1968.49 ± 50.72^{d}	254.24 ± 14.13^{e}	42.36 ± 2.50^{c}
	Methanol	2172.65 ± 13.10^{d}	184.62 ± 10.71^{cd}	69.43 ± 7.80^{e}
	Water	285.66 ± 42.47^{a}	107.18 ± 4.20^{a}	3.788 ± 0.45^{a}
P- value		***<0.001***	***0.002***	***0.002***
Hybrid garlic	Acetone	164.86 ± 26.74^{cde}	75.33 ± 5.84^{b}	7.44 ± 2.19^{ab}
	Ethanol	207.19 ± 12.75^{e}	124.33 ± 3.71^{c}	27.31 ± 3.70^{c}
	Methanol	118.99 ± 12.18^{bc}	64.68 ± 5.57^{b}	16.84 ± 1.63^{abc}
	Water	40.87 ± 2.63^{a}	23.78 ± 1.52^{a}	3.80 ± 1.73^{ab}
Local garlic	Acetone	172.01 ± 16.32^{cde}	64.84 ± 1.93^{b}	6.52 ± 1.07^{ab}
	Ethanol	147.52 ± 13.83^{cd}	88.40 ± 0.69^{b}	22.26 ± 4.45^{c}
	Methanol	191.04 ± 36.60^{de}	84.50 ± 5.90^{b}	17.45 ± 2.19^{bc}
	Water	64.89 ± 3.61^{ab}	26.78 ± 2.72^{a}	3.14 ± 0.78^{a}
P- value		***0.177***	***<.001***	***0.696***
Turmeric	Acetone	1379.94 ± 62.93^{c}	382.66 ± 20.83^{c}	175.86 ± 3.24^{c}
	Ethanol	515.60 ± 37.78^{b}	411.88 ± 29.2^{c}	20.45 ± 3.02^{a}
	Methanol	561.16 ± 8.511^{b}	339.01 ± 18.62^{b}	96.19 ± 0.19^{b}
	Water	307.45 ± 43.91^{a}	58.11 ± 6.44^{a}	71.56 ± 10.31^{b}
P- value	***p***	***< 0.001***	***< 0.001***	***< 0.001***

Values= Mean ± SD (n=3). Values with different superscripts along the column differ significantly for each spice ($p<0.05$).

The values recorded in this study are within the range of those previously reported for ethanol, methanol, and water extracts of turmeric (Nisar et al., 2015). On the contrary, higher values were reported for turmeric extract; 22,790, 17,210, 9,010, 380 mg in GAE/100 g in acetone, ethanol, methanol and water, respectively (Sepahpour et al., 2018); although the trend is similar, being high in acetone extracts and low in water extracts. The variation in total phenolic content is attributed to the extraction method, nature, and concentration of the solvent, as well as the source/nature of the raw material for extraction.

In a previous study, 70% acetone was considered the most efficient solvent for extracting

TPC from a wide range of raw vegetables (Suleiman & Abdallah, 2014). The solubility of phenolic compounds is generally higher in mixtures of aqueous organic solvents compared to the absolute aqueous or organic solvent system (Brglez Mojzer et al., 2016). The high efficiency of acetone in extracting total phenolic content could be due to its ability to prevent protein-polyphenol binding, which forms an insoluble complex in the food matrix (Jakobek, 2015). It is suggested that acetone is able to inhibit the formation of protein-polyphenol complexes during extraction, or possibly breakdown the interaction between the functional groups of polyphenols (-OH) and the carbonyl group of proteins (Hwang & Thi, 2014).

Flavonoid Content

Organic solvent extracts had significantly higher total flavonoid content (TFC) compared to the aqueous ($p<0.05$). The highest TFC was recorded in ethanolic extracts: 254.2 and 205.9 mg QE/100g in local and hybrid ginger; 124.33 and 88.40 mg QE/100 g in hybrid and local garlic, respectively. The TFC were comparable to those found in a variety of spices (Muzolf-Panek & Stuper-Szablewska, 2021). The values observed for ginger (107.2 to 254.2 mg QE/100 g) were lower than previously reported: 379 and 428 mg QE/100 g for ethanol extracts of ginger cultivars in Malaysia (Ghasemzadeh et al., 2016); 655 and 4025 mg QE/100 g for chloroform/methanol and petroleum ether extracts of Sudanese ginger rhizomes (Ali et al., 2018). In a study involving 43 Chinese garlic cultivars, the TFC ranged from 0.15 to 0.60 mg rutin /g (Chen et al., 2013).

In this study the highest TFC was observed in turmeric extracts; with amounts in ethanol and acetone extracts (411.88 and 382.66 mg QE/100 g, respectively) being significantly higher compared to 58.11 mg QE/100 g in water extracts ($p<0.05$). Flavonoid is reported to form the largest proportion (up to 60 %) of dietary polyphenols (González-Vallinas et al., 2013). As a result of its abundant presence in plant materials and good biological functions, flavonoids continue to be investigated as a potential source of drug or food supplements(Brglez Mojzer et al., 2016). Previous reports have presented turmeric as a good source of flavonoids possessing an antioxidant, free radical scavenging ability (Li,

2011). Lower values were reported for turmeric extracts from Malaysia compared to the results of this study (Ali Ghasemzadeh, 2012).

Extracts with high flavonoid content also had a high total phenolic content. Solubility of flavonoids in various solvents is known to vary, and the choice of its extraction solvent is frequently dependent on polarity. Flavonoid content variation could be due to the intrinsic variability in plant materials. High flavonoid content suggests the usefulness of the plant material; since flavonoids have become an essential component in a wide range of nutraceutical, pharmacological, therapeutic, and cosmetic applications (Panche et al., 2016). Also flavonoids are highly effective scavengers of most oxidizing molecules, including singlet oxygen, and various free radicals (Saeed et al., 2012). The antioxidant capacity of flavonoids varies depending on the type of functional group and how it is arranged around the nuclear structure (Dias et al., 2021).

Tannins

Tannin concentration in ginger extracts were significantly different ($p< 0.05$); ranging from 3.79 to 69.43 mg CE/100g in water and methanolic extracts of the local ginger. However, the tannin content of local and hybrid ginger extracts in ethanol, acetone, and aqueous extracts did not differ significantly. Similarly, the tannin content of organic and aqueous extracts of both varieties did not differ significantly. Methanolic extracts of local and hybrid garlic had the highest tannin content (69.43 and 55.47 mg CE/100 g, respectively), whereas water extracts had the lowest. Ethanolic extracts of hybrid and local garlic, had the highest tannin concentration at 22.26 and 27.31 mg CE/100g, respectively. This implies that the most effective solvent for extracting tannins from ginger and garlic are methanol and ethanol, respectively. This is consistent with a recent study which found that tannin concentrations in methanol extracts were significantly greater than in water extracts (Xiang Ng et al., 2020).

On the other hand, tannin content of turmeric extracts was significantly highest in acetone and lowest in the ethanol extracts (175.86 and 20.45 mg CE/100g respectively). Methanol

and water extracts had a non- significant difference in tannin content ($p< 0.05$). This finding is in agreement with previous reports where water extracts of most turmeric varieties contained higher levels of tannins compared to the ethanolic extracts (Tanvir et al., 2017). This implies that turmeric contain high levels of hydrolysable tannins. Tannins are important water-soluble plant secondary metabolites which have been reported to have stringent, antioxidant, anti-inflammatory, and antimicrobial property (Tong et al., 2022). Their strong anti-oxidative action is reflected in the free radical scavenging activity, chelation of transition metals, inhibition of pro-oxidative enzymes and lipid peroxidation (Brglez Mojzer et al., 2016).

Tannin content in this study was lower than previously reported for spices (Ali et al., 2021; Esievo et al., 2020). In earlier reports, ethanol and methanol extracted tannins more effectively than acetone (Shirin Adel & Prakash, 2010). Tannins have hydroxyl groups at various positions in their structure, as well as other functional groups like carboxyl, which are critical for the formation of complexes with macromolecules and proteins, and hence determine their solubility (Bule et al., 2020). Researchers have become more interested in employing tannin-rich plants and plant extracts in the diets of ruminant animals in order to improve the quality of various animal products in recent years (Tong et al., 2022). In several food products, plant tannins have been shown to have antioxidant effects. A study in mutton revealed that tannin supplementation increased the color stability of the longissimus dorsi muscle (LM), with lesser variations in the hue angle in the treatment groups than in the control groups (Luciano et al., 2011).

3.4 Vitamin C, Alkaloid, Saponin and Terpenoids in Ginger, Garlic and Turmeric Extracts

Table 3.2 shows the Vitamin C, alkaloid, saponins and terpenoids content of the spices. Levels of vitamin C varied significantly among ginger and garlic extracts ($p<0.05$); being highest in aqueous extracts (40.80 and 35.24 mg/100 g) of local ginger and hybrid garlic respectively. The high levels of vitamin C in water extracts are expected as it is water soluble vitamin. Ascorbic acid is a polar organic molecule that has many hydroxyl groups

in its structure. For that reason, it is believed that smaller molecules such as water compared to ethanol and other organic solvent achieve equilibrium more easily because the hydroxyl groups exist on vitamin C to establish hydrogen bonds, favoring the increase of its solubility (Ribeiro Neto et al., 2009). Efficient water extraction of phytochemicals is an interesting result due to the general safety of extracts in food applications and the low cost of extraction.

Among the organic solvent extracts, ethanolic extracts of hybrid ginger had higher vitamin C content than local ginger, while methanolic extracts of hybrid garlic had higher vitamin C content than local garlic. This indicates that the vitamin C concentration of extracts is influenced by both the solvent and variety, which is consistent with prior results on the vitamin C content of fruits and vegetables (Galani et al., 2017). Levels of vitamin C in this study were comparable to previous reports; for spice in Ghana (Sheringham Borquaye et al., 2017).

Table 3.2: Vitamin C, Alkaloid, Saponin and Terpenoids of Ginger, Garlic and Turmeric Extracts

Spice/variety	Solvent	Vitamin C (mg AAE/100 g)	Alkaloid (%)	Saponins	Terpenoids
Hybrid ginger	Acetone	8.00 ± 0.13^{a}	12.37 ± 0.89^{c}	++	+
	Ethanol	18.33 ± 0.16^{d}	13.05 ± 1.19^{c}	++	++
	Methanol	15.98 ± 0.07^{b}	9.35 ± 0.66^{ab}	+	++
	Water	40.80 ± 0.12^{f}	13.15 ± 1.09^{c}	++	+
Local ginger	Acetone	7.51 ± 0.07^{a}	9.27 ± 0.01^{ab}	++	+++
	Ethanol	17.25 ± 0.27^{c}	10.37 ± 0.27^{b}	+	+++
	Methanol	15.52 ± 0.63^{b}	8.80 ± 0.95^{a}	+	+++
	Water	38.34 ± 0.75^{e}	12.68 ± 0.27^{c}	+	+++
P value		***<.001***	***0.009***		
Hybrid garlic	Acetone	10.29 ± 0.06^{b}	6.66 ± 0.75^{a}	ND	+++
	Ethanol	13.95 ± 0.11^{d}	11.48 ± 0.29^{c}	ND	+++
	Methanol	16.58 ± 0.08^{f}	10.90 ± 0.27^{bc}	+	+
	Water	35.24 ± 0.05^{h}	13.02 ± 0.60^{d}	+	++
Local garlic	Acetone	9.22 ± 0.11^{a}	6.30 ± 0.74^{a}	+	+++
	Ethanol	13.54 ± 0.67^{c}	10.42 ± 0.36^{b}	+	+
	Methanol	15.65 ± 0.02^{e}	11.00 ± 0.57^{bc}	+	+
	Water	33.65 ± 0.04^{g}	12.67 ± 0.67^{d}	++	+
P value		***<.001***	***0.360***		
Turmeric	Acetone	11.77 ± 0.06^{a}	11.68 ± 1.34^{b}	+	+++
	Ethanol	19.97 ± 0.15^{b}	8.15 ± 0.13^{a}	ND	+++
	Methanol	26.82 ± 1.41^{c}	9.83 ± 0.99^{ab}	+	++
	Water	62.78 ± 0.03^{d}	14.97 ± 0.69^{c}	+	+++
P value		***< 0.001***	***< 0.001***		

Values= Mean ± SD (n=3). Values with different superscripts in the column differ significantly for the variety/spice. Key +: present in low quantity, ++: present in moderate quantity, +++: present in large quantity; ND: Not Detected

The Vitamin C content of aqueous turmeric extracts (62.78 mg /100 g) was higher than both in local and hybrid ginger aqueous extracts (38.34 and 40.80 mg/100 g respectively). Values of 48 mg /100 g was reported for ginger rhizome in India (Manas, 2014), while a range of 4.44 to 31.51 mg/100 g was reported for methanolic extracts of six varieties of garlic produced and harvested under identical conditions (Kopeć et al., 2020). Vitamin C is a potent antioxidant that interacts directly with a wide spectrum of harmful reactive

oxygen species via electron transfer to inhibit the reaction initiated by free radicals; it also contributes in the regeneration of other antioxidants, such as tocopherol, to their functioning state (Manas, 2014; Tanvir et al., 2017).

Alkaloid, Saponins and Terpenoids

The highest alkaloid content (13.05 %) was observed in ethanol extract of the hybrid ginger, which was statistically comparable to aqueous extracts of both varieties and acetone extract of the hybrid ginger. Local and hybrid methanol extracts contained the least amount of alkaloid (8.80 and 9.35%, respectively). Aqueous extracts of garlic had a statistically equivalent high alkaloid quantity; 12.67 and 13.02 % in local and hybrid garlic; alkaloid concentration was least in acetone extracts of local and hybrid ginger (6.30 and 6.66% respectively). In turmeric extracts, the alkaloid content was 8.15% in ethanol and 14.97% in aqueous extracts. Alkaloid concentration was in the range reported for other spices; pepper (13.44%), ginger (11.21%) and 2.54 % in garlic (Otunola et al., 2010); 15.38 and 11.32% in ginger and garlic (Umeh et al., 2021); 0.15 and 0.012 mg AE/g for ginger and garlic (Siddhartha et al., 2017). Variation is attributed to the difference in the method of testing as the previous study employed a spectrophotometric method. Previous screening of phytochemicals in herbs and spices did not detect alkaloids in extracts of ginger (*Z. officinale*) and garlic (*A. sativum*) obtained from Nigeria (Belewu et al., 2009).

Saponins and terpenoids were qualitatively determined, so use on minute, medium and large were description of relative concentrations. Moderate amounts of saponins was detected in the acetone extracts of hybrid and local ginger, and ethanolic and aqueous extracts of the hybrid ginger, however, saponins was not detected in acetone and ethanol extracts of the hybrid garlic. In turmeric, saponins were present in aqueous, acetone and methanol extracts, but not detected in ethanolic extracts. According to Umeh et al. (2021), absolute methanol, ethanol extracts and hot water extracts of ginger contained saponins; organic solvent extracts of garlic contained saponins while the hot water extracts did not contain saponins. This is contrary to previous reports in which moderate quantities were

reported in ginger, while high quantities were reported for garlic extracts (Belewu et al., 2009).

On the other hand, large quantities of terpenoids were present in all the organic and aqueous solvent extracts of the local ginger, while minute quantities were present in the acetone and aqueous extracts of the hybrid ginger. In garlic, acetone extracts of both varieties and ethanol extracts of hybrid garlic contained large quantities of terpenoids; and in turmeric, terpenoids were present in both the aqueous and organic solvent extracts. Umeh et al. (2021) detected terpenoids in both organic solvent and aqueous extracts of ginger and garlic, however, terpenoid concentration was higher in garlic than ginger extracts. In other studies, saponins was not detected in ethanolic and aqueous extracts; where as terpenoids was detected in aqueous extract and not in ethanolic extracts of turmeric (Suman et al., 2018).

Previous research has shown that terpenoids have anticancer, antibacterial, antifungal, antimalarial and anti-inflammatory properties (Ramkumar et al., 2020). Plant saponins and terpenoids are secondary metabolites that are mainly involved in defense mechanisms and are related to the repair of damaged tissues. This qualifies terpenoids as natural antibiotics (Otunola et al., 2010). Saponins are characterized by surface-active foaming properties, bitter taste, and astringency. Tannins are reported to have various health benefits including medicinal and pharmacological applications due to its foaming ability with the production of frothy effect (Marrelli et al., 2016).

3.4.1 Antioxidant Activity of Ginger, Garlic, and Turmeric Solvent Extracts

The results of this study showed that all extracts exhibited free radical scavenging activity in a dose dependent manner from 1-10 mg/ml. The organic extracts of the local ginger had a higher free radical scavenging activity compared to the aqueous extracts (Figure 3.1).

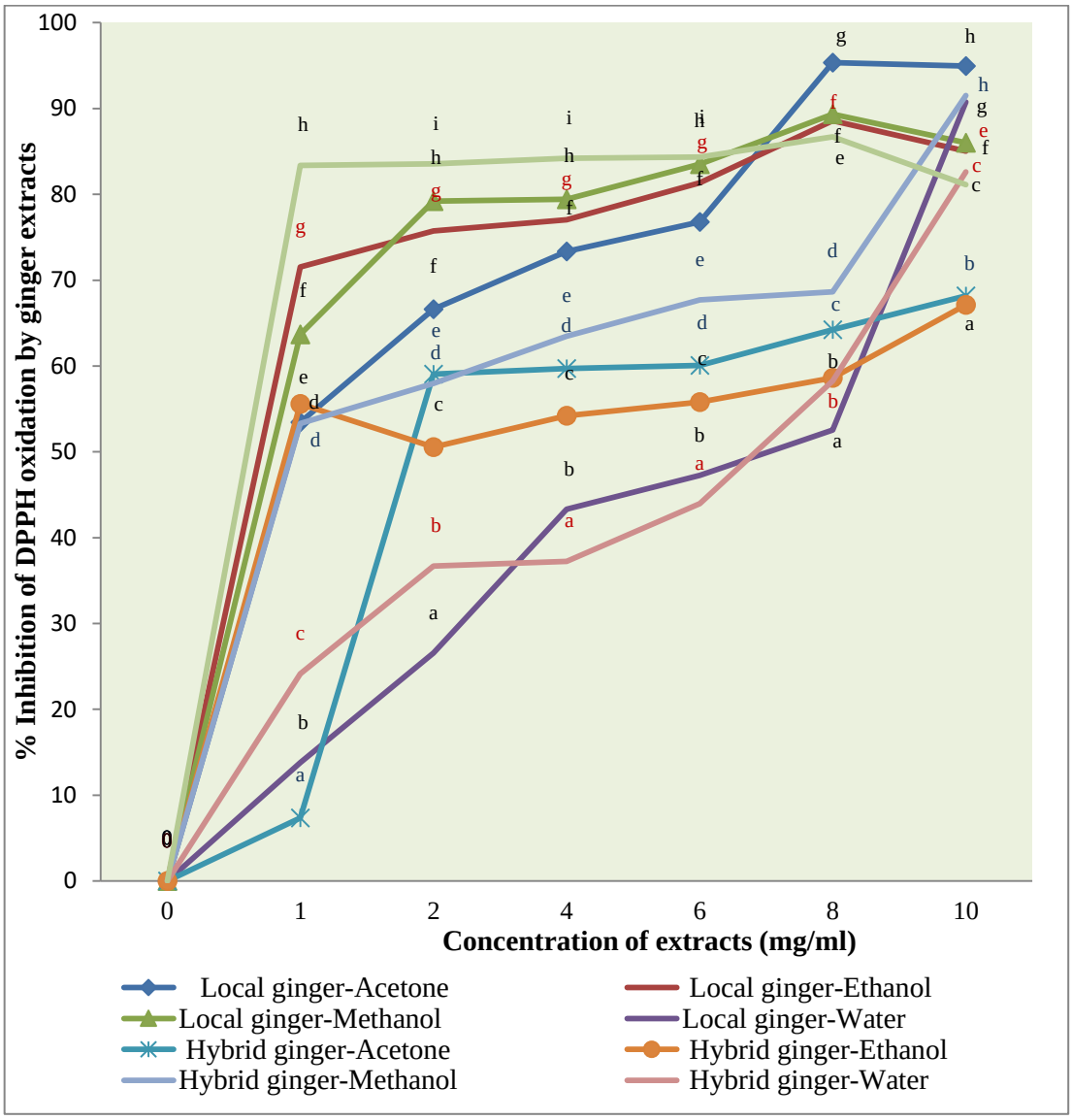


Figure 3. 1: DPPH Free Radical Scavenging Activity of Ginger Extracts

Values plotted is the mean of triplicate determination, different superscripts at the same concentration denote significant difference in inhibition percentage ($P<0.05$)

Ethanol and methanol extracts of the local ginger had the highest 2, 2-diphenyl-1-picryl hydrazyl (DPPH) radical inhibition at 71.54 and 63.7% at 1 mg/ml, and 85.05 and 86.01% at 10 mg/ml, respectively. The inhibition potential of the ethanol and methanol extracts of

the local ginger compared favourably with the standard (vitamin C) across all concentrations; 83.38 at 1 mg/ml and 81.14 % at 10 mg/ml. The organic extracts of the hybrid ginger exhibited moderate inhibition of the DPPH free radical at most concentrations in comparison to the local ginger, while the water extracts of the local and hybrid ginger exhibited the lowest free radical scavenging activity at most concentrations.

The inhibition of ethanol and methanol extracts of hybrid ginger (55.57 and 53.29 %) at 1 mg/ml was lower than that of ethanolic extracts (66.04%) of ginger powder (Mushtaq et al., 2019); and higher than reported (41.7 and 49.7%) for ethanolic extracts of fresh rhizomes of ginger varieties in Malaysia (Ghasemzadeh et al., 2016). Meanwhile, 32.7, 27.0, 28.4, and 11.5% were reported for acetone, ethanol, methanol, and aqueous extracts of torch ginger (Sepahpour et al., 2018). This is consistent with a similar study showing differences in DPPH inhibition and half maximum inhibition concentration (IC50) of various solvent extracts of 16 ginger-like plants (Rachkeeree et al., 2020). This study suggest that the local ginger has a higher free radical scavenging potential when compared to the hybrid ginger and that organic solvent is more efficient in extracting compounds with strong free radical activity when compared to water. Such difference can be attributed to difference in the genetic characteristics of the varieties.

Garlic extracts had significantly lower DPPH free radical scavenging activity than the standard ($P< 0.05$) as shown in Figure 3.2. The free radical scavenging ability of organic extracts were stronger than that of water extracts in both local and hybrid garlic. Ethanol and acetone extracts of the local garlic had a higher inhibition at 1 mg/ml and at 10 mg/ml compared to the hybrid garlic. These results were within the range reported for garlic water-soluble components and garlic oil at concentrations of 1000-5000 g/ml (Dewi et al., 2017); however, they were lower than the 97.95 % reported in aged garlic (Sadrefozalayi et al., 2018). This is due to the nature of the products, which differ in that AGE is made by soaking fresh garlic in aqueous ethanol and maturing it for several months. Our findings are consistent with report on free radical scavenging abilities of different garlic cultivars (Thampi & Jeyadoss, 2015); a range of 3.60% to 45.63% was reported for 43 varieties of garlic (Chen et al., 2013).

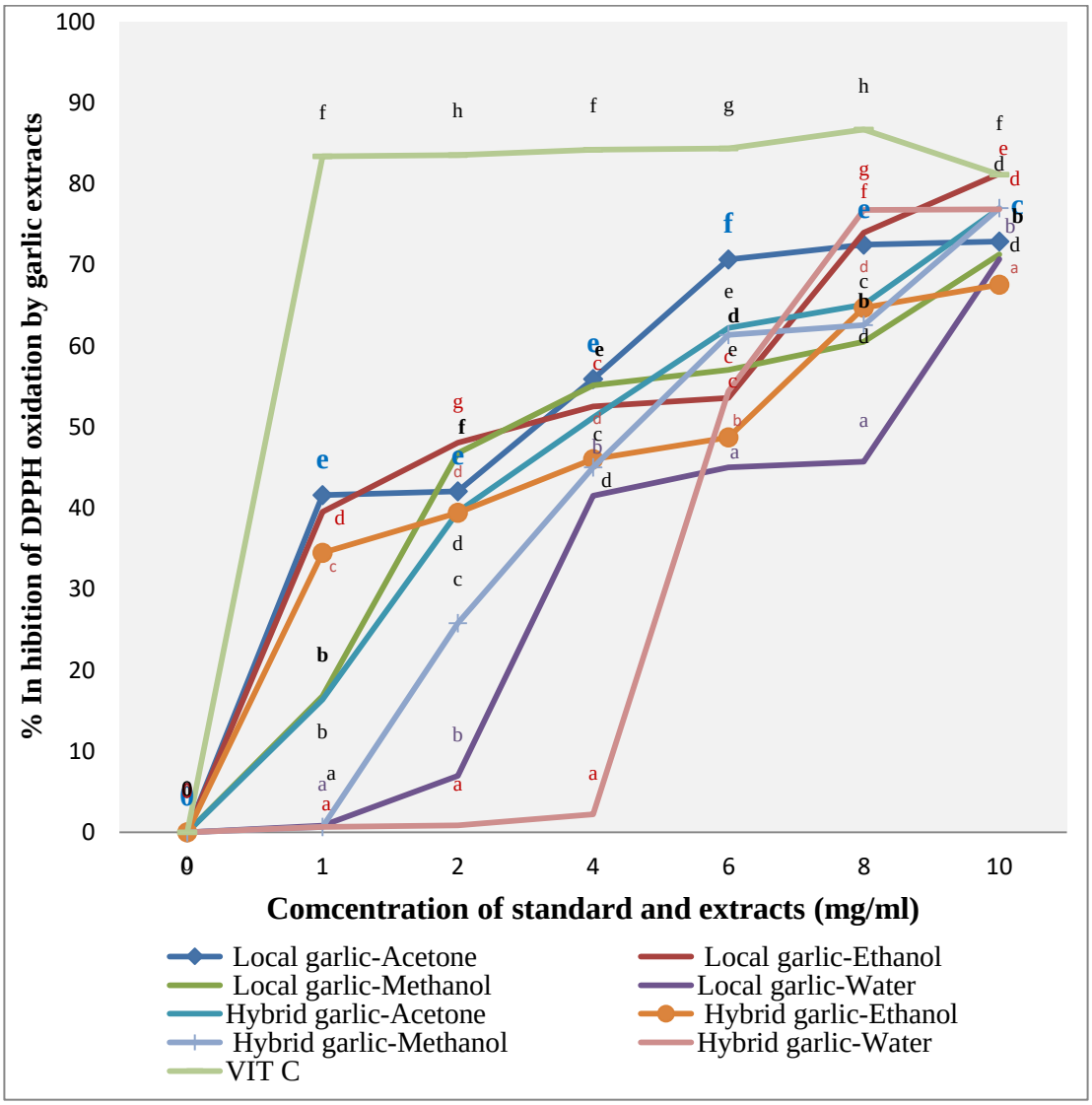


Figure 3.2: DPPH Free Radical Scavenging Ability of Garlic Extracts

Values plotted is the mean of triplicate determination, different superscripts at the same point denote significant difference in inhibition percentage ($P<0.05$).

The DPPH radical scavenging activity of the turmeric extracts also increased in a concentration-dependent manner from 1-10 mg/ml as shown in Figure 3.3. Acetone extract had the significantly highest free radical scavenging ability which compared favorably with Vitamin C; followed by ethanol. This suggests that acetone and ethanol are

more efficient in extracting compounds with strong free radical scavenging activity from turmeric compared to methanol.

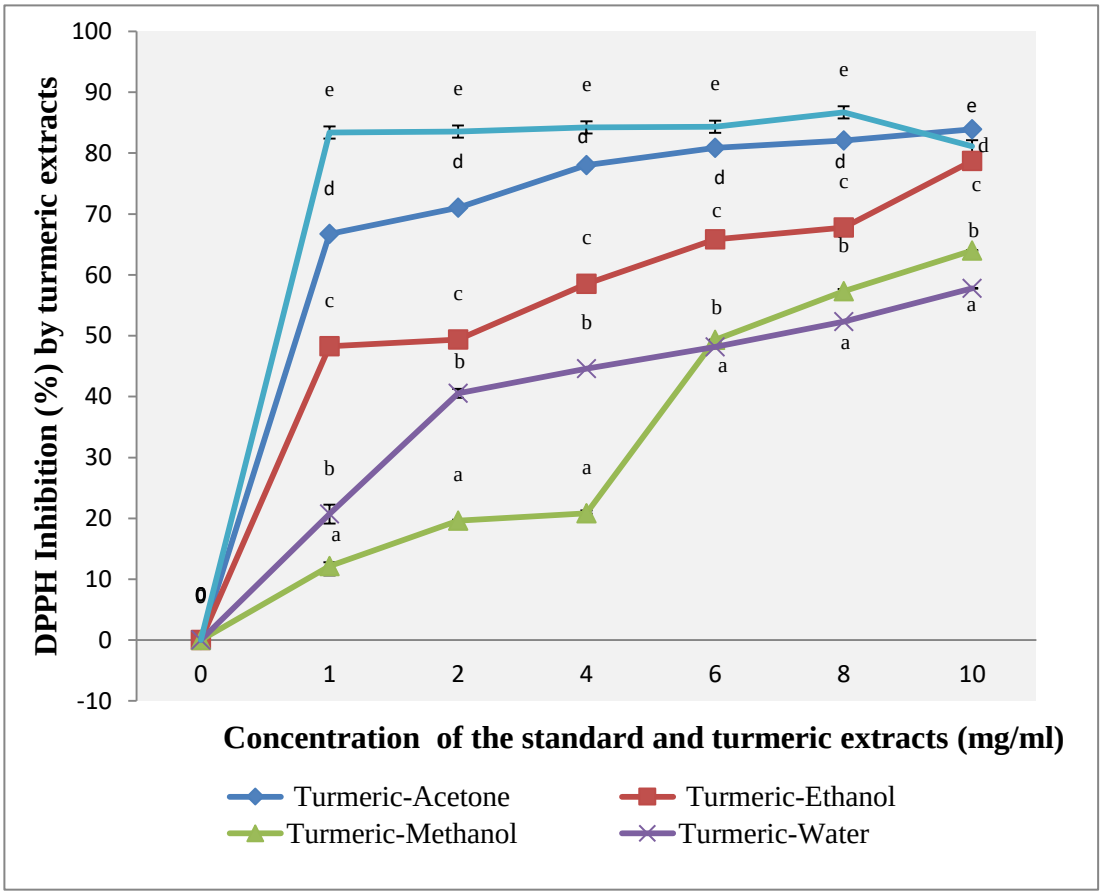


Figure 3.3: DPPH Free Radical Scavenging Ability of Turmeric Extracts

Values plotted is the mean of triplicate determination, different superscripts at the same point denote significant difference in inhibition percentage ($P<0.05$).

3.4.2 IC50 of Ginger, Garlic, and Turmeric Extracts

Free radical scavenging activity is denoted by inhibitory concentration at 50 (IC50), defined as the concentration of the extract required to decrease DPPH free radical absorbance by 50%. A low IC50 value indicates a strong antioxidant activity of the extract. A significant variation ($p<0.05$) existed in the IC50 of ginger, garlic, and turmeric in

organic and aqueous solvents (Table 3.3). IC50 ranged from 0.16 to 0.34 mg/ml in organic extracts of the local ginger and 4.39 to 5.81mg/ml in the hybrid ginger. Non-significantly different low IC50 values were observed among the acetone, ethanol, and methanol extracts of the local ginger. High IC50 values were observed for organic extracts of hybrid ginger, while significantly high values were obtained in the aqueous extracts (8.93 and 6.44 and mg/ml) of both the local and hybrid ginger ($P<0.05$). Other studies report lower IC50 values in ethanolic extracts of fresh ginger rhizomes and dried ginger (65.82 and 14.69 µg/ml respectively) (Mustafa et al., 2019); petroleum ether and chloroform/methanol extracts of ginger rhizomes obtained from Sudan (8.29 and 29.87 µg/ml, respectively (Ali et al., 2018). However, our result is a consistent range of 1C50 values reported for 16 ginger-like plants extracted with different solvents (Rachkeeree et al., 2020).

Table 3.3: IC50 of Ginger, Garlic and Turmeric Solvent Extracts (mg/ml)

	Solvent				
Spice/Variety	**Acetone**	**Ethanol**	**Methanol**	**Water**	**P value**
Hybrid ginger	4.43 ± 0.10^{b}	5.81 ± 0.13^{c}	4.39 ± 0.05^{b}	6.44 ± 0.01^{d}	*0.001*
Local ginger	0.27 ± 0.05^{a}	0.16 ± 0.01^{a}	0.34 ± 0.05^{a}	8.93 ± 0.01^{e}	
Hybrid garlic	4.44 ± 0.019^{c}	4.72 ± 0.02^{e}	4.80 ± 0.06^{e}	5.27 ± 0.02^{f}	*0.001*
Local garlic	3.93 ± 0.013^{a}	4.01 ± 0.028^{b}	4.55 ± 0.031^{d}	5.64 ± 0.05^{g}	
Turmeric	0.10 ± 0.00^{a}	0.17 ± 0.04^{b}	7.25 ± 0.02^{d}	6.98 ± 0.02^{c}	*0.001*

Values= Mean ± SD (n=3). Means with different superscripts along the row differ significantly ($P<0.05$) for each spice

.In garlic extract, IC50 values differed significantly between the organic solvent and aqueous extracts, as well as between local and hybrid varieties ($p<0.05$). Acetone extracts of local and hybrid garlic had the lowest IC50, whereas aqueous extracts of hybrid and local garlic had the highest IC50. The recorded values were within the range of 1.03 to 6.01 mg/ml published for garlic extracts (Bozin et al., 2008); and 4,376 to 8,540 g/ml reported for garlic extracted with various solvents and techniques (Loghmanifar et al., 2020). According to Muzolf-Panek and Stuper-Szablewska (2021), 50% ethanol extracts of spices were more powerful antioxidants than both ethanol alone and water extracts. In *Torilis leptophylla* assays, the half maximal concentration of ethyl acetate fractions was found to be lower than that of methanol and aqueous fractions (62, 189, and 264 g/ml,

respectively) (Saeed et al., 2012); this is in agreement with our results.

Acetone extracts of turmeric had the lowest IC50 comparing favorably with vitamin C (antioxidant standard) (Table 3.3). while methanol and aqueous extracts had the highest IC50. This trend was also reported for most medicinal plant species when acetone extracts were compared to methanol extracts; for instance, the IC50 of *Thymelaea hirsuta* in acetone (0.17mg/ml) was almost 11 times lower than in the methanol (1.90 mg/ml) extracts (Tlili et al., 2019). The free radical scavenging abilities of the ethanolic and aqueous extracts in this study were lower than those reported for ethanolic and aqueous extracts (IC50: 1.08 to 3.03 and 5.31 to 16.55 μg/ml, respectively) of popular turmeric varieties in Bangladesh (Tanvir et al., 2017). Even though the figures are dissimilar, variations in the antioxidant activity of ethanolic and aqueous extracts followed a similar trend, thus indicating the influence of the extraction solvent on the antioxidant properties of extracts.

3.4.3 Relationship between IC50 and Total Phenolic Content Ginger, Garlic, and Turmeric Extracts

Generally, spices extracts with low IC50 had high TPC. However, there was a variation in variety, with methanol and acetone extracts of the hybrid ginger having significantly higher IC50 ($p<0.05$) than ethanol extracts of the local ginger extract, although they had statistically identical total phenolic content as shown in Figure 3.4. The correlation between TPC, TFC, and IC50 of ginger extracts from aqueous and organic solvent extracts of local and hybrid varieties was assessed using Pearson correlation. Results indicate a strong negative correlation between TPC and IC50 ($r=-0.8011$), $p< 0.0001$, TFC and IC50 ($r= -0.6726$), $p<0.005$. This result suggests that the ginger extract contains phytochemical constituents that are capable of donating hydrogen to a free radical to scavenge the potential damage.

The antioxidant capabilities of phenolic compounds are dependent on the presence of OH groups in their chemical structure (Rachkeeree et al., 2020). For instance, when ethanol

extracts of ginger were compared to acetone, methanol, and water extracts of the same sample, ethanol extracts contained the largest amount of chlorogenic acid (Sepahpour et al., 2018). According to Ghasemzadeh et al. (2016), the content of gingerol and shaogoal, which are the main components responsible for antioxidant action in ginger, varied greatly with variety. Moreover, the IC50 of gingerol and shaogoal also differed (Ali et al., 2018).

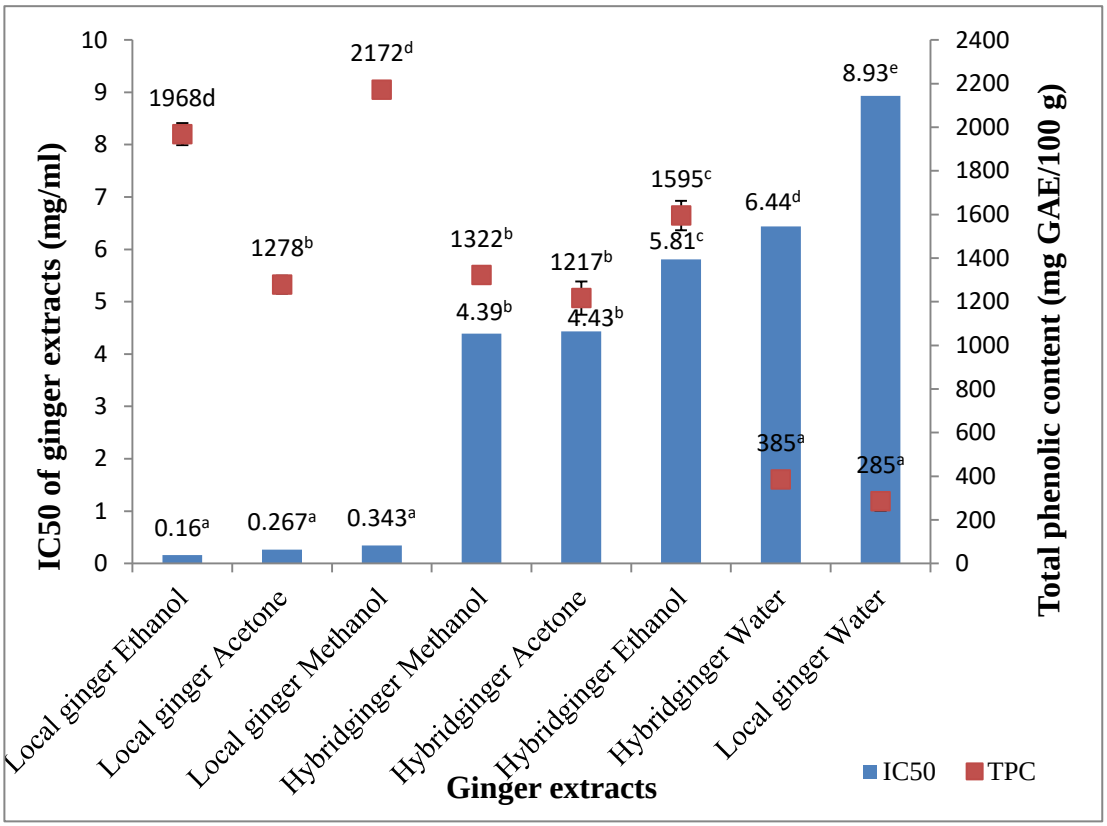


Figure 3.4: Relationship between IC50 and TPC of Ginger Extracts

Values= mean ± SD, (n=3), different superscript show significant difference ($p<0.05$).

The relationship between the IC50 and TPC of garlic extracts is depicted in Figure 3.5. The TPC of organic solvent extracts of the local garlic did not differ significantly, but the IC50 did ($p< 0.05$). Similarly, phenolic content was statistically comparable in methanol extracts of local garlic and ethanolic extracts of hybrid garlic, yet with a variable IC50.

Acetone and water extracts of both varieties also had similar phenolic content with significantly different IC50. Pearson correlation showed that there was a strong negative correlation between the TPC of the extracts and the IC50 ($r = -0.7804$), ($p<0.0001$), TFC and IC50 ($r = 0.5834$), ($p<0.005$). This means that higher TPC and TFC were associated with lower IC50 values, indicating stronger antioxidant potential through the free radical scavenging method. The findings of this study showed that phenolic component levels and antioxidant activity were highly influenced by the solvent and variety.

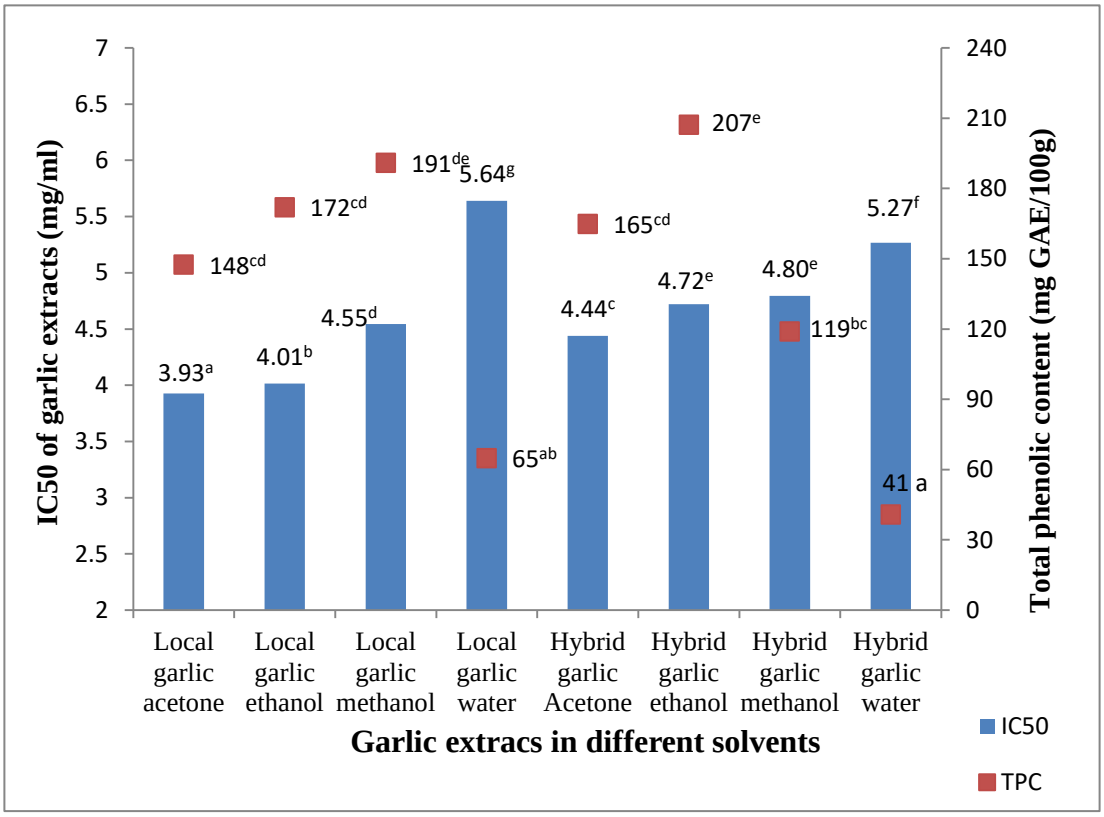


Figure 3.5: Relationship between IC50 and TPC in Garlic Extracts

Values= mean ± SD, (n=3), different superscript show significant difference ($P<0.05$).

This result has previously been observed in a range of spices with varying amounts of specific phenolic compounds (Muzolf-Panek & Stuper-Szablewska, 2021); and different solvents with varying amounts of garlic bioactive components are reported (Cavalcanti et

al., 2021; Loghmanifar et al., 2020). Moreover, the antioxidant activity of garlic components is shown to vary greatly, with each compound exhibiting different patterns of activity as free radical scavenging compounds (Chung, 2006; Dewi et al., 2017; Kopeć et al., 2020).

The relationship between IC50 and TPC of turmeric is shown in Figure 3.6. Higher phenolic content in acetone extracts resulted in a significantly lower IC50, hence higher free DPPH radical scavenging activity. Ethanol extracts had a lower phenolic content, yet a low IC50 (high radical scavenging ability). This is attributed to the effectiveness of ethanol in extracting compounds that possess strong antioxidant activities. A similar trend was observed for ethanolic extracts of turmeric in the DPPH assay, with IC50 of 200 μg/ml (Panpatil et al., 2013).

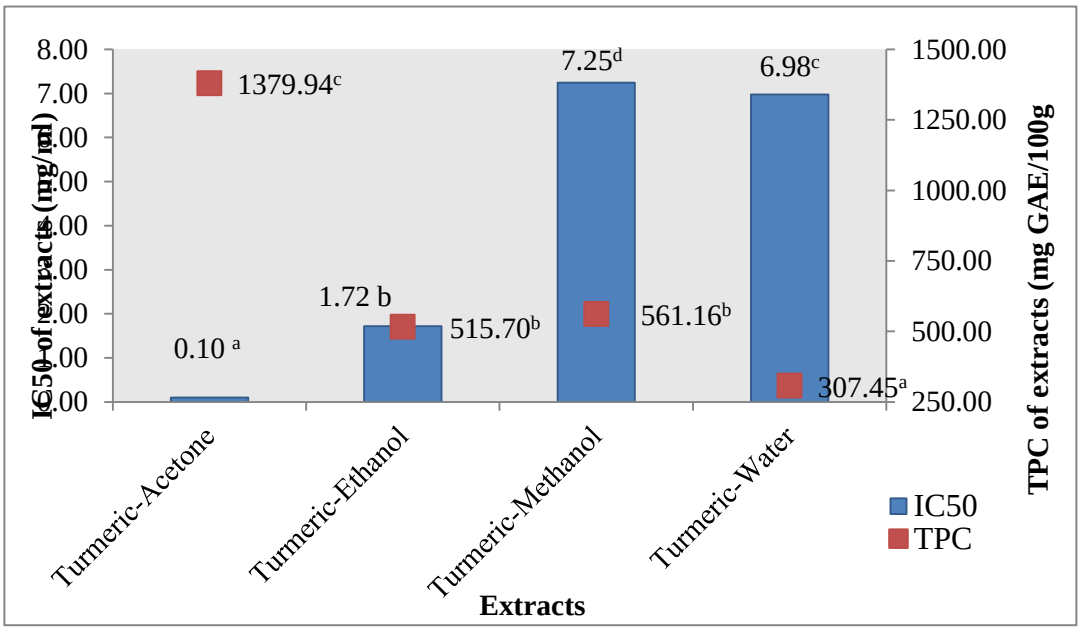


Figure 3.6: Relationship between IC50 and TPC in Turmeric Extracts

Values= mean ± SD, (n=3), different superscript show significant difference ($p<0.05$).

The antioxidant activity of extracts with high phenolic content is attributed to their ability to donate hydrogen atoms or electrons and to capture free radicals; phenolic compounds

in spices and herbs are reported to have significantly contributed to their antioxidant properties (Maizura et al., 2011)

3.5 Conclusion

The phytochemical content and antioxidant activity of the extracts varied with the solvent of extraction. This was attributed to differences in the polarity of the extraction solvent and the solubility of the plant materials in the solvents. Ginger and turmeric extracts had higher total phenolic, flavonoid, and vitamin C content compared to garlic extracts, and the local ginger surpassed the hybrid ginger. The organic solvents were more efficient in extracting the phytochemicals in the spices than water; however, water extracts had higher vitamin content. The antioxidant activity of extracts assayed by DPPH was strongly correlated to the total phenolic and flavonoid content; the strongest antioxidant activity was recorded in the turmeric-acetone extracts and in the acetone and ethanol extracts of the local ginger. The study concluded that the spices examined are good sources of natural antioxidants. Further research is required to identify the bioactive compounds in the extracts and investigate the in vivo efficacy against free radicals to provide the additional information required to promote their use as additives in the food industry.

CHAPTER FOUR

EFFECT OF AQUEOUS AND ORGANIC SOLVENT EXTRACTION ON *IN-VITRO* ANTIMICROBIAL ACTIVITY OF GINGER (*ZINGIBER OFFICINALE*), GARLIC (*ALLIUM SATIVUM L*) AND TURMERIC (CURCUMA LONGA L)

Abstract

The current state of antimicrobial resistance to synthetic antimicrobial drugs and antimicrobial food additives has led to renewed interest in natural antimicrobial compounds. In this study, two varieties (local and hybrid) of ginger and garlic, together with turmeric available in Ugandan, were extracted using acetone, ethanol, methanol and water following standard laboratory procedures. Antimicrobial activity of extracts was investigated using the agar well diffusion method against *Staphylococcus aureus, Escherichia coli,* and *Candida albicans.* The most susceptible microorganism to aqueous and raw extracts of ginger, garlic, and turmeric was *C. albicans*, with inhibition zones ranging from 14.67 to 30.67 mm. However, garlic extracts had a significantly higher inhibition potential against *S. aureus* and *E. coli* compared to ginger and turmeric extracts. Raw juices of garlic exhibited higher antimicrobial activities against *S. aureus, E. coli,* and *C. albicans* (hybrid; 32.67, 33.00, 26.33 and local; 29.67, 29.67, 30.33 mm), respectively. The activity of garlic ethanolic extracts was close to that of the raw extracts against the respective organisms (hybrid: 26.33, 24.33, 25.00, and local: 20.33, 20.33, 27.67 mm). The efficacy of turmeric extracts against *S. aureus* was not significantly different (p>0.05). The minimum inhibitory concentration ranged from 2.5-10 mg/ml in garlic extracts. The study concluded that both varieties of ginger and garlic, and turmeric possess antimicrobial substances, though ginger is more potent as an antifungal agent. The study recommends ethanol extraction for food application basing on efficacy and safety.

Keywords: Antimicrobial activity, Minimum Inhibitory concentration, well diffusion method, pathogens.

4.1 Introduction

Antimicrobial substances of plant origin are secondary metabolites that are produced and used by plants for protection. These secondary metabolites have various uses in medicine and food applications (Tiwari & Rana, 2015). Plant antimicrobial substances have attracted a lot of attention from scientists as ingredients in human drugs (De Zoysa et al., 2019; Maharjan et al., 2012), and of great interest is the control of spoilage and pathogenic microorganisms that cause food borne-infections and intoxications (Sofia et al., 2007; Thongson et al., 2005). This is particularly important due to the current state of antimicrobial resistance encountered in the food chain, which poses a big risk to public health (Bennani et al., 2020; Oniciuc et al., 2019).

Herbs and spices have traditionally been used in many communities to treat a variety of ailments, including as antimicrobial substances (Dini, 2018; Dog, 2006; Leja & Czaczyk, 2016). When compared to conventional antibiotics, herbs and spices are generally regarded as safe for humans, owing to their long term history of use in food preparation. Several herbs and spices are reported to be effective antimicrobials. Among the spices used in the traditional management of diseases are ginger, garlic, and turmeric. Members of the *Zingiberaceae* family, which includes ginger (*Zinger officinale*) and turmeric (*Curcuma longa*), are widely distributed across the tropical and sub-tropical regions of Asia, Africa, America, and Australia, where they are used as a spice and medicinal plant (Ajanaku et al., 2022). In traditional food preparation, ginger is used to impart a pungent aroma to the food, and turmeric is used as a spice to impart a characteristic yellow color to the food along with its flavor and taste (Panpatil et al., 2013). Meanwhile, garlic (*Allium sativum*) is a bulbous perennial herb that belongs to the family *Alliaceae;* a popular remedy for various ailments and physiological disorders. Garlic is basically of two broad types: the hard neck and soft neck (Kshirsagar et al., 2018). Several studies have reported on the antimicrobial activity of ginger and garlic (Akintobi et al., 2013; Indu et al., 2006; Khashan, 2014; Mohammed et al., 2019). There is a wide spectrum of antibacterial activity of ginger rhizomes against a range of Gram negative and Gram positive bacteria (Njobdi et al., 2018; Riaz et al., 2015). However, conflicting reports exist about the

antibacterial effectiveness of ginger against bacteria from different sources (Abdalla & Abdallah, 2018). The antifungal and antiviral activity of ginger is also reported (Gebreyohannes & Gebreyohannes, 2013). Zingiberene in ginger rhizome oils is the most active antibacterial component (El-baky et al., 2010), and the therapeutic effectiveness of garlic is attributed to its oil content and water-soluble organosulfur compounds (Prati et al., 2014). It has been demonstrated that the antimicrobial effects of ginger and garlic are affected by the extraction solvent, method, and concentration of bioactive compounds, respectively (Ali Hasan, 2012; Bakht et al., 2011). It was reported that Chinese garlic demonstrated high potential as ingredient for food applications due to high allicin content (Sommano et al., 2016).

According to Abdalla & Abdallah (2018), the method of plant extraction, antimicrobial assay conditions, genetic variations, bacterial strains, geographic variations, environmental conditions, and physiological factors all have an influence on the phytochemical compounds and hence affect the efficacy of plant extracts. For instance, studies have shown that of the spice extracts evaluated, many exhibited antibacterial activity against foodborne pathogens, with Gram-positive bacteria being more sensitive than Gram-negative bacteria. *S. aureus* was reported as the most sensitive, while *E. coli* was the most resistant (Shan et al., 2007a, 2009).

The antibacterial activity of plant extracts is due to their phenolic content (El-Saber Batiha et al., 2021). Turmeric rhizome extracts from different extraction solvents exhibited strong antibacterial effects against different pathogenic strains of microorganisms (Gupta et al., 2015). The efficacy of turmeric extracts from different solvents has also demonstrated potential for minimizing bacterial food spoilage, fungal related spoilage, and fungal pathogens (Guimarães et al., 2020; Sepahpour et al., 2018). The extraction of bioactive compounds from food and medicinal plants involves the use of several methods, among them the use of organic solvents such as ethanol, acetone, methanol, and petroleum ether (Aludatt et al., 2016; Maizura et al., 2011; Michielin et al., 2009). The suitability of solvents for extraction is based on the chemical nature and polarity of the bioactive components to be extracted (Wong & Kitts, 2006). Groups of compounds such as

polyphenols, flavonoids, and anthocyanins are hydrosoluble. The polar and medium polar solvents, such as water, ethanol, methanol, propanol, acetone, and their aqueous combinations, are commonly used for their extraction (Belwal et al., 2020).

There is limited scientific evidence on the antimicrobial activity of the varieties of ginger, garlic, and turmeric consumed in Uganda, and this may affect their utilization and wide spread industrial application. Additionally, the ability of the different solvents to extract the active ingredients and their influence on the antimicrobial activity of these spices are not well documented. Therefore, this study evaluated the effect of extraction solvents on the antimicrobial activity of two varieties of fresh garlic and ginger as well as turmeric varieties, locally available in Ugandan markets, against common foodborne pathogens (*S. aureus, E. coli,* and *C. albicans*).

4.2 Materials and Methods

4.2.1 Collection of Spices, Processing and Extraction

Two varieties (local and hybrid) of fresh garlic cloves and ginger rhizomes were separately purchased from Lira city market (2.2581° N, 32.8874° E), in Northern Uganda. The market was sub-divided into five (5) zones, and from each zone, 0.5 kg of the local and hybrid variety of fresh ginger and garlic was purchased; hence, a total of 2.5 kg of each variety was procured. The same procedure was used to obtain 2.5 kg of fresh turmeric rhizomes from the same market. The samples were packaged in a cool box and transported to the food biochemistry laboratory at Jomo Kenyatta University of Agriculture and Technology (JKUAT), Kenya. In the laboratory, the respective samples were sorted to remove bad quality, soaked in tap water for 30 minutes, washed, and rinsed under running water. This was followed by the grating and extraction of 25g of sample in 100 ml of appropriate solvent as in section 3.1.2. Raw juices of the spices were made by squeezing out the juice from grated samples

4.2.2 Microbial Culture

Three food borne microorganisms; *S. aureus*, *E. coli,* and *C. albicans* were used to assess the antimicrobial activity of the spice extracts in this study. The microorganisms represented gram-positive bacteria, gram-negative bacteria, and yeasts, respectively. Standard microbial cultures were obtained from the Food Microbiology Laboratory, JKUAT. The microbial solution from the mother culture was streaked using a sterile loop onto selective media plates to ensure the growth of pure colonies; *E. coli* was streaked on Mac-Ckonkey agar; *S. aureus* and *C. albicans* were streaked on nutrient agar. The agar plates were left to dry for about 3-5 minutes, inverted, and incubated overnight at 37°C for 24 hours. Subsequently, a single pure colony was transferred from the streaked plates into separate test tubes containing 10 ml of nutrient broth. The test tubes were then incubated at 37°C for 24 hours to boost the respective microorganisms' growth. The concentration of the actively growing broth cultures was adjusted using standard procedure to obtain a working culture of 10^6cfu/ml. The culturing process was repeated weekly during the study period to obtain viable working cultures.

4.2.3 Evaluation of the Antimicrobial Activity against Bacteria And Yeast

The agar well diffusion method was used to investigate the antimicrobial activity of the crude extracts according to the procedures described (Schumacher et al., 2018). Nutrient agar (NA) was used for the bacteria, and Sabouraud Dextrose Agar (SDA) was used for the yeast. Twenty (20 ml) of the sterile agar media was transferred to the respective sterile petri-dishes and allowed to solidify. Each microbial suspension inoculum (100 µl) was dispensed and evenly spread on the surface of the media in the agar plates. Using a sterile cork borer, four 9 mm wells were cut into the inoculated media in each agar plate, and the bottom of the well was sealed with molten nutrient agar.

In the inoculated plates, 100 µl each of a specific spice extract (25 mg/ml) was dispensed into two wells (treatment); and the same quantity of extract solvent was dispensed in the remaining two wells (negative control). This process was repeated for all the aqueous and

organic solvents of the two varieties on the three test microorganisms in duplicates. Plates were properly labeled to show the media, microorganism inoculated, and spice extract dispensed into the wells; and left to dry for 1 hour prior to aerobic incubation. Plates were then inverted and incubated at 37℃ for 24 hours for bacterial assays and at 25°C for 72 hours for yeast assay. After the incubation period, observed zones of inhibition around the well were measured using a Vernier caliper in mm, and this is the diameter of the growth free zones around the well, which is reported as antimicrobial activity.

4.2.4 Determination of the Minimum Inhibitory Concentration (MIC) of Effective Extracts

The plant extract that exhibited the strongest antimicrobial activity during the screening stage at 25 mg/ml against all the test microorganisms was chosen for the determination of MIC using the agar well diffusion method. Different concentrations (2.5, 5, 10, and 15 mg/ml) of spice extracts were extracted separately using ethanol, methanol, acetone, and water following the previously described methods. Sterile agar plates containing NA were also prepared and inoculated with 100 µl of *S. aureus* and *E. coli* separately, while SDA plates were used for *C. albicans*. As previously described, specific concentrations of the different extracts were added to each well, and the plates were inverted and incubated aerobically. The antimicrobial activity of the different extracts was again assayed by measuring the zone of inhibition in mm for each concentration.

4.2.5 Data Analysis

All measurements were done in triplicates for each microorganism. The values were reported as Mean ± Standard deviation (SD). ANOVA tests were performed using GenStat software to determine significant differences in extracts. Statistical comparisons were separated using Bonferroni adjustments at 5% level of significance ($P \leq 0.05$).

4.3 Results

4.3.1 Antimicrobial Activity of Ginger, Garlic and Turmeric Extracts

The antimicrobial activity of the two varieties of ginger rhizomes and garlic cloves extracted using different solvents is shown in Table 4.1. According to the findings, all extracts of local and hybrid ginger exhibited antimicrobial activity against the tested microorganisms. Susceptibility of the three organisms (*S. aureus, E. coli,* and *C. albicans*) to the solvent extracts differed significantly ($P< 0.05$). The inhibition activity of extracts was generally higher against *C. albicans* in comparison to the bacterial species.

Acetone extracts of the hybrid ginger had the highest activity against *S. aureus* but were not different from methanol, with a Diameter of Inhibition Zone (DIZ) of 16 mm. However, this was lower than the activity of the raw extracts (20.00 mm). There was no significant difference in the antimicrobial activity of ginger extracts against *E. coli*, while *C. albicans* was much more sensitive to the raw extracts (30.67 mm). The antibacterial activities of the local ginger extracts were generally lower than those of the hybrid. Ethanol and acetone extracts exhibited higher (13.67 and 13.00 mm) activity against *S. aureus*. DIZ for methanol, ethanol, and water extracts against *E. coli* was higher than acetone, though not significantly different. Local ginger was highly effective against *C. albicans,* with DIZ ranging from 20.00 to 30.33 mm for methanolic and water extracts, respectively; the activity of the water extracts was higher than that of the raw extracts (29.67 mm), though not statistically different ($P>0.05$).

Table 4.1: Antimicrobial Activity of Ginger and Garlic Extracts (25 mg/ml)

		Inhibition Zones (mm)		
Spice	**Solvent**	***S. aureus***	***E. coli***	***C. albicans***
Hybrid ginger	Raw	20.00 ± 2.00^{c}	13.33 ± 1.15^{b}	30.67 ± 1.16^{d}
	Acetone	16.00 ± 1.00^{b}	11.33 ± 0.58^{a}	18.00 ± 2.00^{b}
	Ethanol	12.00 ± 1.00^{a}	13.00 ± 1.00^{ab}	20.00 ± 2.00^{bc}
	Methanol	15.67 ± 2.08^{b}	13.00 ± 1.00^{ab}	19.33 ± 1.53^{bc}
	Water	12.33 ± 1.52^{a}	12.00 ± 1.00^{ab}	14.67 ± 1.53^{a}
Local ginger	Raw	11.67 ± 0.58^{a}	11.67 ± 0.58^{ab}	29.67 ± 0.58^{d}
	Acetone	13.00 ± 1.00^{a}	11.67 ± 0.58^{ab}	21.67 ± 1.53^{c}
	Ethanol	$13.67 \pm 2.5\ 2^{ab}$	12.00 ± 1.00^{ab}	21.00 ± 2.65^{bc}
	Methanol	12.00 ± 1.00^{a}	12.67 ± 1.52^{ab}	20.67 ± 1.56^{bc}
	Water	11.67 ± 0.58^{a}	12.00 ± 1.00^{ab}	30.33 ± 0.58^{d}
P value		***< 0.001***	***< 0.001***	***< 0.001***
Hybrid Garlic	Raw	32.67 ± 0.58^{e}	33.00 ± 1.00^{e}	26.33 ± 0.58^{bcd}
	Acetone	25.33 ± 1.15^{c}	25.67 ± 0.58^{c}	26.00 ± 1.73^{bcd}
	Ethanol	26.33 ± 2.08^{c}	24.33 ± 1.16^{c}	25.00 ± 1.00^{b}
	Methanol	22.33 ± 0.58^{b}	21.33 ± 1.16^{c}	27.00 ± 1.00^{cd}
	Water	21.33 ± 1.15^{ab}	25.00 ± 1.73^{d}	27.67 ± 0.58^{d}
Local Garlic	Raw	29.67 ± 1.52^{d}	29.67 ± 0.58^{d}	30.33 ± 1.53^{e}
	Acetone	20.67 ± 1.15^{ab}	19.33 ± 2.31^{ab}	20.67 ± 0.58^{a}
	Ethanol	20.33 ± 2.30^{ab}	20.33 ± 1.53^{ab}	27.67 ± 0.58^{d}
	Methanol	19.67 ± 1.52^{a}	18.33 ± 1.53^{a}	19.67 ± 1.16^{a}
	Water	20.33 ± 0.58^{ab}	24.00 ± 1.00^{c}	25.33 ± 0.58^{bc}
P value		***<.0.019***	***<.0.038***	***<.0.001***

Values= Mean ± SD (n=3), including the diameter of the well (9.00 mm).Values with different superscripts in the column differ significantly for the variety and the extraction solvent.

Garlic extracts were very effective against the three microorganisms tested in the study. The susceptibility of the microorganisms to the different solvent extracts of the two varieties of garlic differed significantly (*P<0.05*). DIZ of hybrid garlic extracts ranged from 21.33 to 32.67 mm in aqueous and raw extracts against *S. aureus;* 21.33 to 33.00 mm in methanolic and raw extracts against *E. coli;* and 26.00 to 27.67 mm in ethanolic and water extracts against *C. albicans.* The antibacterial activity of different solvent extracts of local garlic against *S. aureus* did not vary significantly (P>0.05). Water and ethanolic extracts were more effective on *E. coli* (24.00 mm) and *C. albicans* (27.67 mm, respectively). The antimicrobial activity of the raw local garlic was significantly higher

than all the solvent extracts for the three microorganisms, with DIZ of 29.67, 29.67, and 30.33 mm for *S. aureus, E. coli,* and *C. albicans*, respectively. Water and ethanolic extracts of local garlic exhibited significantly higher activity against *E. coli* (24.00 mm) and *C. albicans* (27.67 mm) compared to other extracts. Generally, garlic exhibited effective antimicrobial activity; therefore, it was chosen for the MIC study.

On the other hand, turmeric extracts exhibited varied antimicrobial activity against the tested microorganisms ((Table 4.2); the activity of raw juices against *S. aureus* (17.00 mm) was significantly higher than that of organic solvent extracts (14.33 to 14.67 mm) at 25 mg/ml. Ethanol extract was the most effective against *E. coli,* while water extract had the lowest inhibition zone against *E. coli* and the highest against *C. albicans.*

Table 4.2: Antimicrobial Activity of Turmeric (Curcuma Longa) Solvent Extracts

	Diameter of Inhibition Zone (mm)		
Solvent	***Staphylococcus aureus***	***Escherichia coli***	***Candida albicans***
Raw	17.00 ± 1.00^{b}	16.00 ± 1.00^{b}	17.67 ± 0.58^{a}
Acetone	14.67 ± 1.15^{a}	15.00 ± 1.00^{ab}	19.00 ± 1.00^{a}
Ethanol	14.67 ± 0.58^{a}	18.33 ± 2.08^{c}	25.33 ± 3.51^{b}
Methanol	14.33 ± 0.58^{a}	16.00 ± 1.00^{b}	25.33 ± 0.58^{b}
water	15.67 ± 1.15^{ab}	13.00 ± 1.00^{a}	29.33 ± 1.15^{c}
P value	***0.014***	***0.006***	***<.001***

Values= Mean ± SD (n=3), including the diameter of the well (9.00 mm).Values with different superscripts in the column differ significantly for the extraction solvent ($P < 0.05$).

4.3.3 Minimum Inhibitory Concentration (MIC) of Garlic in Different Solvent Extracts

The MIC of hybrid garlic for different solvent extracts, represented by the DIZ for different concentrations of the solvent extracts, is summarized in Table 3.4. Inhibition against *S. aureus, E. coli,* and *C. albicans* varied significantly (*P< 0.05*); methanol, acetone, and aqueous extracts of hybrid garlic had a MIC of 5.0 mg/ml against *S. aureus,* while ethanolic extracts had a MIC of 10 mg/ml.

Table 4.3: Minimum Inhibitory Concentration (MIC) of Solvent Extracts of Hybrid Garlic

Solvent	Concentration (mg/ml)	Inhibition Zones (mm)		
		S. aureus	***E. coli***	***C. albicans***
Acetone	15	14.33 ± 0.58^{de}	14.33 ± 0.58^{c}	20.67 ± 0.58^{h}
	10	14.44 ± 1.15^{de}	14.67 ± 0.58^{c}	16.67 ± 0.58^{f}
	5.0	13.67 ± 0.58^{cd}	13.67 ± 0.58^{bc}	17.33 ± 0.58^{fg}
	2.5	10.00 ± 0.00^{a}	12.67 ± 0.58^{b}	15.33 ± 0.58^{de}
Ethanol	15	15.67 ± 0.58^{fg}	14.67 ± 0.58^{c}	23.67 ± 0.58^{j}
	10	15.00 ± 0.58^{ef}	13.67 ± 0.58^{bc}	21.33 ± 0.58^{j}
	5.0	10.00 ± 0.00^{a}	10.00 ± 0.00^{a}	16.33 ± 0.58^{ef}
	2.5	10.00 ± 0.00^{a}	10.00 ± 0.00^{a}	10.00 ± 0.00^{a}
Methanol	15	13.67 ± 0.58^{cd}	14.33 ± 0.58^{c}	18.67 ± 0.58^{h}
	10	11.33 ± 0.58^{b}	13.67 ± 0.58^{c}	17.33 ± 0.58^{fg}
	5.0	11.33 ± 0.58^{b}	10.00 ± 0.00^{a}	14.67 ± 1.15^{cd}
	2.5	10.00 ± 0.00^{a}	10.00 ± 0.00^{a}	13.67 ± 0.58^{c}
Water	15	16.33 ± 0.58^{g}	16.67 ± 1.15^{d}	18.33 ± 0.58^{gh}
	10	14.33 ± 0.58^{de}	14.00 ± 1.00^{c}	15.33 ± 0.58^{de}
	5.0	13.00 ± 0.58^{c}	13.67 ± 0.58^{bc}	12.33 ± 0.58^{b}
	2.5	10.00 ± 0.00^{a}	10.00 ± 0.00^{a}	10.00 ± 0.00^{a}
P value		***0.001***	***0.001***	***0.001***

Values= Mean ± SD (n=3), including diameter of the well (9.00 mm). Different superscripts in the column differ significantly for the solvent. DIZ ≤ 10.00 mm is considered no activity

The hybrid garlic extracts of acetone and water were effective against *E. coli* at concentrations of 2.5 and 50 mg/ml, respectively; however, there was no inhibition by ethanol or methanol extracts at the same concentration. The MIC of methanol and acetone extracts of hybrid garlic against *C. albicans* was 2.5 mg/ml and 5.0 mg/ml for aqueous and ethanolic extracts, respectively. The MIC of local garlic in different solvent extracts is presented in Table 4.4 The DIZ of different extracts varied significantly with the concentration; at 2.5 mg/ml, all extracts inhibited the growth of *E. coli* and *C. albicans*.

Table 4.4: Minimum Inhibitory Concentration of Different Solvent Extracts of Local Garlic

Solvent	Concentration (mg/ml)	Inhibition Zones (mm)		
		S. aureus	***E. coli***	***C. albicans***
Acetone	15	16.00 ± 1.00^{fg}	17.67 ± 0.58^{ijk}	18.67 ± 0.58^{c}
	10	15.33 ± 1.53^{ef}	16.67 ± 0.58^{hij}	17.67 ± 0.58^{bc}
	5.0	15.00 ± 1.00^{de}	16.00 ± 1.00^{def}	16.33 ± 0.58^{ab}
	2.5	13.67 ± 0.58^{cd}	15.33 ± 0.58^{bde}	15.33 ± 0.58^{a}
Ethanol	15	20.00 ± 1.00^{i}	18.33 ± 0.58^{k}	25.00 ± 1.00e
	10	17.33 ± 0.58^{h}	17.33 ± 1.15^{ijk}	21.33 ± 1.15^{d}
	5.0	14.33 ± 0.58^{cd}	14.67 ± 1.15^{bc}	18.33 ± 0.58^{c}
	2.5	14.67 ± 0.58^{cd}	13.67 ± 0.58^{ab}	16.33 ± 0.58^{ab}
Methanol	15	15.33 ± 1.15^{ef}	15.00 ± 1.00^{bcd}	18.67 ± 0.58^{c}
	10	13.67 ± 0.58^{cd}	15.00 ± 1.00^{bcd}	16.33 ± 0.58^{ab}
	5.0	10.00 ± 0.00^{a}	14.67 ± 1.53^{bc}	15.33 ± 0.58^{a}
	2.5	10.00 ± 0.00^{a}	14.33 ± 1.15^{abc}	14.67 ± 0.58^{a}
Water	15	16.33 ± 0.58^{gh}	16.33 ± 0.58^{eg}	25.67 ± 1.53^{e}
	10	14.33 ± 0.58^{cd}	15.67 ± 0.58^{de}	22.33 ± 1.53^{d}
	5.0	13.33 ± 0.58^{c}	13.67 ± 0.58^{abc}	21.00 ± 2.65^{d}
	2.5	11.67 ± 0.58^{b}	13.00 ± 0.58^{a}	15.67 ± 0.58^{a}
P value		***0.001***	***0.021***	***0.001***

Values= Mean ± SD (n=3), diameter of the well (9.00 mm). Different superscripts in the column differ significantly for variety and solvent. DIZ ≤ 10.00 mm is considered as no activity.

Generally, the inhibition zones increased with increase in the extract concentration. Higher zones of inhibition for the different extracts at all concentrations were observed against *C. albicans* compared to *S. aureus* and *E. coli.* At a MIC of 2.5 mg/ml, *C. albicans* was highly susceptible, with a DIZ of 14.67, 15.33, 15.67 and 16.33 mm in methanol, acetone, water, and ethanol extracts of the local garlic, respectively. Extracts of water, acetone, and ethanol were effective in suppressing the growth of *S. aureus* at 2.5 mg/ml and methanol extract at 10 mg/ml, respectively. The inhibition effect of acetone extract (15.33 mm) was significantly higher than that of other extracts at 2.5 mg/ml against *E. coli.*

4.4 Discussion

4.3.1 Effect of Solvent Extractions on the Antimicrobial Activity of Ginger Extracts

Hybrid and local ginger exhibited inhibition effects in both aqueous and organic solvent extracts against all the microorganisms tested. This matches previous reports that ginger possesses antimicrobial properties (Abdalla & Abdallah, 2018; Khashan, 2014). The antimicrobial activity could be attributed to the presence of gingerol and shogaol (phenolic compounds), which are active ingredients in ginger (Ali Hasan, 2012). The antimicrobial activity of ginger is reported to depend on the chemical composition, extraction solvent, and method of extraction (Beristain-Bauza et al., 2019; Naji & Jassemi, 2010; Park et al., 2008). This could explain the variations in our results.

The study observed a significant variation in the susceptibility of the three microorganisms tested against the solvent extracts in comparison to the raw ginger juice. Raw, acetone, and methanol extracts of hybrid ginger had very high inhibitory effect at 25 mg/ml, while water and ethanol extracts had a slight inhibition effect on *S.* a*ureus.* This is in line with previous findings: methanolic extracts (16 mm) at 25 mg/ml (Ali Hasan, 2012), and water, and ethanol (9 and 13 mm) at 20 mg/ml (Akintobi et al., 2013). In other studies, ethanol and methanol extracts of ginger were effective against both *E. coli* and *S. aureus* at higher concentrations (Beristain-Bauza et al., 2019). The effectiveness of extracts against *S. aureus* is of great importance in food applications as staphylococcal food borne illness is a serious public health challenge (Kadariya et al., 2014).

It was observed that ethanolic extracts of ginger had antimicrobial effects against some multidrug resistant human pathogens, with the efficacy increasing with concentration; however, the antimicrobial activity of the heated extracts was lower than that of the non-heated extracts (Karuppiah & Rajaram, 2012). This justifies the use of fresh ginger rhizomes and cold extraction for antimicrobial uses, as heat destroys the bioactive compounds in the extracts. In comparison to the organic solvent extracts, the inhibition effect of the aqueous and raw extracts of local ginger was very high against *C. albicans*.

This is consistent with previous research that found that aqueous extract inhibited the growth of *C. albicans* and *E. coli* more effectively than ethanolic extract (Adetunde et al., 2014). These findings need to be investigated further, as the extraction of spices using water is safe, cheap, and could be of interest for industrial food applications.

On the contrary, other than variation resulting from the extraction method and extraction solvent, studies on the diversity of ginger reported that there is variation in rhizomes and a high influence of environmental factors on the content of key compounds (Kizhakkayil & Sasikumar, 2011). This study observed a low inhibitory effect of aqueous and organic solvent extracts of fresh ginger against *E. coli*. Whereas other studies have reported no inhibitory effect of aqueous and ethanolic extracts (Akintobi et al., 2013); and methanolic extracts (25 mg/ml) of fresh ginger against *E. coli* (Ali Hasan, 2012). This could confirm reports that Gram negative microorganisms are more resistant to ginger extracts than Gram positive microorganisms.

The antifungal activities of all extracts were higher than the antibacterial activities of both hybrid and local varieties. This is consistent with previous reports that fungi are more sensitive to compounds in ginger than bacteria (Beristain-Bauza et al., 2019). In another study, the MIC values of ginger oil against *C. albicans* and *Aspergillus niger* were much lower than those for bacteria in the same study (Sharma et al., 2013). The high inhibitory effect observed against *C. albicans* could be attributed to the presence of monoterpene, which is reported to have a wide range of antifungal activity (Ali Hasan, 2012).

4.4.2 Effect of Solvent Extractions on Antimicrobial Inhibition Activity of Garlic Extracts

This study confirmed that garlic possesses a strong antimicrobial potential. Aqueous and organic solvent extracts of hybrid and local garlic exhibited high antimicrobial activity against all the tested micro-organism at a concentration of 25 mg/ml. This is in agreement with previous reports in which garlic exhibited a strong inhibitory effect against 20 different serotypes of *E. coli*, including the enterohemorragic and enterotoxigenic *E. coli*

(Indu et al., 2006). This finding could be of great interest to the food industry as *E. coli* is a major food borne pathogen.

Organic solvent extracts of the hybrid variety were more effective on *S. aureus* compared to the aqueous extracts. Similar findings were reported when white and purple skin garlic cultivars delivered in organic solvents were compared to water- based emulsions (El-Sayed et al., 2017). These results could inform the choice of extracts to be used in food applications. On the other hand, aqueous extracts produced the best inhibitory activity against *E. coli*. In a previous study, water extraction of garlic at low pH produced the best antimicrobial activity compared to ethanol and methanol (Chen et al., 2018). Antimicrobial activity of garlic is due to its ability to destroy the structural integrity of the cell membrane, which is easily achieved at low pH. Moreover, cell-wall structure differentiating gram-positive from gram-negative species was previously suggested to influence the effectiveness of plant extracts (Michielin et al., 2009).

Generally, the inhibition effect of the raw garlic was much higher than the aqueous and organic solvent extracts of the hybrid and local varieties against *S. aureus*, *E. coli*, *and C. albicans.* This may be attributed to the nature of allicin (the active ingredient in garlic) which is reported to be unstable during processing of garlic because of exposure to varying temperatures, pH, light, and extraction medium (Haiping Wang et al., 2015). However, inhibition effect on *C. albicans* was much higher than that on bacteria for the extracts of the two varieties. This is in line with previous studies that investigated the antifungal activity of garlic against *C. albicans* (Suleiman & Abdallah, 2014). High inhibition to fungi is attributed to the activity of allicin, which is known to curb the performance of some enzymes that is important to fungal growth and activity (Muhsin et al., 2001).

4.4.3 Effect of Solvent Extractions on Antimicrobial Inhibition Activity of Turmeric Extracts

It can be envisaged that the use of turmeric extracts in food applications can aid in preventing the growth of food-borne spoilage organisms. These results were in the range

reported in which all organic and aqueous extracts of turmeric effectively inhibited the growth of the clinical *Staphylococcus* strains and standard *S. aureus*; with inhibition zones ranging from 10-20 mm and 10-19 mm at 50 mg/ml for organic and aqueous extracts, respectively (Gupta et al., 2015).

Ethanol extract exhibited significantly high antibacterial activity (18.33 mm) compared to acetone (15.00 mm) and methanol (16.00 mm) against *E. coli*, whereas water extract was partially active. This is in line with previous reports where organic solvent extracts of turmeric were more active: methanol (26 to 28 mm) and ethanol (22 to 24 mm) against *E. coli* compared to aqueous extracts (17 to 18 mm) (Ramkumar et al., 2020). The efficacy of organic solvent extracts is attributed to the ability of the solvent to dissolve organic compounds in turmeric; hence liberating the antimicrobial components for activity. There are reports that phenols, alkaloids, and flavonoids are responsible for the activity. In a previous study, alkaloids inhibited the growth of *S. aureus* and *E. coli* (Ramkumar et al., 2020). Other reports on the activity of turmeric extracts against *E. coli* have suggested that the activity is due to the presence of curcumin and other curcuminiods, which are phenolic compounds (Maharjan et al., 2012).

The effectiveness of turmeric extracts against *E. coli* and *S. aureus* is remarkable for controlling food borne pathogens in the food industry; which are reported to cause several diseases with a significant effect on human health (Gourama, 2020). Additionally, this provides an alternative for handling the current challenge of antimicrobial resistance reported in the food chain (Bennani et al., 2020). Moreover, most Gram-positive bacteria are known to be more resistant to synthetic antibiotics than Gram-negative bacteria (Shan et al., 2007, 2009).

In this study, turmeric extracts exhibited very high activity against *C. albicans.* Water extract had the highest inhibition zone (29.33 mm), followed by ethanol and methanol extracts (25.33 mm). The activity of curcumin, a major component in turmeric, against clinical and standard strains of Candida was previously reported (Khan et al., 2012). The antifungal activity of aqueous extracts is probably due to the anionic components such as

thiocyanate, nitrate, chlorides, and sulphates, along with other water-soluble components, naturally occurring in the plant material. Among the mechanisms suggested for the antifungal effect of spices are cytoplasmic granulation, cytoplasmic membrane rapture, and inactivation and/or inhibition of intracellular and extracellular enzymes (Ramkumar et al., 2020).

4.4.4 Minimum Inhibitory Concentration (MIC) of Garlic Extracts

MIC is the lowest concentration of an antimicrobial agent that inhibits microbial growth in an appropriate medium after incubation (Mostafa et al., 2018). The antimicrobial effects of extracts depended on the concentration. Based on the high inhibition effect of aqueous and organic solvent extracts of garlic at 25 mg/ml, it was appropriate to determine the minimum inhibitory concentration against the tested microorganisms.

The MIC varied significantly among extracts against the tested microorganisms, ranging from 2.5 to 10 mg/ml. Aqueous, acetone, and ethanol extracts of local garlic were effective in suppressing the growth of *S. aureus* at 2.5 mg/ml. At the same concentration, all extracts of local garlic inhibited the growth of *E. coli* and *C. albicans*. Contrary to these, hybrid garlic extracts did not inhibit the growth of *S. aureus* at 2.5 mg/ml. In other studies, MIC of 2 mg/ml was reported for *S. aureus* and *E. coli*, while a range of 0.5-2 and 1-5mg/ml was reported for Gram-positive and Gram-negative bacteria, respectively (Kim et al., 2002). MICs of 0.5-32 and 8.0-64 mg/ml were reported for extracts of white and purple garlic against different strains of streptococci (Groppo et al., 2007), while 5 mg was reported for fresh garlic extract on *E. coli* isolates (Vishal Gaekwad, 2013).

All extracts of local garlic and hybrid garlic extracts in methanol and acetone significantly suppressed the growth of *C. albicans* at 2.5 mg/ml. This is similar to previous reports in which a MIC of 2.5 mg/ml was reported for crude garlic powder against a range of fungi (Suleiman & Abdallah, 2014). Deviation in MIC is attributed to the chemical variation within the different extracts and the concentration of bioactive compounds within extracts due to the nature of the solvents. For instance, chemical characteristics and antimicrobial

activity variation were noted when three different cultivars of Australian-grown garlic were compared (Phan et al., 2019). Moreover, Chinese garlic was reported to have a high potential as an ingredient in food supplement products due to its high alliin content (Sommano et al., 2016).

It is confirmed that allicin, the main antimicrobial component of garlic, varies in concentration between varieties and the extraction/processing and handling of garlic (Cutler & Wilson, 2004; Prati et al., 2014; Shobana et al., 2009). The sensitivity of the microorganisms to aqueous and organic garlic solvent extracts increased with concentration. This trend was observed by other researchers (Indu et al., 2006; Vishal Gaekwad, 2013). Less active, moderately active, and highly active were reported for 10-20, 40-60, and 80-100 mg/ml of garlic extracts on *S. aureus* respectively (Khashan, 2014). Increasing the concentration of the extract also increases the concentration of the antimicrobial compounds in the extracts, resulting in a positive influence on microbial sensitivity.

4.5 Conclusion

Our study indicates that ginger, garlic (local and hybrid) varieties and turmeric possess antimicrobial activities against *S. aureus*, *E. coli*, and *C. albicans*. The antifungal activities of the aqueous and organic solvent extracts were higher than the antibacterial activities, probably due to the bioactives in the spice extracts disrupting the performance of key enzymes that are important to fungal growth and activity. Solvent extracts varied in their antimicrobial effect; raw juices of garlic and ginger were more effective than the aqueous and organic solvent extracts. However, the study recommends ethanol extraction based on its efficacy and safety in food applications. Generally, garlic exhibited high antimicrobial activity compared to ginger and turmeric, although ginger and turmeric were more potent antifungal agents. The MIC of garlic extracts varied from 2.5 to 10 mg/ml, and the inhibition effect was concentration-dependent. Therefore, ginger, garlic, and turmeric consumed in Uganda have promising antimicrobial compounds that can be used in

therapeutic and food applications. However, further research is required to document the bioactive compounds in the spices and enhance their application.

CHAPTER FIVE

OIL CHARACTERISTICS AND LIPID STABILITY OF CRICKET (*GRYLLUS BIMACULATUS*) FLOUR PRESERVED USING GINGER AND GARLIC EXTRACTS

Abstract

The study evaluated the fatty acid composition and oxidative stability of the flour produced from blanched crickets treated with spice extract. Blanched crickets were treated with ginger, garlic, or ginger and garlic mixed extracts, with treatment with sodium benzoate serving as the positive control and distilled water serving as the negative control. Samples were oven dried, milled into flour, and stored at ambient conditions for evaluation on days 0, 30, and 60. The major fatty acids in the flour were palmitic, stearic, oleic, and linoleic. The palmitic acid and stearic acid increased from a range of 24.62 to 25.38% and 8.72 to 8.89% at initial storage to a range of 24.93 to 25.40% and 8.76 to 9.04% at the end of the storage period, respectively. While oleic and linoleic acid decreased in both treated and untreated samples, ranging from 29.75 to 29.01% and 32.85 to 32.38% at day 0 and 29.75 to 29.01% and 32.38 to 32.21% at day 60 of flour storage, respectively. A significant decrease was recorded in the untreated samples ($p<0.05$). The saturated fatty acids increased from 35.12 to 35.25% and 35.28 to 35.78% at day 0 and 60 of flour storage, respectively. While Total unsaturated fatty acids decreased by 0.52, 0.37, 0.36, 0.32, and 0.15% in untreated, garlic, ginger-garlic, ginger, and sodium benzoate-treated cricket flour, respectively. The ratio of polyunsaturated fatty acid to saturated fatty acid varied significantly ($p<0.05$) among the samples, ranging from 0.99 to 0.94. The acid value (AV), peroxide value (PV), and thiobarbituric acid reactive substances (TBARS) of flour all stayed within safe levels. Flour preserved with ginger-garlic mixture showed minimal changes, compared favorably with sodium benzoate treatment.

Key words: blanched cricket, ginger, garlic, fatty acids, peroxide value, TBARs

5.1 Introduction

The Food and Agriculture Organization (FAO) has reported that global food production must rise by 70% by the year 2050 in order to meet the food demand of the increasing global population (FAO, 2017). In this regard, FAO recommends adopting sustainable diets that minimize negative environmental effects while ensuring food and nutrition security for both present and future populations. Due to their sustainability, nutritional value, ease of rearing, low environmental footprint, and animal welfare, eating edible insects is regarded as an effective alternative for achieving this goal (Babarinde et al., 2021; Ishara et al., 2022; Oonincx et al., 2010; Van Huis, 2003; Wegier et al., 2018). It is estimated that at least two billion people consume insects globally, and more than 2000 species of insects are used as food (Jongema, 2017; van Huis et al., 2022).

Among the edible insect species, crickets (*Acheta domesticus* and *Grylus bimaculatus*) are probably one of the most widely farmed insects with modern mass production techniques in many regions of the world (Ayieko et al., 2016; Mitchaothai et al., 2022; Ngonga et al., 2021). Crickets have long been regarded as promising candidates to produce an affordable and sustainable source of protein for human food and animal feed because of their high protein quality and quantity, excellent feed conversion efficiency, prolific breeding habits, short life cycle, and rapid growth, among other factors (Akinyi Orinda et al., 2017; Mitchaothai et al., 2022; Oibiokpa et al., 2018; Tanga et al., 2021). The nutritional value of several edible cricket species has been the subject of numerous kinds of research. According to Magara et al. (2021), *G. bimaculatus* has a protein and lipid content of 57–70 and 15–33 g/100 g dry weight, respectively, and the lipids contain more than 60% unsaturated fatty acids (Gan et al., 2022).

Processing insects into flour is one method for introducing insects as culinary ingredients and enhances the acceptability of insect foods. However, it is critical to know that the use of edible insects in the food industry depends greatly on our ability to comprehend how changes that could occur throughout the processing and storage of flour could affect its nutritional and sensory qualities (Lucas-González et al., 2019). Current research on edible

insects has mainly focused on utilizing them to enrich other foods or extracting components (like protein from crickets) to enhance the nutritional value of other foods (Akullo et al., 2018; Homann et al., 2017; Kinyuru et al., 2021; Kowalski et al., 2022; Osimani et al., 2018). Few studies have highlighted the changes in the fatty acid composition and lipid stability of edible insect products during storage.

One of the major causes of insect food deterioration is oxidative rancidity resulting from the high level of unsaturated fatty acids (Kinyuru, 2021). The lipid oxidation process produces low-molecular off-flavor compounds and leads to the loss of unsaturated fatty acids (Embuscado, 2015; Semeniuc et al., 2016); it is an important reaction that affects the nutritional, sensory, and storage stability of food (Perez-Santaescolastica et al., 2022; Sun et al., 2011). The primary oxidation products, hydroperoxides, are formed and accumulated during the early stages of lipid oxidation. These products are further degraded into secondary oxidation products, such as alcohols, aldehydes, free fatty acids, and ketones, which cause rancidity (Turhan et al., 2009). The rate of lipid oxidation is influenced by a number of variables, including the composition of fatty acids and external factors like heat, light, and enzymes.

Therefore, minimizing oxidation is a crucial step in the processing and storage of food. In order to limit the oxidation process, synthetic antioxidants, particularly butylated hydroxyanisole, butylated hydroxytoluene, and propyl gallate, are widely used (Brewer, 2011). However, due to their toxicity and carcinogenicity, the use of these synthetic antioxidants continues to be limited (Jessica Elizabeth et al., 2017; Ribeiro et al., 2022). Therefore, there is an increasing interest in finding new, natural antioxidants that could replace synthetic preservatives (Gottardi et al., 2016; Maizura et al., 2011; Panpatil et al., 2013). Some plant extracts have been proven to function as potent antioxidants. In Uganda, ginger and garlic are among the spices that are both farmed and consumed, and they serve a variety of food and therapeutic uses. The phenolic chemicals gingerol and shogaol in ginger (Ali et al., 2018; Baliga et al., 2011; Riaz et al., 2015) and allicin in garlic (Loghmanifar et al., 2020; Shang et al., 2019; Shobana et al., 2009) are principally responsible for their antioxidative activity. The utilization of ginger and garlic in the

preservation of meat and fish food products has been reported. However, their use in insect-based foods is not sufficiently studied; moreover, information on the effect of their use on the fatty acid composition and lipid stability of cricket flour has not been attempted. Therefore, this study was undertaken to treat crickets (*G. bimaculatus*) with ginger and garlic extract and examine the changes in the fatty acid profile and lipid stability of the resultant flour during storage.

5.2 Materials and Methods

5.2.1 Spice Selection and Extract Preparation

Following the high antioxidant and antimicrobial activity exhibited by the ethanolic extract of the hybrid variety of ginger and garlic, these were chosen for treatment of cricket for preservation. Turmeric was left out because the ethanol extracts did not have good antibacterial activity and yet bacteria are always a common microflora in food.

Therefore, fresh hybrid ginger rhizomes and garlic cloves were bought from a local market in Uganda and transported to the Jomo Kenyatta University of Agriculture and Technology (JKUAT, Kenya) laboratory for processing and extraction. Samples were cleaned, washed, and rinsed using tap water. The skin was then peeled before being crushed for extraction in accordance with previously established protocols (Tanvir et al., 2017). A quantity of 200 ml of 99.9% ethanol was added to forty (40) g of either ginger, garlic, or a combination of the two in 500 ml conical flasks. The flasks were shaken for 24 hours at 300 rpm on a mechanical shaker (KS 250 basic, Ika Labortechnik-Japan) while being wrapped in aluminum foil to minimize the reaction of their contents to light. Whatman Filter Paper No. 1 was used to filter the solution afterward, and rotary evaporation at 50°C was used to concentrate the filtrate. For the purpose of treating crickets thereafter, the concentrated extracts were reconstituted in distilled water to make 200 ml.

5.2.2 Cricket Acquisition and Preparation

Adult crickets (*Gryllus bimaculatus*) were procured from the insectPro farm in Limuru, Kenya. In order to rid their intestines of the ingested material, crickets were fasted for 48 hours prior to harvest and given only water. The live crickets were transported to the laboratory in aerated boxes, transferred into buckets, and frozen at -20 °C. Frozen crickets were first allowed to thaw at room temperature for two hours prior to being washed three times in tap water in order to remove dirt and other foreign materials according to the procedures of Fröhling et al. (2020). The washing procedure involved swirling the crickets in a large bowl for five minutes at a cricket-to-water ratio of 1:3 (w/v) and then removing the crickets from the water using a sieve.

5.2.3 Treatment and Processing of Cricket Flour

Adequately cleaned crickets were blanched in hot water for 1 minute and then divided into 5 batches of approximately 1000 g each. Three batches were chosen at random, and each was treated with either ginger (C+G), garlic (C+Ga), or a mixture of the two (C+GGa) at a ratio of 1:4 (v/w). The remaining two batches were either treated with 0.1% sodium benzoate (C+SB) as a positive control or distilled water as a negative control (C). The samples were soaked for 30 minutes in the respective solutions, drained of any excess, and then dried for two hours at 105 °C (until crisp dry) in an oven. This drying condition were necessary to rapidly dehydrate and dry the samples prior to cooling to room temperature and grinding into flour using a laboratory grinder; samples were and then sealed in zip-top low-density polyethylene bags (10 µm thickness). The packed samples were analyzed for changes in the fatty acid composition and lipid stability at days 0, 30, and 60 of storage under ambient conditions of room temperature (232°C) and relative humidity (60%). Choice of the assessment period was to determine changes taking place in the flour in an interval of one month.

5.2.4 Extraction of Lipids

Lipid was extracted from 1.0 g of dried cricket flour with chloroform/methanol according to the Folch method (1957) with modifications. One gram of cricket flour was dissolved in 20 parts of a chloroform/methanol mixture (2:1) and shaken on an orbital shaker KJ-201BD for 120 rpm. The mixture was kept in the freezer overnight and then filtered using Whatman filter paper No.1. Subsequently, 4 ml of 0.9% NaCl solution was added to the filtrate, followed by vortexing using the autovortex SA6 for 30 seconds and centrifuging at 30 rpm for 5 minutes. The upper layer was siphoned off and disposed, while the lower layer was transferred into a pre-weighed vial and dried under a stream of nitrogen to a constant weight. The fat content of the flour was determined gravimetrically, and the results were expressed as g lipid per 100 g flour.

5.2.5 Preparation of Fatty Acid Methyl Esters (FAME) and Analysis by Gas Chromatography

Derivatization of the fatty acid was done using basic esterification following the procedures of Wychen et al. (2015). About 10-15 mg of the lipid extract was weighed into Reacti-vial and 2 ml of hexane, followed by 4 ml of 4 mol/L potassium hydroxide/methanol was added. The reacti-vial was capped and incubated for 30 minutes at 50 ℃ with gentle swirling after every 10 minutes. The mixture was cooled to room temperature, and the upper layer was siphoned into clean, labeled centrifuge tubes. Subsequently, 2 ml of distilled water was added, followed by centrifugation at 4000 rpm for 5 minutes. The upper layer was siphoned into a new set of freshly labeled centrifuge tubes containing a 1 mm bed of Sodium sulfate powder. The mixture was centrifuged as previously described, and the FAME was transferred into a clean GC vial, labeled and kept in the freezer, ready for GC analysis.

The components were separated by Gas chromatography on a ZB-FAME, Zebron Capillary column with a length of 30 m, an internal diameter of 25mm, and a film thickness of 0.25 (um), Part No. 7HG-G033-10, fitted with an FID detector at 260 ℃. The

FAME (1 µl) was injected at 240 ℃ in a split injection (50:1). The temperature of the column was kept at 100 °C for 2 min after injection and thereafter increased to 150 °C at a rate of 25°C/ min, followed by an increase of 3 °C/min to 200 °C and held for 2 min. The temperature was then increased at a rate of 8 °C and maintained at 280 °C for 10 min; the total run time was 132 min. Identification of fatty acid methyl esters was performed by comparing the retention time of the standard mixture of fatty acid methyl esters, Sigma-Aldrich CRM47885 (Supleco 37 component FAME mix). The proportion of each fatty acid was calculated as the ratio of each fatty acid to the total fatty acid content, and the result was expressed as a percentage of total fatty acids.

5.2.6 Determination of Acid Value

The acid value of oil or fat is the number of mg of potassium hydroxide required to neutralize the free fat acidity in 1 g of sample. Acid value was determined according to the AOAC method 940.28 (AOAC, 1996). Neutral solvent was prepared by mixing 25 ml of diethyl ether with 25 ml ethanol and 1 ml of 1% phenolphthalein indicator and carefully neutralized using 0.1 M sodium hydroxide/potassium hydroxide solution. About 1 g of each oil sample was dissolved in the neutral solvent and titrated against aqueous 0.1 M solution of sodium hydroxide/potassium hydroxide while shaking until the pink colour that lasted about 15 seconds was obtained.

5.2.7 Determination of Peroxide Value and Thiobarbituric Acid Reactive Substance (TBARS) Assay

The peroxide value was determined according to the IFRA Analytical Method for the determination of peroxide value (IFRA Method, 2019) with some modifications. Weighted portions of 1 g of cricket oil from each treatment and storage period were combined with 10 ml of chloroform. The mixture was immediately shaken to dissolve the oil in the chloroform. To the chloroform-oil mixture, 15 ml of acetic acid and 1 ml of potassium iodide were added, respectively. The mixture was then agitated for a minute and allowed to stand at room temperature in a dark environment for 5 minutes.

Subsequently, 75 ml of distillate water and 1 ml of starch were added to the solution. The mixture was then titrated against standardized 0.01M sodium thiosulphate using starch solution as an indicator until the blue black colour disappeared. The peroxide value was calculated using the formula; Peroxide value

$$PV\ (\frac{mEq}{kg}) = S - B \times N\ Sample\ weight\ (g) \times 1000$$

Where: S = volume of titrant (ml) for sample, B = volume of titrant (ml) for blank, N = normality of $Na_2S_2O_3$ solution (mEq/ml), 1000 = conversion of units (g/kg).

TBARS was measured according to the procedures of Papastergiadis et al. (2012). One (1) gram of oil was weighed in a test tube, to which 5 ml of distilled water was added. The mixture was vortexed for 2 min and centrifuged at 5000 g for 5 min. The aqueous layer was collected, and the procedure was repeated twice. The supernatant was collected and 2.5 ml of the extract was mixed with 2.5 ml of TBA reagent (46 mM in 99% glacial acetic acid) and heated in a boiling water bath for 35 min. The reaction mixture was chilled, and the absorbance was measured at 532 nm using a UV-Vis spectrophotometer (Shimadzu model UV–1601 PC, Kyoto, Japan). For quantification, standard solutions of malondialdehyde (MDA) in 7.5% trichloroacetic acid (TCA) were prepared and used for making calibration curves. The TBARS were expressed as milligrams of malondialdehyde/kilogram.

5.2.9 Data Analysis

Data on the fatty acid composition of the different samples was analyzed using Genstat version 12.0 analytical software. Analysis of Variance (ANOVA) was performed and difference between mean was separated using Bonferroni adjustments at 5% level of significance, and results were reported as means ± standard deviations.

5.3 Results and Discussion

The effect of plant extract preservation on the fatty acid composition of edible insects, particularly cricket-based foods, has only been conducted in a few research studies so far. Prior studies have concentrated on the impact of processing, particularly conventional processing techniques, on the nutrient profile of insect food products. This study compared the fatty acid profile of the flour produced from cricket treated with ginger and garlic extract to the control, which was produced from sodium benzoate treated and the untreated cricket flour.

5.3.1 Fatty Acid Profile of Cricket Flour Preserved With Spice Extracts

The most prevalent saturated fatty acids in the cricket flour were palmitic (C16:0) and stearic acids (C18:0), which constituted 24.62 to 25.40% and 8.70 to 9.04% of the total fatty acids, respectively, in treated and untreated cricket flour (Table 5.1). This is consistent with previous research that has reported on the fatty acid profile of different cricket species (Akullo et al., 2018; Dobermann et al., 2019; Kowalski et al., 2022). Other saturated fatty acids detected in low quantities were lauric (C12:0), myristic (C14:0), pentadecanoic (C15:0), heptadecanoic (C17:0), arachidic (C20:0), and behenic (C22:0). This finding was in agreement with previous reports (Ghosh et al., 2017; Kinyuru, 2021; Raksakantong et al., 2010). Generally, there was an increase in the relative percentages of palmitic acid and stearic acid between day 0 and 60 of flour storage. This was attributed in the decrease in the proportion of the unsaturated fatty acids in the flour during storage.

At the end of the 60-day storage period, the untreated sample had the highest content of stearic acid (9.04%), indicating a significant increase of 0.29% from day 0 to day 60 of storage ($p<0.05$). These results are consistent with a previous study by Gan et al. (2022), which studied how different antioxidants affected the fatty acid composition of deep-fried crickets (*Gryllus bimaculatus*) and discovered that palmitic acid content increased after 60 days of storage in all samples. However, the control group that did not receive the antioxidant treatment showed a greater increase.

The two main unsaturated fatty acids were oleic (C18:1 cis-9) and linoleic acid (C18:2cis-9, 12), which made up 28.82 to 29.75% and 32.07 to 32.93% of the total fatty acid content in cricket flour (Table 5.2, Table 5.3), respectively. Osimani et al. (2018) reported a value of 27.10 and 35.25% of oleic acid and linoleic acid, respectively, in cricket (*Acheta domesticus*) powder; while 33.53% and 30.18% for oleic acid and linoleic acid were reported for thermally dried cricket flour of the same species (Lucas-González et al., 2019). In another study, Khatun et al. (2021) reported 20.95% and 27.14% oleic acid and 30.76% and 32.58% linoleic acid in oven-dried crickets *(A. domesticus* and *G. assimilis).* Linolenic acid (C18:3) in the samples consisted of two isomers: Gamma linolenic acid (C18:3 cis-6, 9, 12) and alpha linolenic acid (C18:3 cis-6, 9, 15), which made up 0.69 to 0.78 and 0.39 to 0.46% of the fatty acids, respectively.

The other omega-3 fatty acid, detected in the sample in small quantities was eicosatrieonic acid (C20:3 cis-11, 14, 17, and C20:3 cis 8, 11, 14). This is consistent with other studies that reported low quantities of omega- 3 fatty acids in insect-based foods (Ghosh et al., 2017; Kinyuru, 2021). All previous reports show similar trends to our results, with slight variations in relative percentages that could have resulted from species differences, processing methods, and geographical location.

At the end of the 60-day storage period, oleic acid decreased by 0.02 to 0.07% in treated samples and 0.93% in untreated samples. The proportions of linoleic acid also decreased from day 0 to day 60 of storage, with 0.17% reduction observed in the untreated samples and 0.04, 0.06, and 0.07% in the garlic, ginger, and ginger-garlic extract treated samples, respectively. The reduction in proportions of both oleic acid and linoleic acid varied significantly with treatment ($p<0.05$). However, the interaction of storage and treatment was not significant ($p>0.05$).

Previous research has shown that the unsaturated fatty acids in cricket flour decrease over time during storage. For instance, Gan et al. (2022) reported a reduction of 2.86% (from 40.29 to 37.43%) of oleic acid after 150 days of storage of deep-fried crickets (*G .bimaculatus*). However, Kim et al. (2016) observed that during a 6-month period of

storage of cricket (*G. bimaculatus*) flour at varied temperatures, the proportions of the key fatty acids (oleic and linoleic) remained unchanged.

Other than insects, it has been noted that the quantities of specific unsaturated fatty acids decreased during the storage of other high-fat foods such as fish (Cyprian et al., 2017); goose meat (Orksuz et al., 2021); peanut (Liu et al., 2019) and Soybean (Prabakaran et al., 2018). Moreover, edible insects are more prone to lipid oxidation due to their increased USFA content. In this study, changes in major fatty acids varied with treatment rather than storage duration. Generally, the sodium benzoate treated samples had a higher content of saturated fatty acids, followed by the spice extract treated samples, when compared with the untreated samples.

Table 5.1: Fatty Acid Profile of Cricket Flour Preserved With Ginger and Garlic Extracts

Treatment	Storage (Days)	C12:0	C14:0	C14:1 cis-9	C15:0	C15:1 cis-10	C16:0	C16:1 cis-9
C+G	0	0.03±0.00^a	0.57±0.00^a	0.02±0.00^a	0.12±0.00^a	0.03±0.00^a	25.06±0.01^a	0.78±0.00^a
	30	0.04±0.00^a	0.59±0.01^a	0.03±0.02^a	0.12±0.00^a	0.02±0.01^a	25.34±0.00^a	0.97±0.01^a
	60	0.03±0.00^a	0.58±0.00^a	0.02±0.00^a	0.13±0.00^a	0.03±0.00^a	25.27±0.07^a	0.84±0.00^a
C+Ga	0	0.04±0.00^a	0.59±0.01^a	0.02±0.00^a	0.12±0.00^a	0.03±0.00^a	25.38±0.01^a	0.97±0.01^a
	30	0.04±0.01^a	0.58±0.00^a	0.02±0.00^a	0.12±0.00^a	0.03±0.00^a	25.31±0.01^a	0.91±0.10^a
	60	0.04±0.00^a	0.57±0.00^a	0.02±0.00^a	0.13±0.00^a	0.03±0.00^a	25.40±0.15^a	0.97±0.00^a
C+Gga	0	0.04±0.00^a	0.57±0.00^a	0.02±0.00^a	0.13±0.00^a	0.03±0.00^a	25.05±0.00^a	0.97±0.00^a
	30	0.05±0.01^a	0.57±0.02^a	0.02±0.00^a	0.12±0.00^a	0.03±0.00^a	25.00±0.04^a	0.77±0.09^a
	60	0.04±0.00^a	0.58±0.00^a	0.02±0.00^a	0.13±0.00^a	0.03±0.00^a	25.28±0.13^a	0.72±0.00^a
C+SB	0	0.03±0.00^a	0.57±0.00^a	0.02±0.00^a	0.12±0.00^a	0.03±0.01^a	24.91±0.16^a	0.80±0.05^a
	30	0.04±0.00^a	0.57±0.01^a	0.02±0.00^a	0.12±0.00^a	0.03±0.00^a	24.86±0.01^a	0.72±0.00^a
	60	0.04±0.00^a	0.57±0.00^a	0.04±0.02^a	0.13±0.00^a	0.03±0.00^a	24.93±0.31^a	0.78±0.07^a
C	0	0.04±0.00^a	0.59±0.04^a	0.02±0.00^a	0.07±0.08^a	0.09±0.07^a	24.62±0.34^a	0.80±0.07^a
	30	0.03±0.00^a	0.58±0.01^a	0.02±0.00^a	0.13±0.00^a	0.04±0.00^a	25.06±0.20^a	0.80±0.06^a
	60	0.050.00^a	0.32±0.31^a	0.03±0.03^a	0.07±0.07^a	0.03±0.00^a	25.11±0.72^a	0.89±0.12^a
P value		(0.20, 0.25)	(0.338, 0.27)	(0.854, 0.796)	(0.145, 0.195)	(0.177, 0.24)	(0.053, 0.69)	(0.177, 0.24)
		0.070	**0.227**	**0.885**	**0.83**	**0.434**	**0.55**	**0.434**

Results are mean ± SD, different superscripts along the column show significant difference. P values in bracket are main effect of treatment and storage, respectively, and outside bracket is the interaction effect. G: ginger, Ga: garlic, GGa: ginger-garlic mixed, SB: sodium benzoate, C: cricket only; 0, 30, 60 are days of flour storage.

Table 5.2: Fatty Acid Profile of Cricket Flour Preserved with Ginger and Garlic Extracts-Continued

Treatment	Storage(Days)	C17:0	C17:1 cis-10	C18:0	C18:1 trans-9	C18:1 cis-9	C18:2 trans-9,12
C+G	0	0.25±0.00^{b}	0.03±0.00^{a}	8.74±0.02^{ab}	0.38±0.01^{b}	29.48±0.04^{ab}	0.06±0.00^{b}
	30	0.24±0.00^{ab}	0.11±0.01^{a}	8.73±0.04^{ab}	0.38±0.00^{b}	29.20±0.12^{ab}	0.06±0.01^{b}
	60	0.24±0.00^{ab}	0.03±0.00^{a}	8.82±0.02^{ab}	0.37±0.00^{b}	29.41±0.06^{ab}	0.06±0.00^{b}
C+Ga	0	0.24±0.00^{ab}	0.03±0.00^{a}	8.73±0.04^{ab}	0.28±0.00^{ab}	29.34±0.01^{ab}	0.04±0.00^{a}
	30	0.09±0.00^{a}	0.04±0.01^{a}	8.78±0.06^{ab}	0.28±0.01^{ab}	29.35±0.20^{ab}	0.04±0.00^{a}
	60	0.09±0.00^{a}	0.11±0.11^{a}	8.76±0.03^{ab}	0.29±0.01^{ab}	29.32±0.13^{ab}	0.04±0.00^{a}
C+Gga	0	0.09±0.00^{a}	0.03±0.00^{a}	8.89±0.01^{ab}	0.27±0.00^{ab}	29.25±0.06^{ab}	0.05±0.00^{ab}
	30	0.17±0.11^{a}	0.11±0.10^{a}	8.90±0.12^{ab}	0.36±0.12^{b}	29.25±0.03^{ab}	0.06±0.01^{b}
	60	0.25±0.00^{b}	0.19±0.00^{a}	8.99±0.04^{ab}	0.38±0.01^{b}	29.21±0.27^{ab}	0.05±0.00^{ab}
C+SB	0	0.24±0.00^{ab}	0.17±0.00^{a}	8.72±0.01^{ab}	0.22±0.01^{a}	29.01±0.01^{ab}	0.03±0.00^{a}
	30	0.24±0.00^{ab}	0.19±0.01^{a}	8.74±0.03^{ab}	0.22±0.01^{a}	29.08±0.02^{ab}	0.04±0.00^{a}
	60	0.14±0.14^{a}	0.18±0.00^{a}	8.78±0.01^{ab}	0.22±0.01^{a}	28.98±0.05^{ab}	0.04±0.00^{a}
C	0	0.24±0.01^{ab}	0.11±0.11^{a}	8.75±0.22^{ab}	0.30±0.01^{ab}	29.75±0.40^{b}	0.06±0.01^{b}
	30	0.24±0.00^{ab}	0.11±0.11^{a}	8.70±0.07^{a}	0.30±0.01^{ab}	29.29±0.01^{ab}	0.05±0.00^{ab}
	60	0.17±0.12^{a}	0.03±0.00^{a}	9.04±0.13^{b}	0.30±0.02^{ab}	28.82±0.04^{a}	0.05±0.00^{ab}
		(0.032, 0.328)	(0.553, 0.525)	(0.007,0.292)	(<.001, 0.292)	(0.04,0.119)	<.001, 0.60)
P value		**0.026**	**0.483**	**0.041**	**0.27**	**0.056**	**0.547**

Table 5.3: Fatty Acid Profile of Cricket Flour Preserved with Ginger and Garlic Extracts-Continued

Treatment	Storage(Days)	C18:2cis-9,12	C20:0	C18:3 cis-6,9,12	C18:3 cis-6,9,15	C20:1 cis-11	C22:0
C+G	0	32.44±0.04^{ab}	0.06±0.00^{a}	0.69±0.00^{a}	0.41±0.00^{a}	0.09±0.00^{a}	0.26±0.19^{a}
	30	32.07±0.12^{a}	0.06±0.00^{a}	0.69±0.00^{a}	0.39±0.00^{a}	0.15±0.10^{a}	0.33±0.01^{a}
	60	32.24±0.00^{ab}	0.06±0.00^{a}	0.72±0.03^{a}	0.41±0.03^{a}	0.15±0.11^{a}	0.25±0.20^{a}
C+Ga	0	32.38±0.02^{ab}	0.06±0.00^{a}	0.69±0.01^{a}	0.39±0.00^{a}	0.16±0.10^{a}	0.23±0.16^{a}
	30	32.36±0.23^{ab}	0.06±0.00^{a}	0.75±0.01^{ab}	0.43±0.00^{a}	0.08±0.00^{a}	0.24±0.16^{a}
	60	32.34±0.03^{ab}	0.06±0.00^{a}	0.69±0.00^{a}	0.38±0.00^{a}	0.15±0.11^{a}	0.12±0.00^{a}
C+Gga	0	32.57±0.04^{ab}	0.06±0.00^{a}	0.69±0.01^{a}	0.41±0.00^{a}	0.07±0.03^{a}	0.37±0.00^{a}
	30	32.56±0.36^{ab}	0.04±0.02^{a}	0.74±0.04^{ab}	0.46±0.03^{a}	0.25±0.02^{a}	0.09±0.06^{a}
	60	32.50±0.08^{ab}	0.06±0.00^{a}	0.71±0.00^{a}	0.38±0.00^{a}	0.24±0.04^{a}	0.25±0.19^{a}
C+SB	0	32.85±0.22^{b}	0.06±0.00^{a}	0.74±0.04^{ab}	0.39±0.02^{a}	0.23±0.01^{a}	0.38±0.01^{a}
	30	32.89±0.11^{b}	0.07±0.02^{a}	0.74±0.03^{ab}	0.40±0.05^{a}	0.23±0.02^{a}	0.25±0.016^{a}
	60	32.93±0.07^{b}	0.06±0.00^{a}	0.78±0.02^{b}	0.43±0.00^{a}	0.23±0.00^{a}	0.24±0.17^{a}
C	0	32.38±0.35^{ab}	0.05±0.02^{a}	0.70±0.05^{a}	0.41±0.03^{a}	0.23±0.02^{a}	0.25±0.18^{a}
	30	32.28±0.13^{ab}	0.06±0.00^{a}	0.72±0.05^{a}	0.41±0.04^{a}	0.24±0.00^{a}	0.36±0.01^{a}
	60	32.21±0.07^{ab}	0.06±0.01^{a}	0.73±0.01^{ab}	0.40±0.03^{a}	0.09±0.01^{a}	0.15±0.01^{a}
		(<.001, 0.746)	(0.784, 0.73)	(0.022, 0.100)	(0.397, 0.286)	(0.043, 0.443)	(0.766, 0.316)
P value		**0.648**	**0.409**	**0.255**	**0.031**	**0.04**	**0.571**

Results are mean ± SD, different superscripts along the column show significant difference. P values in bracket are main effect of treatment and storage, respectively, and outside bracket is the interaction effect. G: ginger, Ga: garlic, GGa: ginger-garlic mixed, SB: sodium benzoate, C: cricket only; 0, 30, 60 are days of flour storage.

5.3.2 Dietary Indices of Cricket Oil

Five dietary indices of the cricket oil were assessed: total saturated fatty acids (SFA), unsaturated fatty (UFA) acids, monounsaturated fatty acids (MUFA), essential fatty acids (EFA), and the ratio of polyunsaturated to saturated fatty acids (P/S) and n-6/n-3and n-3/n-6. The proportion of total saturated fatty (SFA) acid was in the range of 35.12 to 35.25% at day 0 and 35.28 to 35.78% after day 60 of cricket flour storage. There was a gradual increase in the quantity of SFA, which did not differ significantly among samples during storage ($p>0.05$). This increase was attributed to the increase in palmitic and stearic acids, which were the major SFAs in cricket flour. The total unsaturated fatty acid (TUFA) was 64.71 to 64.85% among samples at day 0 of storage then decreased ($p<0.05$) to a range of 64.23 to 64.71% after 60 days of sample storage at ambient conditions (Table 5.4). A reduction of 0.52, 0.37, 0.36, 0.32, and 0.15% was recorded in the untreated, garlic, ginger-garlic, ginger, and sodium benzoate-treated cricket flour, respectively. The untreated samples exhibited greater losses than the treated samples. However, percentage reductions were statistically similar among treatments and the storage durations ($p>0.05$). Decrease in the relative percentage of the UFA is caused by lipid oxidation that breaks down unsaturated fatty acids more easily than the saturated fatty acids.

Similarly, the treatments exhibited a non-significant change in their MUFA during flour storage, with proportions in the range of 30.36 to 31.38% of the total fatty acids. During 150 days of storage of deep-fried crickets, Gan et al. (2022) showed that the SFA increased from 44.32% to 46.06% and the UFA declined from 55.36% to 53.94% in the untreated samples, while an increase of 44.47 to 46.64% (SFA) and a decline of 55.53 to 53.36% (UFA) was recorded in the rosemary extract treated samples stored under vacuum conditions. Our study samples were processed by oven drying and kept at room temperature in contrast to the prior study samples, which were deep-fried in palm oil that is low in UFA and high in SFA and stored under vacuum.

The proportion of polyunsaturated fatty acids (PUFA) was significantly different among samples during storage ($p<0.05$). The lowest quantity (33.98%) was recorded in the ginger treated sample at day 0 of storage, and the highest (34.69%) was recorded in the sodium benzoate treated sample at day 0 of storage. A gradual decrease was observed during storage, reducing the PUFA to a range of 33.61 to 34.42%. The same trend was observed for the essential fatty acids, consisting of linoleic acid (C18:2 cis-9, 12) and alpha linolenic acid (C18:3 cis-6, 9, 15), where a range of 32.79 to 33.34% and 32.41 to 33.26% was recorded for samples at day 0 and day 60 of flour storage, respectively. Proportions of EFA were significantly higher in sodium benzoate preserved samples, followed by the combination of ginger and garlic treated samples.

Khatun et al. (2021) reported a proportion of SFA (32.01 and 35.37%), MUFA (22.29 and 29.86%), and PUFA (45.70 and 34.86%) in oven dried crickets A. *domesticus* and A. *assimilis*, respectively; this is comparable to the findings of the current study. Kamau et al. (2017) reported that the proportion of MUFA and PUFA in adult house cricket meal gradually reduced throughout the course of storage, with reductions in the groups being between 16.2 to 52.1% at 90 days and 10.73 to 67.0% at 180 days of storage. This loses were greater than the values detected in our studies probably due to the variation in the nature of products, packaging material, storage condition, and long storage duration of products in the previous study.

The P/S ratio varied significantly among the samples in the range of 0.94 in the control and ginger treated samples at day 60 of storage and 0.99 in the SB treated samples at day 0 of storage (Table 5.5). The p/s ratio in samples treated with a combination of ginger and garlic compared favorably with the SB treated samples. Lucas-González et al. (2019) reported a P/S ratio of 1.30 and 0.98 in lyophilized and thermally dried cricket (*A. domesticus*) flour, while Kowalski et al. (2022) and Paul et al. (2017) reported a ratio of 0.89 and 1.32 in the same species, respectively. One of the most important markers of the lipid composition of a healthy diet is the polyunsaturated to saturated fatty acid (P/S) ratio (Zhu et al., 2022). It is recommended to consume food with a P/S ratio close to 1 (Paul et al., 2017).

The proportions of n-6 and n-3 fatty acids in the samples varied significantly among the treatments ($p<0.05$). The n-6 fatty acids were composed of linoeliadic acid (C18:2 trans-9, 12), linoleic acid (C18:2cis-9, 12), gamma linolenic acid (C18:3 cis-6, 9, 12), and arachidonic acid (C20:4 cis 5, 8, 11, 14), which made up 33.05 to 33.78% of the total fatty acid methyl esters. Samples treated with SB (33.78 to 33.62%),) and a combination of ginger and garlic extracts (33.76 to 33.36%) had higher proportions of n-6. On the other hand, n-3 fatty acids consisting of alpha linolenic acid (C18:3 cis-6, 9, 15) and eicosatrienoic acid (C20:3 cis-11, 14, 17; C20:3 cis-8, 11, 14) constituted 0.72 to 0.78% of the total fatty acid. The n-3 proportion was significantly higher in the ginger-garlic preserved samples followed by the SB preserved samples. Singh et al. (2020) reported a range of 35.5 to 39.8% and 1.92 to 2.80 for n-6 and n-3 fatty acids in cricket flour, respectively. Diets with n-6/n-3 fatty acid ratio close to 6 has been associated with cardiovascular health (Fereidoon Shahidi & Ambigaipalan, 2018; Wijendran & Hayes, 2004). Previous studies on cricket flour have reported n-6/n-3 rations of 38.55 (Osimani et al., 2018); 37.05 (Paul et al., 2017); 248.92 (Kowalski et al., 2022); and 14.9 to 18.8 (Singh et al., 2020). While the n-3/n-6 of cricket flour was reported between 0.02 and 0.05 (Khatun et al., 2021; Paul et al., 2017). In this study, ratios of n-6/n-3 and n-3/n-6 did not differ significantly with both the treatment and storage duration ($p>0.05$), being in the range of 43.13 to 46.15 and 0.02, respectively. Variations in n-6/n-3 and n-3/n6 were attributed to the difference in the total n-6 and n-3 in the sample which is in turn affected by factors such as; nutrition, species, stage of the insects, and environmental factors and method of processing (Raksakantong et al., 2010).

The results indicated that ginger and garlic extract treatments had certain effects on the fatty acid composition of cricket flour during storage, and the effects gradually increased with the length of storage. Lipids are oxidized and hydrolyzed by oxygen and lipase during the processing and storage of food goods. The stability of different fatty acids varies, and SFAs are the most stable. The simplicity of generating fatty acid alkyl radicals is necessary for autoxidation. Unsaturated fatty acids have double bonds from which hydrogen can be abstracted, making them more susceptible to autoxidation than saturated fatty acids.

Polyunsaturated fatty acids are more susceptible to oxidation, followed by monounsaturated fatty acids (Fereidoon & Ying, 2010). Due to safety issues, the use of natural antioxidants in food is much preferred compared to synthetic antioxidants (Santos-Sánchez et al., 2018; Shah & Mir, 2021; Xu et al., 2017). Ginger and garlic are among the most widely used natural antioxidants (Kumari et al., 2018; Sepahpour et al., 2018; Sofia et al., 2007). The strong antioxidant activity of ginger is due to the presence of compounds such as gingerol and shogaol (Baliga et al., 2011; Mushtaq et al., 2019; Tanweer et al., 2020), with the former being abundant in fresh ginger and the latter in dried ginger. In garlic, the active ingredients are organosulfur compounds such as allicin and allin (Feriedoon Shahidi & Hossain, 2018; Shang et al., 2019; Haiping Wang et al., 2015). These compounds exhibit antioxidant functions because they possess strong free radical scavenging activities.

Table 5.4: Fatty Acid Profile (Dietary Indicators) of Cricket Flour Preserved with Ginger and Garlic Extracts

Treatment	Storage (Days)	C20:3 cis-11,14,17	C20:3 cis-8, 11,14	C20:4 cis 5,8,11,14	C24:1	Σ SFA	Σ USF	ΣMUFA
C+G	0	0.21±0.00^{a}	0.12±0.01^{a}	0.06±0.00^{a}	0.04±0.00^{a}	35.13±0.07^{a}	64.74±0.07^{a}	30.75±0.03^{a}
	30	0.21±0.00^{a}	0.12±0.01^{a}	0.12±0.09^{a}	0.04±0.00^{a}	35.41±0.06^{a}	64.62±0.05^{a}	30.81±0.09^{a}
	60	0.21±0.00^{a}	0.13±0.02^{a}	0.05±0.01^{a}	0.03±0.00^{a}	35.61±0.14^{a}	64.42±0.14^{a}	30.81±0.16^{a}
C+Ga	0	0.21±0.01^{a}	0.12±0.01^{a}	0.06±0.00^{a}	0.02±0.03^{a}	35.18±0.02^{a}	64.71±0.01^{a}	30.68±0.02^{a}
	30	0.22±0.00^{ab}	0.12±0.01^{a}	0.12±0.10^{a}	0.02±0.02^{a}	35.34±0.20^{a}	64.55±0.22^{a}	30.65±0.08^{a}
	60	0.22±0.00^{ab}	0.13±0.02^{a}	0.11±0.10^{a}	0.04±0.00^{a}	35.70±0.18^{a}	64.34±0.18^{a}	30.84±0.09^{a}
C+Gga	0	0.22±0.01^{ab}	0.12±0.01^{a}	0.05±0.01^{a}	0.04±0.00^{a}	35.25±0.00^{a}	64.73±0.01^{a}	30.36±0.04^{a}
	30	0.20±0.02^{a}	0.16±0.05^{a}	0.06±0.01^{a}	0.03±0.00^{a}	35.35±0.09^{a}	64.53±0.10^{a}	30.64±0.22^{a}
	60	0.23±0.00^{b}	0.13±0.01^{a}	0.10±0.14^{a}	0.04±0.00^{a}	35.63±0.02^{a}	64.37±0.03^{a}	30.54±0.24^{a}
C+SB	0	0.22±0.01^{ab}	0.13±0.01^{a}	0.06±0.00^{a}	0.04±0.00^{a}	35.12±0.18^{a}	64.86±0.18^{a}	30.44±0.08^{a}
	30	0.23±0.00^{b}	0.12±0.01^{a}	0.12±0.09^{a}	0.04±0.00^{a}	35.20±0.16^{a}	64.79±0.17^{a}	30.43±0.05^{a}
	60	0.22±0.00^{ab}	0.12±0.00^{a}	0.18±0.00^{a}	0.03±0.00^{a}	35.28±0.02^{a}	64.71±0.04^{a}	30.39±0.11^{a}
C	0	0.22±0.00^{ab}	0.13±0.01^{a}	0.06±0.01^{a}	0.04±0.00^{a}	35.17±0.73^{a}	64.75±0.80^{a}	31.38±1.24^{a}
	30	0.21±0.01^{ab}	0.12±0.01^{a}	0.12±0.10^{a}	0.04±0.00^{a}	35.53±0.14^{a}	64.44±0.15^{a}	30.74±0.04^{a}
	60	0.23±0.00^{b}	0.13±0.01^{a}	0.06±0.02^{a}	0.00±0.00^{a}	35.78±0.31^{a}	64.23±0.28^{a}	31.06±0.31^{a}
P value		(0.017, 0.12)	(0.144, 0.30)	(0.739, 0.238)	(0.187, 0.46)	(0.07, 0.935)	(0.03, 0.88)	(0.053, 0.846)
		0.075	**0.094**	**0.85**	**0.064**	**0.434**	**0.284**	**0.707**

Results are mean ± SD, different superscripts along the column show significant difference. P values in bracket are main effect of treatment and storage, respectively, and outside bracket is the interaction effect. G: ginger, Ga: garlic, GGa: ginger-garlic mixed, SB: sodium benzoate, C: cricket only; 0, 30, 60 are days of flour storage.

Table 5.5: Fatty Acid Profile (Dietary Indicators) of Cricket Flour Preserved With Ginger and Garlic Extracts- Continued

Trt	Storage (Days)	ΣPUFA	ΣEFA	P/S	Σn-6	Σn-3	n-6/n-3	n-3/n-6
C+G	0	33.98±0.04^{abc}	32.85±0.05^{ab}	0.97±0.00^{abc}	33.73±0.05^{bc}	0.74±0.00^{ab}	45.71±0.34^{a}	0.02±0.00^{a}
	30	33.65±0.03^{a}	32.56±0.12^{a}	0.95±0.00^{a}	33.25±0.04^{a}	0.74±0.01^{ab}	45.66±0.57^{a}	0.02±0.00^{a}
	60	33.61±0.03^{ab}	32.46±0.03^{a}	0.94±0.00^{a}	33.07±0.01^{a}	0.72±0.05^{ab}	44.04±0.28^{a}	0.02±0.00^{a}
C+Ga	0	34.05±0.02^{ab}	32.81±0.02^{ab}	0.97±0.00^{abc}	33.29±0.03^{abc}	0.76±0.02^{b}	46.15±1.25^{a}	0.02±0.00^{a}
	30	33.97±0.14^{abc}	32.76±0.22^{ab}	0.96±0.01^{ab}	33.15±0.13^{ab}	0.72±0.01^{a}	43.75±0.13^{a}	0.02±0.00^{a}
	60	33.90±0.10^{ab}	32.71±0.02^{ab}	0.95±0.01^{a}	33.18±0.13^{ab}	0.72±0.03^{a}	43.13±1.91^{a}	0.02±0.00^{a}
C+Gga	0	34.11±0.03^{abc}	32.97±0.04^{ab}	0.97±0.00^{abc}	33.76±0.04^{bc}	0.78±0.01^{bc}	44.47±0.44^{a}	0.02±0.00^{a}
	30	34.04±0.32^{abc}	32.92±0.33^{ab}	0.96±0.01^{ab}	33.41±0.38^{abc}	0.75±0.06^{ab}	45.41±0.59^{a}	0.02±0.00^{a}
	60	34.00±0.21^{abc}	32.88±0.08^{ab}	0.95±0.01^{a}	33.36±0.22^{abc}	0.73±0.01^{a}	43.31±0.90^{a}	0.02±0.00^{a}
C+SB	0	34.69±0.26^{bc}	33.34±0.03^{b}	0.99±0.01^{c}	33.78±0.26^{c}	0.76±0.05^{b}	45.07±0.20^{a}	0.02±0.00^{a}
	30	34.53±0.12^{bc}	33.29±0.03^{b}	0.98±0.01^{bc}	33.67±0.18^{bc}	0.75±0.05^{ab}	45.17±3.80^{a}	0.02±0.00^{a}
	60	34.42±0.07^{c}	33.26±0.08^{b}	0.98±0.00^{bc}	33.62±0.06^{abc}	0.75±0.01^{ab}	44.35±0.42^{a}	0.02±0.00^{a}
C	0	34.00±0.44^{ab}	32.79±0.38^{ab}	0.97±0.01^{abc}	33.60±0.04^{abc}	0.76±0.04^{b}	45.26±1.93^{a}	0.02±0.00^{a}
	30	33.91±0.10^{abc}	32.59±0.17^{a}	0.95±0.01^{a}	33.10±0.07^{ab}	0.74±0.03^{ab}	43.76±1.80^{a}	0.02±0.00^{a}
	60	33.76±0.03^{ab}	32.41±0.05^{a}	0.94±0.01^{a}	33.05±0.06^{a}	0.72±0.03^{a}	43.48±1.35^{a}	0.02±0.00^{a}
P value		(<.001, 0.82)	(<.001,0.83)	(<.001, 0.899)	(<.001,0.925)	(0.036, 0.268)	(0.06, 0.376)	(0.046, 0.31)
		0.387	**0.532**	**0.11**	**0.508**	**0.013**	**0.056**	**0.018**

Results are mean ± SD, different superscripts along the column show significant difference. P values in bracket are main effect of treatment and storage, respectively, and outside bracket is the interaction effect. G: ginger, Ga: garlic, GGa: ginger-garlic mixed, SB: sodium benzoate, C: cricket only; 0, 30, 60 are days of flour storage

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5.3.3 Changes in Lipid Stability of Cricket Flour during Storage

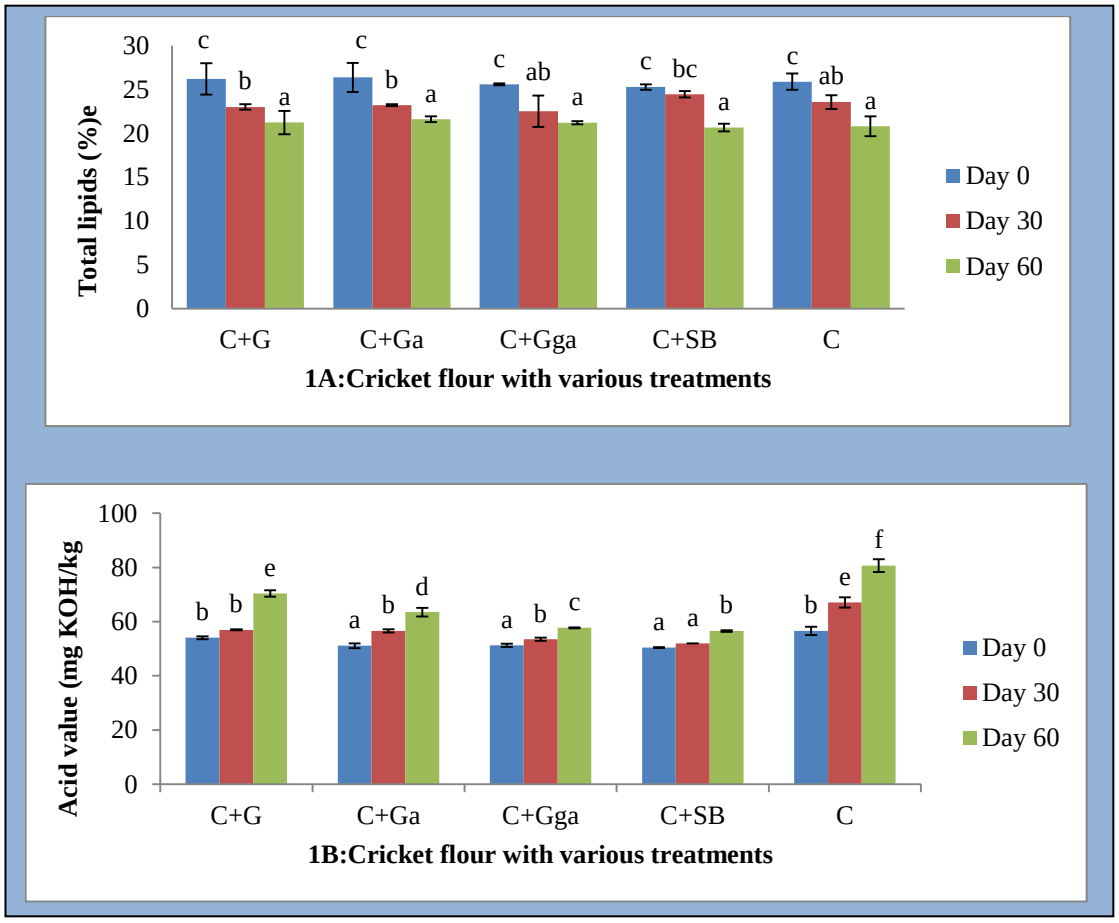
Lipid Content and Acid Value

The changes in lipid content and acid value of cricket flour during the 60 days period of storage are shown in Figure 5.1. The lipid content of the cricket flour varied with the storage duration, from 26.36 to 25.27% at the start of the storage to 21.23 to 20.20% after 60 days of storage. The decrease in fat content was significant in both the treated and the untreated flour ($p<0.05$). Previous studies reported different quantities of fat in different cricket species. Osimani et al. (2018) reported 28.75% in powder, while Lucas-González et al. (2019) reported 24.91% in thermally dried crickets *A. domesticus*. Jeong et al. (2021) reported 17.4% in *G. bimaculatus*, while 21.62% was registered in *G. assimilis* (Khatun et al., 2021). It is known that the amount of fat in edible insects varies depending on factors including the stage of development, species, habitat, feeding habits, processing techniques, and storage (Fombong et al., 2017; Lucas-González et al., 2019; Raksakantong et al., 2010). In this study, the decrease in lipid content during storage in ginger-garlic treated (4.38%), ginger treated (4.97%), SB (4.62%), garlic (5.06%), and untreated flour (5.1%) was consistent with the decreasing proportion of the unsaturated fatty acid registered during storage, which is indicative of fat degradation during storage.

The acid value increased gradually during the period of storage, ranging from 50.42 to 70.41 mg KOH/kg in the treated samples and from 56.60 to 80.66 mg KOH/kg in the untreated samples on days 0 and 60 of storage, respectively. The difference in the acid value was significant among treatments and across the storage period ($p<0.05$). Lower values were observed in samples treated with a combination of ginger and garlic (51.21 to 57.74) and SB (50.42 to 56.55) mg KOH/kg. Ssepuuya et al. (2016) reported acid value of 8-10 mg KOH/kg of sautéed ready-to-eat *Ruspolia nitidula* at the initial storage period which decreased during storage. The quantity of free fatty acids (FFA) in fats or oils is determined by the acid value. FFA content increasing in oil is an indication of fat degradation (Gan et al., 2022). In this study, the initial acid value was attributed to the

effect of thermal processing with the increase during storage as a result of the reaction of the flour with light since the products were kept on shelf, under ambient conditions. FFA is a source of flavors and aromas; they are used to monitor the oil stability during storage; unsaturated FFA serves as a substrate for autoxidation (Fereidoon & Ying, 2010).

Figure 5.1: Lipid Content and Acid Value of Cricket Flour during Storage



Peroxide Value and TBARS

The peroxide value of a sample is the measure of the milliequivalents of peroxide contained in a kilogram of the sample. It is often used as an indicator of the primary products of lipid oxidation (Javadian et al., 2017). The UFAs are unstable and are hydrolyzed to hydroperoxides as a result of lipid oxidation. When the generation of peroxides exceeds their breakdown, the peroxides progressively accumulate, increasing PV. This study showed a significant increase in the peroxide value among treatments and storage periods ($p<0.05$); with 1.65 to 3.63 and 1.94 to 3.91 mEq/kg among the treated samples and the untreated flour, respectively (Figure 5.2). At the beginning of storage, the peroxide value is low as there is minimal lipid oxidation. However, as the product spends more days in storage, the lipid oxidation increases and there is an accumulation of the hydroperoxides, hence increasing the PV. However, in both the treated and untreated flour, the peroxide value was below the acceptable limits (10 mEq/kg) for food (Liu et al., 2019).

The peroxide value of an insect-blended extruded flour increased from 2.80 to 5.32 mEq/Kg during 9 weeks of storage (Shabo et al., 2022). During the storage of fish, the peroxide value increased from 1.23 to 10.01 mEq/kg from day 0 to day 15 of storage of samples with different treatments (Javadian et al., 2017). In our study, PV spice-treated samples were significantly lower than the untreated samples at all points in storage. This was attributed to the antioxidant ability of the spice extracts as a result of their high phenolic content. According to Turhan et al. (2009), phenolic antioxidants prohibit the formation of fatty acid free radicals, which do react with or absorb oxygen in the autoxidation process; hence slowing down the autoxidative processes.

Thiobabituric Acid Reactive Substance (TBARS)

TBARS measures the secondary oxidative products of lipid peroxidation (Papastergiadis et al., 2012). Malondialdehyde (MDA), a small molecule generated from hydroperoxides (the first product of polyunsaturated fatty acids reacting with oxygen), combines with

thiobarbituric acid (TBA) to form MDA-TBA2 adducts, known as TBARS (Tsai & Huang, 2015). The initial TBARS in both treated and untreated cricket flour was in the range of 0.45 to 0.57 mg MDA/kg. The levels significantly increased to a range of 0.82 to 1.05 mg MDA/kg after day 60 of cricket flour storage. MDA is commonly considered the final product of lipid oxidation and is what causes an unpleasant odor to develop (Zhang et al., 2019). With longer storage time, the MDA concentrations in all of the study samples increased slightly ($p < 0.05$), which is consistent with earlier research on foods derived from insects (Gan et al., 2022; Singh et al., 2020; Ssepuuya et al., 2016) and other kinds of food (Liu et al., 2019; Temba et al., 2017).

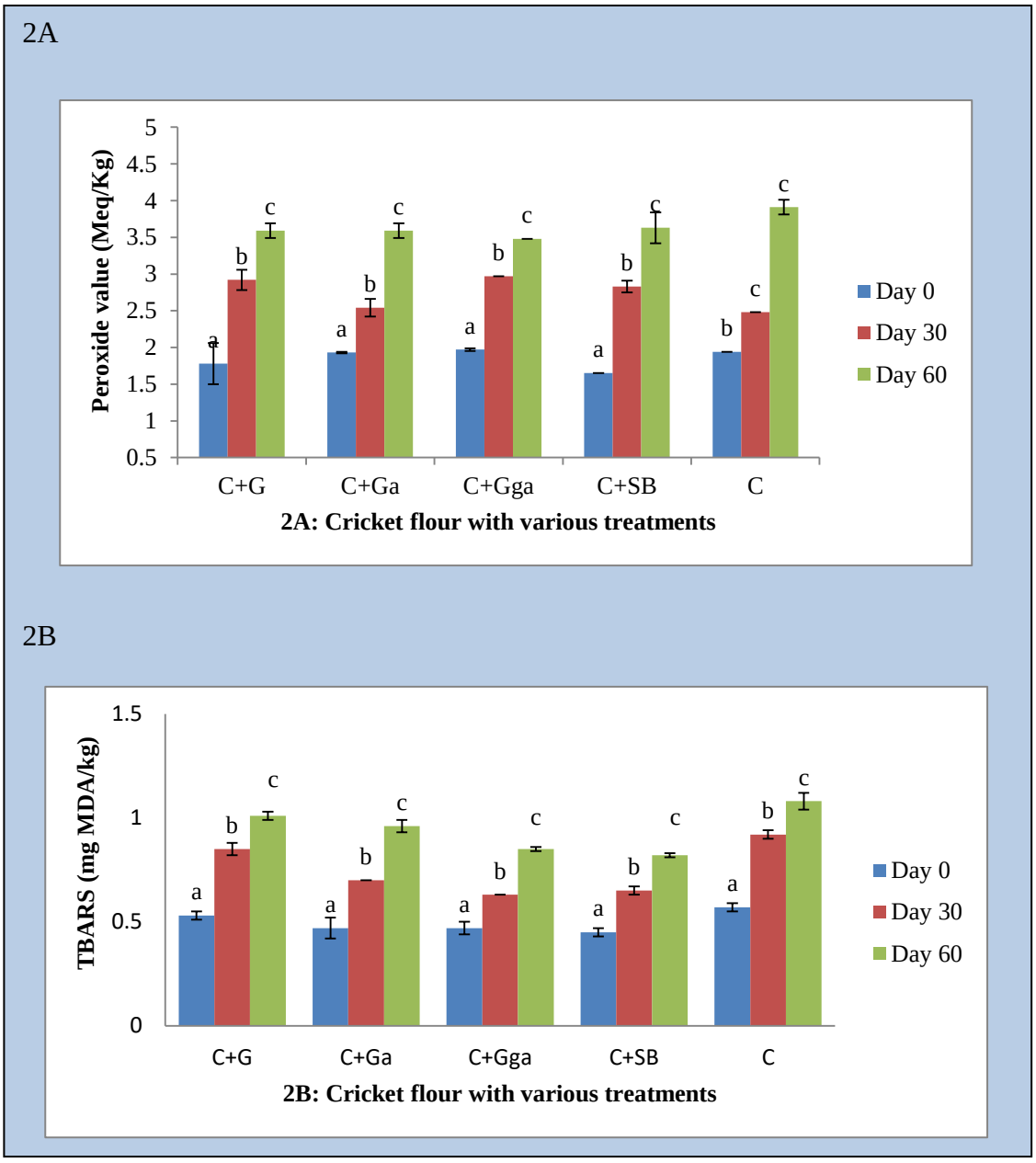


Figure 5.2: Peroxide Value and TBARs of Cricket Flour during storage

5.4 Conclusion

In the current study, it was shown that both treated and untreated cricket flour exhibited some limited reduction in UFAs and an increase in SFAs, implying that the cricket flour was susceptible to oxidation during storage. The beneficial effect of the spice

extracts in preservation is shown, especially by the minimal decrease in UFA and little increase in SFA in samples treated with a combination of ginger and garlic extracts, which compared favorably with the positive control (SB preserved) samples. The PV and TBARs values of cricket flour increased during the 60 days of storage but remained within acceptable limits for a safe food. Ginger-garlic preserved flour was the most stable compared with ginger and garlic treatments used singly. This is consistent with the changes in the fatty acid composition. The study recommends the use of a combination of ginger and garlic in the preservation of cricket flour during storage at ambient conditions. Further research is needed on the effects of different packaging materials, storage conditions, and lengthy storage of cricket flour (exceeding 60 days) on the lipid characteristics.

CHAPTER SIX

COLOR, PH, MICROBIOLOGICAL AND SENSORY QUALITY OF CRICKETS (*GRYLLUS BIMACULATUS*) FLOUR PRESERVED WITH GINGER AND GARLIC EXTRACTS

Abstract

Although spices have been used in food for centuries, little is known about their use to preserve insect-based foods. This study assessed the flour produced from blanched crickets treated with ginger, garlic, or both extracts at a ratio of 1:4 (v/w) for color, pH, microbiological profile, sensory quality, and acceptability. Sodium benzoate treated and untreated cricket flour were used as positive and negative controls, respectively. The flour was stored at ambient conditions and analyzed after 0, 30, and 60 days of storage. The pH, moisture content, and color change increased during storage but remained within acceptable limits. The total microbial count, yeast, and molds significantly decreased with storage duration ($p<0.05$), while fecal coliforms and *Escherichia coli* were not detected in any of the samples. At the end of the 60-day storage period, cricket flour treated with sodium benzoate and garlic extracts both had a significantly lower population of yeast and molds (1.91 log cfu/g). On a five-point hedonic scale, (1. dislike extremely and 5. like extremely), color (3.84±0.86 to 2.55±0.99), aroma (3.59 ± 1.09 to 2.40 ± 1.01), texture (4.11 ± 0.97 to 3.11 ± 0.97) and overall acceptability (3.77 ± 0.64 to 2.83 ± 1.01) sensory scores were all significantly high on day 0 and low on day 60 of storage, respectively. The study concluded that preserving crickets with garlic extracts significantly reduced the population of yeast and molds. Cricket flours were microbiologically safe and acceptable to consumers. Therefore, storing cricket flour preserved with garlic and ginger extracts for longer periods is recommended. In addition, utilization of the preserved flour as an ingredient in different food applications is recommended to determine its suitability and sensory acceptability.

Key words: Spice extracts, insect-based foods, cricket flour, storage, acceptability

6.1 Introduction

Many societies around the world consume insects as an essential component of their diet (Raubenheimer & Rothman, 2013). In terms of nutrition, insects are an excellent source of proteins, fat, vitamins, and minerals (Akullo et al., 2018; Maiyo et al., 2022). In Africa, 212 insect species from eight orders are consumed as food (Hlongwane et al., 2020). In East Africa, widely consumed edible insects include winged termites (*Macrotermes* spp.), grasshoppers (*Ruspolia* spp.), and different species of crickets (Kinyuru et al., 2013; Maiyo et al., 2022; Malinga et al., 2018). Among the different edible insects, crickets (*Gryllus bimaculatus*) are a promising insect species since they have a high nutritional quality and can also be successfully reared under farm conditions (Kinyuru & Kipkoech, 2018; Ng'ang'a et al., 2020; Sorjonen et al., 2019).

The current rapid world population growth and low food production have caused a shortage of protein supplies and widespread malnutrition. Among the possible approaches to closing the gap between current and future levels of food production and consumption are: exploiting new, unconventional food sources such as insects; and enriching low-quality meals with high-quality protein sources such as those derived from insects. However, seasonality and perishability are major barriers to utilizing insects as food (Ayieko et al., 2010), in addition to considerable post-harvest losses. Moreover, insects are a highly perishable food due to their high water activity, free amino acids, polyunsaturated fatty acids, and nutritional value.

The biological processes that result in insect spoilage include the oxidation of lipids, the activity of their intrinsic enzymes, and their metabolic processes. These biological processes affect insect-based food products by altering their physical, organoleptic, and nutritional qualities (Mokhtar et al., 2014). The addition of antioxidants is the most effective way to prevent oxidation and preserve insects, and currently, using natural plant extracts as preservatives is a popular trend because consumers are concerned about the safety of synthetic antioxidants and chemical preservatives (Shah & Mir, 2021). Furthermore, some herbs and spices, including ginger and garlic, have been utilized to

successfully preserve other perishable foods, such as fish and meat, respectively (Brewer, 2011). Several studies have also reported on the food preservation potential of ginger and garlic (Akintobi et al., 2013; Indu et al., 2006; Khashan, 2014; Mohammed et al., 2019).

The preserved products were reported to have good nutritional and sensory quality owing to the action of the antioxidant and antimicrobial components of the extracts. Spices have been used to enhance the physical appearance of food, thereby influencing its consumer acceptability. For instance, pepper and turmeric change the color, appearance, and taste of most foods, with many health benefits (Pop et al., 2019). The influence of several natural antioxidants on the sensory qualities of beef patties during storage was examined (Mokhtar et al., 2014). The results revealed significantly higher sensory scores for odor, color, taste, and overall acceptability than the control. However, the same study showed no substantial difference in the textures of treated and untreated samples. The color parameters of the raw pork samples treated with spice extracts changed slightly during storage, but the control samples exhibited significant changes (Shan et al., 2009). Procyanidin treatment resulted in reduced pH and lightness values in pork patties, while the redness was enhanced in contrast to the untreated controls (Jeong et al., 2015).

According to Goyal et al. (2017), lengthy storage can completely destroy the quality of the flour. The main issues reducing the shelf life of flour are microbiological growth, color change, and oxidative rancidity. Previous research suggests that crickets are contaminated with both harmful and spoilage microorganisms (Fröhling et al., 2020; Grispoldi et al., 2021). Furthermore, the sensory acceptability of a traditionally processed meat-based product was marginally lowered after storage for 12 weeks (Mgbemere et al., 2011). Sensory acceptability has become a vital part of food production. A consumer's first impression of a food product, primarily determined by color, is what causes them to accept or reject it (Pathare et al., 2013). As much as plant extracts have been used to preserve food, as aforementioned, their use to preserve insects has not been attempted previously, and it is not known whether insect preservation with plant extracts can extend the shelf life and improve the sensory acceptability of the resultant insect food products. In this study, we used extracts from garlic and ginger to preserve raw crickets, which were later

processed into flour. The storage quality (changes in color, moisture, PH, microbial profile, and sensory acceptability) of the flour was evaluated during 60 days of storage. The goal was to provide an affordable, safe, and acceptable alternative for the preservation of insects, making insect flour available for a wide range of food applications.

6.2 Materials and Methods

6.2.1 Preparation of Spice Extracts, Treatment of Crickets and Processing of Cricket Flour

Fresh spices (garlic cloves and ginger rhizomes) were purchased from a local market in Uganda and extracts were prepared according to the procedure in section 5.1.1. Live adult crickets were also acquired, treated with spice extracts and processed into flour following the procedure detailed in section 5.1.2. The samples were stored as previously described and subjected to physicochemical, microbiological, and sensory evaluation at days 0, 30, and 60 of storage.

6.2.2 Determination of pH, Moisture Content and Cricket Flour Colour

The pH was determined using a previously validated method (Kalra, 1995). In brief, the pH was tested by homogenizing 10 g of the sample in 100 ml of distilled water. After filtering, the pH was measured in triplicate. Moisture content was determined by weight loss upon drying at 105 °C for 2 hours, following the procedures of Thiex (2009). The colour of flour was monitored following the method adopted by Wang et al. (2015), using a handheld Minolta colour meter (Model CR-200, Osaka, Japan) according to the International Commission on Illumination (CIE) colour space. All measurements were made at ambient temperature upon calibration of the meter using a white plate/surface and reported using the L*, a*, b* colour systems. L* represents whiteness/blackness (100 = white and 0 = black); a* represents redness/greenness (a* positive = red, a* negative = green); and b* represents yellowness/blueness (b* positive = yellow, b* negative = blue). Measurement was performed six times on approximately 10 grams of flour in a small

container. Color changes (ΔL*, Δa*, Δb*) were calculated using the samples at zero as a reference. The total color difference (ΔE*) which shows the extent of color change in parameters between the initial and final color values during storage, was calculated using the equation by (Pathare et al., 2013).

$$\Delta E * = \sqrt{\Delta a *^2 + \Delta b *^2 + \Delta L *^2}$$

Where ΔL*= changes in lightness, Δa*= changes in redness/greenness, Δb*= changes in yellowness/blueness, ΔE*= Total colour change.

6.2.3 Microbiological Analysis

The spread plate method was used to measure total viable counts (TVC) utilizing nutrient Agar (NA) as the medium, according to previous procedures (Sanders, 2012). Prior to plating, all tools, solutions, and media were prepared and sterilized in accordance with the Standard Operating Procedures (SOPs) necessary for minimal microbiological contamination. From each of the storage bags, ten (10) g of cricket flour was removed aseptically and placed in a sample bottle containing 90 ml of sterilized physiological saline. This was followed by homogenization, threefold dilutions, and plating on nutrient agar for each of the dilutions. In brief, 0.1 ml of each dilution was aseptically taken using a micropipette onto a pre-poured, solidified agar plate, and the inoculum was spread using a sterile, bent metallic rod. After cooling, the plates were inverted and incubated at 35± 2°C for 24 ± 2 hours. In accordance with the methods of Feng et al. (2002), fecal coliforms and *Escherichia coli* were identified by plating on Violet Red Bile Glucose Agar (VRBGR) and incubated at 35± 2°C for 24 ± 2 hours. Total yeasts and molds were determined on Sabouraud Dextrose Agar (SDA) plates incubated at 27 °C for 48 hours, as recommended (Tournas et al., 2001). Plating was conducted in triplicate, and microbial counts were expressed as log colony-forming units per gram of sample (log cfu/g).

6.2.4 Sensory evaluation

The sensory/organoleptic properties of flour during storage were evaluated for color, aroma, texture/fineness, and overall acceptability on days 0, 30, and 60 of storage. Sensory quality was evaluated following the principles and practices previously described (Sharif et al., 2017). Processed samples were served to a panel and rated for the mentioned parameters using the hedonic test on a 5-point scale (1=dislike extremely, 2=dislike moderately, 3=neither like nor dislike 4 = like it moderately, 5 = like it extremely). Each testing session consisted of 60 untrained panelists, both males, and females, randomly recruited from among employees and students from the School of Food and Nutrition Sciences, JKUAT. Following the briefing, coded samples were given to each participant at random for rating. Color was judged by sight, aroma was by smelling the flour, while texture/consistency was by feeling the particles of the flour between the fingures.

6.1.5 Statistical Analysis

Data was entered in Microsoft Excel for storage and analyzed using STATA version 12 (StataCorp LP, Texas, USA). Analysis of variance (ANOVA) was performed, and means were separated using Bonferroni as a post hoc test at a 95 % confidence level. Results were reported as mean ± standard deviations. PCA was applied after the ANOVA test to a set of data that was obtained from the physiochemical properties, microbiological analysis, and sensory evaluation of samples from various treatments and storage intervals. PCA is a widely used multivariate analytical statistical method for reducing complexity in high-dimensional data without distorting trends and patterns; this is accomplished by condensing the data into fewer dimensions that serve as feature summaries (Lever et al., 2017, 2021). The original data was transformed into a smaller set of linear combinations called principal components (PC). Two PCs were chosen, and their combination described the best treatment and storage time.

6.3 Results

6.3.1 Changes in Color of Cricket Flour Treated with Ginger and Garlic Extracts

There was a significant interaction between sample treatment and storage duration for total color change and Lightness values (Table 6.1). As the samples were stored, their lightness (L*) values declined from day 0 to day 60. In both the treated and untreated samples, lightness at day 0 was significantly higher than at days 30 and 60 in storage ($p<0.05$). However, lightness values across samples at the same stage of storage did not differ significantly. Measurement for redness/greenness (a*) did not vary significantly among samples ($p> 0.05$). The highest value was observed on day 0, while the lowest value was on day 60 for all the samples. The flour from cricket preserved with garlic and garlic mixed with ginger had the highest values at day 0 ($a^* = 4.97 \pm 0.12$ and 4.73 ± 0.29, respectively). In the garlic/ginger-treated, sodium benzoate-treated, and untreated samples, the measurement for yellowness/blueness (b*) of the cricket flour samples varied significantly at 0 and 30 days of storage ($p< 0.05$). The total color change (ΔE) of samples from the initial storage to the final storage period differed significantly ($p<0.05$). In all samples, the highest color change was noted between 0 and 60 days of storage in the untreated samples ($\Delta E = 11.71 \pm 0.55$). The color change between days 0 and 30 was minimal among samples, ranging from 2.09 ± 1.01 to 2.87 ± 1.09 in flour preserved with garlic and untreated flour.

Table 6.1: Color Changes in Cricket Flour Preserved with Ginger and Garlic Extracts

Sample (flour)	Storage (Days)	L*	a*	b*	ΔE
C+ G	0	31.90 ± 0.20^{d}	4.70 ± 0.62^{bc}	16.10 ± 0.44^{b}	0.00 ± 0.00^{a}
	30	26.67 ± 0.32^{bc}	3.60 ± 0.17^{abc}	10.00 ± 1.31^{a}	2.29 ± 0.19^{b}
	60	25.07 ± 0.06^{abc}	3.23 ± 1.12^{abc}	8.97 ± 1.10^{a}	10.05 ± 0.72^{cd}
C+ Ga	0	32.00 ± 0.26^{d}	4.97 ± 0.12^{c}	16.30 ± 0.53^{b}	0.00 ± 0.00^{a}
	30	25.43 ± 0.21^{bc}	4.30 ± 0.96^{abc}	9.53 ± 1.05^{a}	2.09 ± 1.01^{ab}
	60	26.07 ± 0.75^{bc}	3.73 ± 0.51^{abc}	10.23 ± 1.05^{a}	8.59 ± 1.06^{c}
C+GGa	0	31.20 ± 0.79^{d}	4.73 ± 0.29^{bc}	16.57 ± 0.61^{b}	0.00 ± 0.00^{a}
	30	26.83 ± 0.76^{c}	4.00 ± 0.95^{abc}	10.27 ± 0.85^{a}	2.27 ± 1.23^{b}
	60	25.80 ± 1.54^{bc}	2.63 ± 0.51^{a}	9.67 ± 0.23^{a}	9.04 ± 0.80^{c}
C+SB	0	31.07 ± 0.31^{d}	4.40 ± 0.26^{abc}	14.40 ± 0.46^{b}	0.00 ± 0.00^{a}
	30	25.57 ± 0.15^{bc}	3.80 ± 0.26^{abc}	10.00 ± 0.95^{a}	2.18 ± 0.45^{ab}
	60	24.67 ± 0.12^{ab}	2.87 ± 0.61^{ab}	8.50 ± 0.26^{a}	8.86 ± 0.35^{c}
C	0	32.90 ± 0.52^{d}	4.33 ± 0.31^{abc}	15.07 ± 0.31^{b}	0.00 ± 0.00^{a}
	30	25.97 ± 1.44^{bc}	3.47 ± 0.32^{abc}	9.00 ± 1.15^{a}	2.87 ± 1.09^{b}
	60	23.30 ± 0.40^{a}	2.83 ± 0.55^{ab}	8.63 ± 1.36^{a}	11.71 ± 0.55^{d}
P value		*<.001*	*0.905*	*0.365*	*0.018*

Values = Mean ±SD, (n=6); different superscripts along column = significant difference ($p<0.05$). P values are the interaction effects of sample treatment and storage duration. C: cricket; G: ginger; Ga: garlic; GGa: ginger + garlic; SB: sodium benzoate; L*: lightness; a*: redness or greenness; b*: yellowness or blueness; ΔE: total color change.

6.3.2 Moisture Content and pH of Cricket Flour Preserved with Ginger and Garlic Extracts

The moisture content and pH of the samples increased with the increase in the number of days that the samples were in storage (Table 6.2). However, the rise was more significant ($p<0.05$) across the various storage stages than it was within samples. The moisture level ranged from 1.21 ± 0.32 to 1.48 ± 0.11 % on day 0; by day 60 of storage, it was 2.82 ± 0.08 to 3.02 ± 0.46 %. At day 0, the cricket flour preserved with ginger-garlic mixed extracts had the lowest pH (6.39 ± 0.00), while the cricket flour treated with sodium benzoate had the highest (6.43 ± 0.00). The sodium benzoate-preserved samples had the lowest pH at the end of the storage period, whereas the ginger-preserved cricket flour had the highest pH (6.75 ± 0.01 and 6.90 ± 0.03, respectively).

Table 6.2: Changes in Moisture and ph Of Cricket Flour Preserved with Extracts of Ginger and Garlic

Sample	Storage (days)	Moisture Content	pH
C+G	0	1.47 ± 0.00^{a}	6.42 ± 0.01^{bcd}
	30	2.34 ± 0.33^{ab}	6.46 ± 0.01^{e}
	60	2.9 ± 0.4^{b}	6.90 ± 0.03^{i}
C+Ga	0	1.21 ± 0.32^{a}	6.40 ± 0.01^{ab}
	30	1.90 ± 0.16^{ab}	6.41 ± 0.01^{abc}
	60	2.9 ± 0.4^{b}	6.85 ± 0.01^{h}
C+GGa	0	1.42 ± 0.51^{a}	6.39 ± 0.00^{a}
	30	2.14 ± 0.27^{ab}	6.45 ± 0.01^{de}
	60	2.9 ± 0.3^{b}	6.84 ± 0.01^{h}
C+SB	0	1.44 ± 0.93^{a}	6.45 ± 0.01^{de}
	30	2.33 ± 0.65^{ab}	6.43 ± 0.00^{cde}
	60	2.8 ± 0.1^{b}	6.75 ± 0.01^{f}
C	0	1.48 ± 0.11^{a}	6.42 ± 0.01^{abc}
	30	2.53 ± 0.14^{ab}	6.43 ± 0.01^{cde}
	60	3.0 ± 0.5^{b}	6.81 ± 0.01^{g}
P value		***0.9714***	***<.001***

Values = Mean (n=3) ±SD. Different superscripts along the column are significantly different ($p<0.05$). P values are the interaction effects of sample treatment and storage duration; C: cricket flour; G: ginger; Ga: garlic; GGa: ginger + garlic; SB: sodium benzoate.

6.3.3 Microbiological profile and safety of spice preserved cricket flour

While *E. coli* and fecal coliforms were not detected (ND), the total microbial load and yeast and mold counts decreased with storage duration (Table 6.3). Total microbial load of garlic preserved flour (1.91 log cfu/g) was the lowest at the end of the storage period, while interaction between treatment and storage duration was not significant ($p>0.05$). However, a significant decline in yeast and molds was observed ($p<0.05$); at the end of the 60-day storage period, sodium benzoate and garlic treated samples had a significantly low population of yeast and molds (1.91 log cfu/g).

Table 6.3: Effect of Storage on Microbial Profile of Spice Extract Preserved Cricket Flour

Sample (flour)	Storage (Days)	Total plate count (log cfug)	Fecal coliforms	*Escherichia coli*	Yeast & molds (log cfug)
C+G	0	3.62 ± 0.02^{bcd}	ND	ND	3.03 ± 0.11^{c}
	30	3.19 ± 0.22^{bcd}	ND	ND	2.30 ± 0.12^{abc}
	60	2.50 ± 0.16^{ab}	ND	ND	2.06 ± 0.21^{ab}
C+Ga	0	3.07 ± 0.29^{abc}	ND	ND	2.65 ± 0.06^{abc}
	30	2.54 ± 0.21^{abc}	ND	ND	2.63 ± 0.34^{abc}
	60	1.91 ± 0.00^{a}	ND	ND	1.91 ± 0.00^{a}
C+GGa	0	3.18 ± 0.13^{bcd}	ND	ND	2.93 ± 0.09^{c}
	30	3.37 ± 0.76^{bcd}	ND	ND	2.81 ± 0.08^{bc}
	60	2.45 ± 0.34^{ab}	ND	ND	2.06 ± 0.21^{ab}
C+SB	0	3.72 ± 0.02^{cde}	ND	ND	$2.56 \pm .07^{abc}$
	30	3.34 ± 0.07^{abc}	ND	ND	2.26 ± 0.49^{abc}
	60	2.56 ± 0.07^{abc}	ND	ND	1.91 ± 0.00^{a}
C	0	4.80 ± 0.04^{e}	ND	ND	4.66 ± 0.01^{d}
	30	4.23 ± 0.05^{de}	ND	ND	3.89 ± 0.10^{d}
	60	2.74 ± 0.18^{abc}	ND	ND	2.91 ± 0.00^{c}
P value		***0.088***			***<0.001***
Standard (KEBs, 2020)		10^5 cfu/g		<10 cfu/g	10^2 cfu/g

Values = Mean (n=3) ± SD, different superscripts along the column are significantly different ($p<0.05$).
P values are the interaction effects of sample treatment and storage duration

6.3.4 Sensory Evaluation of Cricket Flour Preserved with Spice Extracts

The sensory attributes of treated and untreated cricket flour are shown in Table 6.4. The color and aroma of the cricket flour was significantly different among samples during storage ($p<0.05$), decreasing with the length of time in storage.

Table 6.4: Effect of Treatment and Storage on Sensory Quality and Acceptability of Cricket Flour

Sample	Storage (Days)	Color	Aroma	Texture	Acceptability
C+G	0	3.67±0.94^{c}	3.59±1.09^{c}	3.89±0.78^{cd}	3.77±0.64^{d}
	30	3.41±0.90^{c}	2.74±1.13^{ab}	3.55±1.06^{bc}	3.36±0.85^{bc}
	60	2.74±0.98^{ab}	2.75±1.02^{ab}	3.30±1.03^{ab}	3.25±0.9^{bc}
C+Ga	0	3.84±0.86^{c}	3.44±1.09^{c}	4.07±0.85^{de}	3.72±0.76^{d}
	30	3.31±0.88^{bc}	2.81±0.92^{ab}	3.47±1.10^{abc}	3.24±0.86^{bc}
	60	2.68±0.98^{a}	2.40±1.11^{a}	3.13±1.14^{a}	2.94±1.05^{ab}
C+GGa	0	3.72±0.92^{c}	3.54±1.03^{c}	4.11±0.97^{e}	3.77±0.76^{d}
	30	3.31±0.80^{bc}	2.92±1.16^{b}	3.43±0.99^{abc}	3.29±0.82^{bc}
	60	2.62±0.97^{a}	2.69±0.98^{a}	3.15±0.89^{a}	3.02±1.01^{ab}
C+SB	0	3.67±0.85^{c}	3.41±1.09^{c}	4.10±0.96^{e}	3.72±0.80^{d}
	30	3.40±0.77^{c}	2.72±1.20^{ab}	3.59±1.01^{bc}	3.50±0.78^{c}
	60	2.70±1.01^{a}	2.79±0.99^{ab}	3.11±0.97^{a}	3.08±0.87^{b}
C	0	3.72±0.99^{c}	3.52±0.99^{c}	3.90±0.98^{cd}	3.59±0.84^{cd}
	30	3.31±0.92^{bc}	2.52±1.10^{a}	3.52±1.06^{bc}	3.43±0.73^{c}
	60	2.55±0.99^{a}	2.40±1.01^{a}	3.26±1.08^{ab}	2.83±1.01^{a}
P value		*(0.898,0.000*)*	*0.189, 0.000*)*	*(0.994, 0.000*)*	*(0.222, 0.000*)*
		0.965	***0.479***	***0.710***	***0.535***

Values= Mean ± SD, N= 60, 58, 53 for Day 0, 30 & 60. Different superscript along the columns= significant difference (P<0.05). P values in the brackets are the main effects of treatment and storage, respectively; outside the bracket is the interaction effect. C: cricket; G: ginger extracts; Ga: garlic extracts; GGa: ginger and garlic extracts; SB: sodium benzoate.

The aroma of the treated and untreated cricket flour decreased significantly with the length of time in storage ($p<0.05$), and the aroma was the least rated of all the sensory parameters. Texture was rated higher than other sensory attributes in all the samples, scoring highest on day 0 in samples treated with ginger-garlic mixed extract (4.11 ± 0.97) and sodium benzoate (4.10 ± 0.96). The sodium benzoate and spice-treated samples were all accepted by the panelists up to 60 days of storage with differing levels of acceptability ($p<0.05$); but the untreated sample was not appreciated at day 60 of storage, with a score of 2.83 ± 1.01. In the sensory parameters, the interaction effect of two factors (sample treatment and storage duration) was not significant ($P>0.05$). The main effect of sample treatment was not significant, but storage duration was significant ($P<0.05$). The sensory evaluation of samples by male and female panelists is shown in Table 6.5. In general, the average color score of the male panellists was higher than that of the females. Male and female panelists significantly differed in their assessments of the color of ginger-preserved cricket flour at days 0 and 60 of storage ($p<0.05$). Additionally, males scored the color of sodium

benzoate and garlic-preserved cricket flour substantially higher than females at day 0 and 60 of storage ($p<0.05$), respectively.

Male and female panelists significantly differed ($p<0.05$), in their opinions of the aroma after 30 days of storage of cricket flour preserved with a combination of ginger and garlic extracts, as well as after 60 days of storage of sodium benzoate preserved cricket flour. Scores of texture and overall acceptability ratings of the treated and untreated cricket flour samples did not significantly vary between the genders during storage. However, after 60 days of storage of the sodium benzoate preserved samples, the average overall acceptability of the male panelists (3.47 ± 0.92) was statistically higher than that of the female panelists (2.92 ± 0.82).

6.3.5 Principle Component Analysis from Variables of Spice Preserved Cricket Flour Samples

There were strong negative correlations seen between the moisture content, pH, and sensory qualities, while strong positive correlations were seen between the sensory attributes and overall acceptability (Table 6.6). The eigenvectors of the PCA are shown in Table 6.7. The contribution of each PC is shown in Table 6.8. Out of the 12 components generated, components 1 and 2 explained most of the variations (89.1%) in the characteristics of the products.

Table 6.5: Sensory Rating of Spice Preserved Cricket Flour and Acceptability among Male and Female Panelists

Parameter	Storage Days	Gender	Samples				
			C+G	**C+Ga**	**C+GGa**	C+SB	C
colour	0	Male	4.04±0.52*	4.19±0.62*	3.78±0.89	3.81±0.79	3.78±1.01
		Female	3.38±1.10*	3.56±0.93*	3.68±0.95	3.56±0.89	3.68±0.98
		P value	***0.006***	***0.004***	***0.671***	***0.246***	***0.693***
	30	Male	3.55±1.15	3.45±1.05	3.40±0.82	3.55±0.76	3.35±0.99
		Female	3.34±0.75	3.24±0.79	3.26±0.79	3.32±0.77	3.29±0.90
		P value	***0.407***	***0.387***	***0.540***	***0.275***	***0.814***
	60	Male	3.27±0.70*	3.00±0.85	3.00±0.76	3.13±0.64*	2.80±1.01
		Female	2.53±1.01*	2.55±1.01	2.47±1.01	2.53±1.08*	2.45±0.98
		P value	***0.012***	***0.134***	***0.073***	***0.048***	***0.247***
Aroma	0	Male	3.56±1.09	3.33±1.04	3.56±0.89	3.37±1.08	3.63±0.93
		Female	3.62±1.10	3.53±1.13	3.53±1.10	3.44±1.11	3.44±1.05
		P value	***0.827***	***0.489***	***0.922***	***0.803***	***0.466***
	30	Male	2.85±1.23	2.45±0.89	3.10±1.17*	2.75±1.41	2.50±1.05
		Female	2.68±1.09	2.37±0.94	2.47±1.11*	2.71±1.09	2.53±1.13
		P value	***0.601***	***0.750***	***0.049***	***0.906***	***0.932***
	60	Male	3.00±0.93	2.87±0.92	2.87±0.92	3.40±1.06*	2.60±0.74
		Female	2.66±1.05	2.79±1.19	2.79±1.19	2.55±0.86*	2.32±1.09
		P value	***0.274***	***0.822***	***0.200***	***0.004***	***0.360***
Texture	0	Male	3.96±0.81	4.04±0.90	4.11±0.97	4.22±0.93	3.93±1.24
		Female	3.82±0.76	4.09±0.83	4.12±0.98	4.00±0.98	3.88±0.73
		P value	***0.491***	***0.818***	***0.979***	***0.374***	***0.865***
	30	Male	3.55±1.00	3.50±0.89	3.45±0.89	3.551.00±	3.50±0.83
		Female	3.55±1.11	3.45±1.20	3.42±1.06	3.61±1.03	3.53±1.18
		P value	***0.993***	***0.864***	***0.917***	***0.845***	***0.9210***
	60	Male	3.33±0.90	3.33±1.05	3.47±0.74	3.27±0.88	3.07±0.88
		Female	3.29±1.09	3.05±1.18	3.03±0.91	3.05±1.01	3.34±1.15
		P value	***0.891***	***0.426***	**0.104**	***0.476***	***0.407***
Acceptability	0	Male	3.89±0.51	3.81±0.62	3.81±0.74	3.78±0.58	3.56±0.93
		Female	3.68±0.73	3.65±0.85	3.74±0.79	3.68±0.94	3.62±0.76
		P value	***0.202***	***0.394***	***0.689***	***0.627***	***0.778***
	30	Male	3.45±1.00	3.35±1.04	3.35±0.88	3.65±0.67	3.55±0.76
		Female	3.32±0.77	3.18±0.77	3.26±0.79	3.42±0.83	3.37±0.71

Parameter	Storage Days	Gender	Samples				
			C+G	**C+Ga**	**C+GGa**	C+SB	C
		P value	***0.573***	***0.493***	***0.704***	***0.291***	***0.371***
	60	Male	3.53±0.92	3.33±0.98	3.33±1.05	3.47±0.92*	2.93±0.96
		Female	3.13±0.88	2.79±1.04	2.89±0.98	2.92±0.82*	2.79±1.04
		P value	***0.143***	***0.088***	***0.156***	***0.039***	***0.646***

Values =Mean ± SD, N = 60, 58, 53 for Days 0, 30, and 60; males = 27, 20, 15; females = 34, 38, 38, respectively. Superscripts (*) show a significant difference between gender groups ($p<0.05$). (C: cricket only; G: ginger extracts; Ga: garlic extracts; GGa: ginger and garlic extracts; SB: sodium benzoate)

Table 6.6: Correlation Matrix of Variables from Analysis of Cricket Flour Preserved with Ginger and Garlic Extracts

Variable	mc	ph	L*	a*	b*	ΔE*	TVC	y& m	color	aroma	texture	acceptance
Moisture	1.000											
pH	0.835	1.000										
L*	-0.904	-0.627	1.000									
a*	-0.932	-0.784	0.827	1.000								
b*	-0.896	-0.585	0.966	0.847	1.000							
ΔE*	0.912	0.961	-0.780	-0.871	-0.728	1.000						
TVC	-0.440	-0.569	0.457	0.278	0.361	-0.502	1.000					
y&m	-0.457	-0.534	0.497	0.358	0.393	-0.488	0.880	1.000				
color	-0.945	-0.932	0.836	0.895	0.799	-0.980	0.544	0.513	1.000			
aroma	-0.918	-0.624	0.938	0.796	0.933	-0.753	0.399	0.397	0.811	1.000		
texture	-0.941	-0.789	0.886	0.865	0.898	-0.864	0.539	0.473	0.932	0.873	1.000	
acceptance	-0.898	-0.806	0.851	0.833	0.832	-0.896	0.486	0.416	0.939	0.863	0.937	1.000

Mc= moisture content, L*: lightness, a*: redness/greenness, b*: yellowness/blueness, ΔE*: total color change, y&m: yeast and molds

Table 6.7: Principal Components (Eigenvectors) from Analysis of Cricket Flour Preserved with Ginger and Garlic Extracts

Variable	Comp1	Comp2	Comp3	Comp4	Comp5	Comp6	Comp7	Comp8	Comp9	Comp10	Comp11	Comp12
mc	-0.3201	0.1179	0.0197	0.1392	0.1487	0.4373	-0.1367	0.1033	0.3629	0.0263	0.6143	0.3339
ph	-0.2835	-0.1391	0.5394	0.0381	0.2051	0.2116	0.1266	0.233	-0.4076	-0.0507	0.1599	-0.5064
L^*	0.3026	-0.1057	0.3768	-0.0992	-0.1464	0.3147	-0.4926	0.1149	-0.3371	-0.0179	-0.1848	0.4702
a^*	0.2973	-0.213	-0.1383	-0.5255	0.4052	-0.0291	0.2621	0.5449	0.0853	-0.0785	0.0857	0.1397
b^*	0.2942	-0.204	0.3958	-0.0934	0.1993	-0.0255	-0.315	-0.2213	0.6298	0.0731	-0.0545	-0.336
ΔE^*	-0.3076	-0.01	0.3755	0.0396	0.2519	-0.2194	0.3017	-0.0432	0.0922	0.526	-0.3138	0.4179
TVC	0.1886	0.6537	0.1356	0.3469	0.1472	-0.2774	-0.1832	0.4972	0.0765	-0.0453	-0.0782	-0.0696
Y&m	0.1868	0.6341	0.2153	-0.4719	-0.0571	0.2614	0.3112	-0.3446	-0.0348	0.0035	0.0676	-0.0306
color	0.3197	0.0049	-0.244	0.0657	0.0324	0.1204	-0.1055	0.0282	-0.2186	0.8079	0.2468	-0.2122
aroma	0.2959	-0.172	0.3438	0.1513	-0.5775	-0.2623	0.3664	0.1378	0.1078	0.0157	0.4029	0.1096
texture	0.3162	-0.0531	0.0532	0.279	0.5305	-0.2529	0.0549	-0.4323	-0.2982	-0.2185	0.3217	0.2028
acceptance	0.3096	-0.081	-0.0678	0.4845	0.0779	0.5681	0.4299	0.0379	0.1442	-0.074	-0.3417	-0.0094

Mc = moisture content, L*: lightness, a*: redness or greenness, b*: yellowness or blueness, ΔE*: Total color change. TVC: total viable count; Y&M: yeast and mold; Comp: principal component.

Table 6.8: The Contribution of Principle Components

Component	Eigenvalue	Difference	Proportion	Cumulative
Comp1	9.2763	7.8550	0.7730	0.7730
Comp2	1.4213	0.6972	0.1184	0.8915
Comp3	0.7241	0.4777	0.0603	0.9518
Comp4	0.2464	0.1299	0.0205	0.9724
Comp5	0.1165	0.0331	0.0097	0.9821
Comp6	0.0834	0.0211	0.0070	0.9890
Comp7	0.0623	0.0282	0.0052	0.9942
Comp8	0.0341	0.0107	0.0028	0.9971
Comp9	0.0234	0.0172	0.0020	0.9990
Comp10	0.0062	0.0023	0.0005	0.9995
Comp11	0.0040	0.0023	0.0003	0.9999
Comp12	0.0017	.	0.0001	1.0000

Principal Component (PC) 1 had 77.3% of the variance and PC2 had 11.8% of the variance (Table 6.9). The samples were clustered, with the day 0 samples on the right side of PC1; the day 30 samples on the intersection between PC1 and PC2-negative and positive sides; the day 60 samples on the PC2-positive side; and the control sample at day 60 on the PC2-negative side. Color measurements and all sensory attributes are located on the positive side of PC 1, while moisture content and pH are located on the negative and positive sides of PC 2, respectively. On PC1, total viable count, yeast, and molds were all negatively correlated. Day 0 samples had a higher predicted value (Figure 6.1).

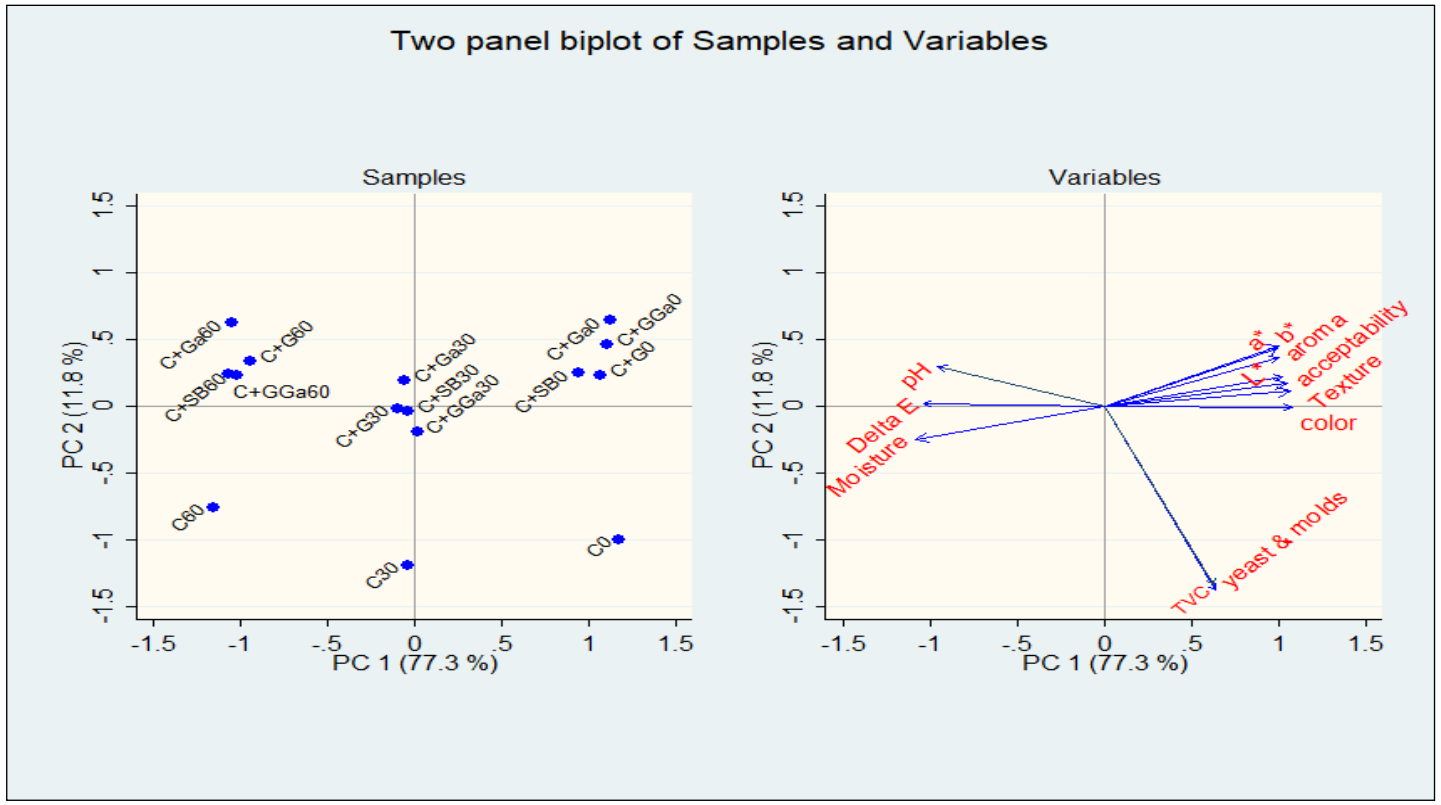


Figure 6.1: Biplot of Loading and Sample Scores of Cricket Preserved Flours

C: Cricket, G: ginger extracts, Ga: garlic extracts, GGa: ginger+garlic extracts, SB: sodium benzoate; 0, 30, 60 are days of storage

Table 6.9: PCA Variables and Predicted Values on Cricket Flour Preserved with Ginger and Garlic Extract

Samples	PCA variables												Predicted values		
	Mc	pH	L*	a*	a*	ΔE	TVC	Y&m	Color	Aroma	Texture	Accept	PC1	PC2	ID
C+G0	1.47	6.42	31.9	4.7	16.1	0	3.62	3.03	3.67	3.59	3.89	3.77	3.616727	-0.48943	3.070928
C+G30	2.34	6.46	26.67	3.6	10	2.29	3.19	2.3	3.41	2.74	3.55	3.36	-0.30753	0.03268	-0.26231
C+G60	2.87	6.9	25.07	3.23	8.97	10.05	2.5	2.06	2.74	2.75	3.3	3.25	-3.16103	-0.71311	-2.83565
C+Ga0	1.21	6.4	32	4.97	16.3	0	3.07	2.65	3.84	3.44	4.07	3.72	3.800794	-1.36499	3.114147
C+Ga30	1.9	6.41	25.43	4.3	9.53	2.09	2.54	2.63	3.31	2.81	3.47	3.24	-0.1771	-0.41281	-0.20843
C+Ga60	2.93	6.85	26.07	3.73	10.23	8.59	1.91	1.91	2.68	2.4	3.13	2.94	-3.52552	-1.31598	-3.23183
C+GGa0	1.42	6.39	31.2	4.73	16.57	0	3.18	2.93	3.72	3.54	4.11	3.77	3.732486	-0.97096	3.107294
C+GGa30	2.14	6.45	26.83	4	10.27	2.27	3.37	2.81	3.31	2.92	3.43	3.29	0.059678	0.413315	0.106684
C+GGa60	2.88	6.84	25.8	2.63	9.67	9.04	2.45	2.06	2.62	2.69	3.15	3.02	-3.61329	-0.50232	-3.19978
C+SB0	1.44	6.43	31.07	4.4	14.4	0	3.72	2.56	3.67	3.41	4.1	3.72	3.175631	-0.53161	2.682857
C+SB30	2.33	6.45	25.57	3.8	10	2.18	3.34	2.26	3.4	2.72	3.59	3.5	-0.12288	0.078326	-0.09613
C+SB60	2.82	6.75	24.67	2.87	8.5	8.86	2.56	1.91	2.7	2.79	3.11	3.08	-3.42499	-0.48578	-3.0343
C0	1.48	6.42	32.9	4.33	15.07	0	4.8	4.66	3.72	3.52	3.9	3.59	3.97544	2.12326	3.729244
C30	2.53	6.43	25.97	3.47	9	2.87	4.23	3.89	3.31	2.52	3.52	3.43	-0.13609	2.526708	0.217852
C60	3.02	6.81	23.3	2.83	8.63	11.71	3.74	2.91	2.55	2.4	3.26	2.83	-3.89232	1.612691	-3.16059

Mc = moisture content, L*: lightness, a*: redness or greenness, b*: yellowness or blueness, ΔE*: Total color change. TVC: total viable count; Y&M: yeast and mold; PC: principal component; ID: predictor value.

6.4 Discussion

6.4.1 Color, Moisture and pH of Cricket Flour Samples during Storage

In all samples, L* (lightness) was greatest on day 0 and lowest on day 60 of storage. Similarly, a* was highest on day 0 and lowest on day 30, though there was a non-significant difference among samples and storage duration, and the samples' tendency to yellowness (b*) declined over the course of storage with no significant variation between samples. This could show the impact of long-term storage on flour color. Cricket flour had lower lightness values than reported for other kinds of flour (Deepa & Umesh, 2017; Gerardi et al., 2022; Uchechukwu-Agua et al., 2015). Differences in color values L* a* b* of flour from previous studies were attributed to the color of the raw materials and other ingredients that are used in the flour blend. In this study, ginger and garlic extracts were used to preserve crickets rather than powder or paste, and this did not significantly impact the color. According to Kaur et al. (2013), differences in the color characteristics of flour could be attributed to differences in the colored pigments of the flour and the level of oxidation, which in turn depend on the composition of the flour.

During storage, there was a general decrease in the lightness, a tendency towards the redness and yellowness of the flour. This could be attributed to the chemical changes that occur during storage as a result of the interaction of the nutrients in the flour with the environment. The total color change (ΔE*) was highest in untreated flour samples on day 60 (11.71 ± 0.55) and lowest in garlic (8.59 ± 1.06) and sodium benzoate (8.86 ± 0.35) preserved flour. This indicates the potential of the garlic and sodium benzoate to reduce chemical reactions taking place in food as a result of the interaction of nutrients in the flour with the environment. Deterioration of color during storage may also indicate a loss of nutritional content as a result of the auto-oxidation reactions (Uchechukwu-Agua et al., 2015).

Color features in a color space with their respective indices are widely used in food product inspection (Siswantoro, 2019). Specific applications of color measurements in food inspection include grading, detection of anomalies or damage, detection of specific content, and evaluation of color changes. According to Pathare et al. (2013), the automated visual inspection systems for food products applying computer vision have essentially substituted the role of traditional inspection, which is frequently costly, labor-intensive, and unreliable. In the food industry, color is a crucial quality element that affects consumers' tastes and choices (Wu et al., 2013).

During the 60 days of ambient storage in polyethylene bags, all samples showed a non-significant rise in moisture content. Similarly, after 90 days of storage in polyethylene bags, flour samples from infant food maintained a constant moisture level (Forsido et al., 2021). The ability of the packaging material to reduce moisture exchange with the storage environment was associated with moisture stability. Polyethylene bags have a water vapour permeability of 86 g·μm/m2 ·day·kPa at 27°C and 100 % RH, which is lower than reported for other kinds of packaging materials at the same storage conditions (Wang et al., 2018). Since flour is a product that is moisture-sensitive, long-term storage in less permeable packaging material is a requirement for increasing shelf life and maintaining product quality (Li et al., 2018). Moisture levels greater than 12% permit microbial growth and product degradation (Kaur et al., 2013). Therefore, the low moisture content reported in this study suggests that ambient storage conditions (23± 2°C, 60% RH) are sufficient to maintain the quality attributes and sustain the shelf life of cricket flour past 60 days of storage.

The pH of the cricket flour in this investigation varied significantly between samples and over the storage period, rising gradually from day 0 to day 30, and drastically from day 30 to day 60. This trend was attributed to the pattern of formation of the chemical substance responsible for influencing pH in the sample. This is consistent with a pattern that was previously observed for meat-based products in storage (Kakimov et al., 2019; Mgbemere et al., 2011). In this study, the likely accumulation of slightly alkaline components due to autolytic breakdown or microbial activity was thought to cause the

increase in pH from 6.39 ± 0.00 to 6.90 ± 0.03 of cricket flour during the 60-day storage period, which is in agreement with previous reports (Liu et al., 2010). It is asserted that flours with pH levels in these ranges are acceptable (Apea-Bah et al., 2011).

6.4.2 Microbial Profile and Safety of Cricket Flour Samples

The microbial population of samples decreased as storage time increased, with spice extract-treated and sodium benzoate-treated samples exhibiting a greater reduction. This was attributed to components in the extract being able to inhibit certain key metabolic processes in the microbes, reducing their ability to reproduce. The results were similar to those that were reported for several brands of cricket powder on the retail market in the United States of America. However, lower counts of 1.98 ± 0.28 and 1.64 ± 0.01 log cfu/g for TVC and yeast and molds were reported for house cricket (*A. domesticus*) flour (Fröhling et al., 2020). The disparity could be attributed to species variation and a difference in the processing conditions in the previous studies. Klunder et al. (2012) reported a significant reduction in TVC from 5.4 log cfu/g on day 0 to a non-detectable level on days 10–16 of storage of house cricket (*A. domesticus*) flour. At the end of 12 weeks of storage, a TVC of < 6 log cfu/g was reported in cricket (*A. domesticus*) powder, while yeast and molds were not detected (Messina et al., 2019).

During the 60 days of storage, this study did not detect *E. coli* or fecal coliforms in the cricket powder. This was attributed to the heat treatment (drying at 105 °C for two hours), which was sufficient to destroy the fecal coliforms during the flour preparation, coupled with hygienic preparation processes. Live crickets are associated with a variety of pathogenic organisms, including fecal coliforms and *E. coli* (Grispoldi et al., 2021). Therefore, their absence from a food product is indicative of quality and safety (Woh et al., 2017). Moreover, the East African standard for insect foods requires <1 log cfu/g for ready-to-use insect products (KEBS, 2020). The general reduction in the microbial population during the storage period is probably due to the low moisture content resulting in low water activity (Jakab et al., 2020); also, the packaging in polyethylene material

limited the interaction of the insect powders with the environment, hence limiting the microbial growth requirements (Forsido et al., 2021).

Cricket flour treated with garlic extracts had the lowest TVC, yeast, and mold counts (1.91 ± 0.00 log cfu/g) at the end of the 60-day storage period. This was linked to the activity of garlic antimicrobial compounds, such as allicin, which is reported to have stronger antimicrobial activity than ginger (Adetunde et al., 2014; El-Sayed et al., 2017; Panpatil et al., 2013). The relatively high TVC, yeast and mold counts in the untreated sample are indicative of the effectiveness of the garlic and ginger extracts used in this study to treat the crickets prior to the flour processing. This is in agreement with earlier research on the ability of spice extracts to preserve food (Beristain-Bauza et al., 2019).

6.4.3 Sensory Quality and Acceptability Cricket Flour

Sensory quality is one of the most important features of any food product, including insects. The panelists liked the color of both treated and untreated cricket flour, but the scores dropped as the number of days the flour was stored increased. According to Pathare et al. (2013), the processing, handling, and storage of food induce a variety of chemical, biological, microbial, and physical changes that impact the color of the food. In this study, the color of the flour produced by treating crickets with ginger and garlic extracts and processing them into flour did not change noticeably. However, both treated and untreated samples' color ratings were at their lowest on day 60 of storage, suggesting possible biochemical changes such as oxidation that occur during prolonged storage. Color could be the first sensory indication that consumers use to create their expectations of the overall quality of a product (Chonpracha et al., 2020).

The mean aroma score of treated and untreated samples was less than 3 (neither liked nor disliked) from 30 to 60 days of storage, receiving the lowest evaluation of all the sensory qualities that were evaluated. The natural aroma of the compounds in the cricket, which were released during the oven drying, was thought to be the source of the aroma of flour. There has been evidence of significant volatile compound variation among insect species

(Perez-Santaescolastica et al., 2022). In a previous study, sensory panelists poorly scored the aroma of the oven- and microwave-dried locust and silkworm in comparison to the samples that had been freeze-dried (Mishyna et al., 2020). Thermally treated foods, including insects, exhibit greater concentrations of lipid oxidation compounds and Maillard reaction byproducts (Liceaga, 2021; Lund & Ray, 2017; Perez-Santaescolastica et al., 2022). Therefore, it is crucial to select the ideal roasting or drying conditions for aroma quality. In a previous report, the characteristic aroma of roasted insects was shaped by 11 odor-active compounds, with insects roasted at 160 ℃ exhibiting the characteristic aroma of baked bacon (Żołnierczyk & Szumny, 2021). The aroma of food allows an initial evaluation of the taste that consumers can expect. However, enzymatic reactions, microbiological growth, or chemical changes in the food product may result in the production of new volatile compounds inside the food package during storage (Han et al., 2018). These changes can be directly related to the sensory quality of the food during storage.

The panelists appreciated the texture of both the treated and untreated cricket flour samples. Even though the texture rating decreased with storage, its mean score was higher than 3. This qualifies the flour for use in later food applications since texture influences other functional properties of the flour such as solubility, water binding capacity, swelling power, and pasting properties. In earlier research, consumers had a positive response to the textural qualities of foods enriched with cricket flour, including crackers (Akullo et al., 2018; Ardoin et al., 2021; Bartkiene et al., 2022; Duda et al., 2019). The acceptability of the treated and untreated cricket flour samples did not significantly differ. However, after 60 days of storage, a considerable decline was observed. This was attributed to the color and aroma's decreasing acceptability at that time. The same trend was observed when extruded composite flour was stored (Forsido et al., 2021). However, the type of packaging and duration of storage had an impact on how much the score decreased. Acceptability of cricket flour is a pre-requisite for its subsequent food application. Recent research has reported that cricket flour is acceptable to consumers when blended with other ingredients or in cooperation with other products (Burt et al., 2020; Liceaga, 2021;

Simeon et al., 2022; Zebib et al., 2020). In this study, male panelists scored sensory characteristics higher than female panelists, including overall acceptability. This demonstrates that males liked the cricket flour more than females did.

6.4.4 PCA of Color, pH, Microbial and Sensory Quality of Spice Preserved Cricket Flour

Two components were extracted from the PCA results, which accounted for 89.1% of the variability in the original data. On PC1 and PC2, samples were grouped based on how long they had been in storage. Furthermore, samples of cricket flour treated with spice extracts and samples treated with sodium benzoate clustered together, whereas samples of untreated cricket flour were independent. This is consistent with earlier results, in which PCA of meat stored for varying lengths of time revealed separate clusters based on the length of storage (Arsalane et al., 2017). Additionally, PCA was used to distinguish between fish samples from various storage times based on the volatile chemicals accumulated during storage (Zhao et al., 2021). Both the spice-treated and sodium benzoate-treated cricket flour had good color, aroma, and texture right after flour processing, which accounted for the relatively high acceptability at day 0 of storage. While the sensory quality slightly deteriorated after 30 days of storage, this in turn decreased consumer preference. Cricket flour had a high correlation between moisture, pH, and color change after 60 days of storage, indicating that the product quality had decreased as a result of possible autolytic or microbiological degradation, which explains the reduced sensory and overall acceptability. Compared to the spice-treated and sodium benzoate-treated samples, this shift was even more pronounced in the untreated samples. In contrast to the treated samples, the total viable count, yeast, and molds were strongly correlated with the untreated cricket flour. This could explain how the spice extracts influenced microbial multiplication in the cricket flour. Previous studies have shown that spices like garlic and ginger have antimicrobial properties (Abdalla & Abdallah, 2018; Sommano et al., 2016). Considering all the parameters, the Day 0 samples were the best, as revealed by the high predicted values.

6.5 Conclusion

The color change (ΔE), pH, and moisture content of cricket flour increased with the length of storage, while the microbial load decreased during storage. When compared, the untreated cricket flour had a higher total microbial load and more yeast and molds compared to the treated flour, with garlic extract-preserved flour having the lowest microbial load. Consumers liked both the spice-treated and control cricket flour samples, but acceptability significantly declined with storage time. A strong negative correlation existed between the ΔE, pH, and moisture content of the cricket flour and the sensory score, while there was a significant positive correlation between sensory scores and overall acceptability. Therefore, the study concluded that treating crickets with ginger and garlic extracts produces flour that is safe, shelf-stable and liked by consumers, although sensory score and acceptability decrease with prolonged storage. Further research is recommended on the bioactives, anti-nutrients, and nutrient composition of spice-preserved cricket flour to promote its use in the food industry.

CHAPTER SEVEN

CONCLUSION AND RECOMMENDATIONS

7.1 Conclusion

The phytochemical profile and antioxidant activity of two varieties of ginger, garlic, and turmeric that are often consumed in Uganda were determined using conventional methods. Ginger and turmeric extracts had higher total phenolic, flavonoid, and vitamin C content compared to garlic extracts, and the local ginger was superior to the hybrid ginger. The organic solvents were more efficient in extracting the phytochemicals in the spices than water; however, water extracts contained more vitamin C. The antioxidant activity of extracts assayed by DPPH was strongly correlated to the total phenolic and flavonoid content; the strongest antioxidant activity was recorded in the turmeric-acetone extracts and in the acetone and ethanol extracts of the local ginger.

The antimicrobial activity of ginger, garlic (local and hybrid) varieties, and turmeric extracts was investigated using the agar well diffusion method. The three spices exhibited good antimicrobial activities against *S. aureus*, *E. coli*, and *C. albicans* in a concentration-dependent manner. Garlic antimicrobial activities were generally stronger compared to ginger and turmeric, although ginger and turmeric were more potent antifungal agents. Raw extract/ juices of garlic and ginger were more effective than the aqueous and organic solvent extracts. Activity of garlic ethanolic extracts compared favorably with raw extracts against the respective organisms. MIC of garlic extracts varied from 2.5 to 10 mg/ml.

The hybrid ginger and garlic were chosen for the treatment of blanched crickets for flour production based on their availability in the market, phytochemical composition, antioxidant and antimicrobial activity. This was an attempt to minimize the process of lipid oxidation in cricket flour during storage. The results of the analysis showed that the most abundant fatty acids in the cricket flour were linoleic, oleic, and palmitic, with more unsaturated than saturated fatty acids.

There was a slight decrease in USFA and a little increase in SFA in samples treated with a combination of ginger and garlic extracts and stored for 60 days under ambient conditions, which compared favorably with flour from crickets treated with a synthetic preservative (SB preserved) samples. The PV and TBARs values of cricket flour increased during the 60 days of storage but remained within acceptable limits for safe food.

Treatment of blanched crickets with ginger and garlic extracts resulted in flour that was microbiologically safe, shelf-stable, and liked by consumers, although sensory score and acceptability decreased with prolonged storage. The pH, moisture content, and color change increased during storage but remained within acceptable limits after 60 days of storage at ambient conditions. The total microbial count, yeast, and mold significantly decreased with storage duration, while fecal coliforms and *E. coli* were not detected in any of the samples. Garlic-preserved cricket flour had the lowest total microbial load and yeast and molds compared to other treatments.

7.2 Recommendations

7.2.1 General Recommendations

1. Ginger, garlic, and turmeric have strong antioxidant and antimicrobial activity; hence, their use as natural antioxidants and antimicrobials in food is recommended as an alternative to chemical preservatives.
2. Among the solvents to use in extraction, the study recommends ethanol extraction of spices for food applications based on efficacy and safety.
3. The study recommends the use of a combination of ginger and garlic extracts for the treatment of blanched crickets in order to minimize the oxidation of fats in the flour during storage at ambient conditions.
4. Cricket flour packed in polyethylene bags can be stored for 60 days under ambient conditions without major changes in the fatty acid profile.

5. The study recommends the utilization of cricket flour as a food ingredient since it contains high levels of unsaturated fatty acids, especially the essential fatty acids that must be supplied to humans from their diet.
6. Utilization of cricket flour preserved with ginger and garlic extracts as an ingredient in various food applications to assess its suitability and sensory acceptability in blended flours is recommended.

7.2.2 Suggestions for further research

1. It is necessary to identify the bioactive compounds in the extracts of ginger, garlic, and turmeric and investigate the *in vivo* efficacy against free radicals to provide the additional information required to promote their use as additives in the food industry.

2. Research on the bioactivity, individual nutrient bioavailability, and anti-nutrient composition of spice-preserved cricket flour is necessary to promote its use in the food industry.

3. The effects of different packaging materials, storage conditions, and durations exceeding 60 days need to be studied to determine the exact shelf life of the cricket flour under different conditions.

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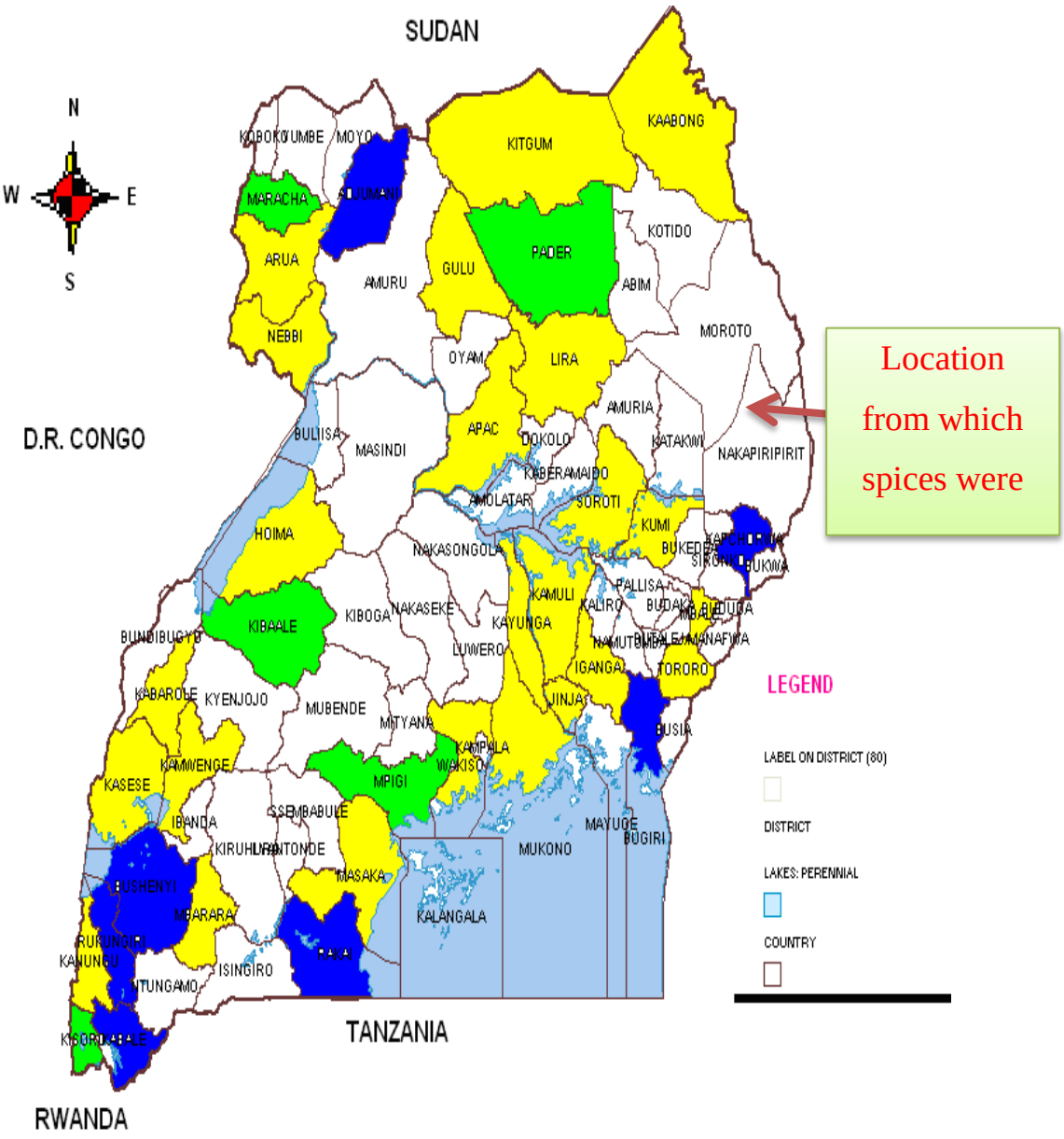
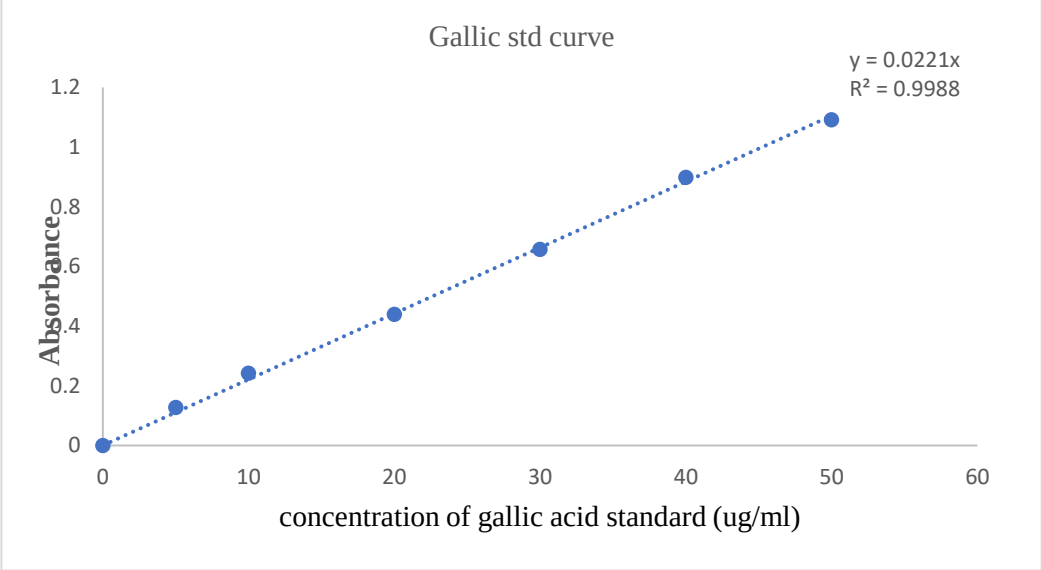
Zia-Ur-Rehman, Salariya, A. M., & Habib, F. (2003). Antioxidant activity of ginger extract in sunflower oil. *Journal of the Science of Food and Agriculture*, *83*(7), 624–629. https://doi.org/10.1002/jsfa.1318

APPENDICES

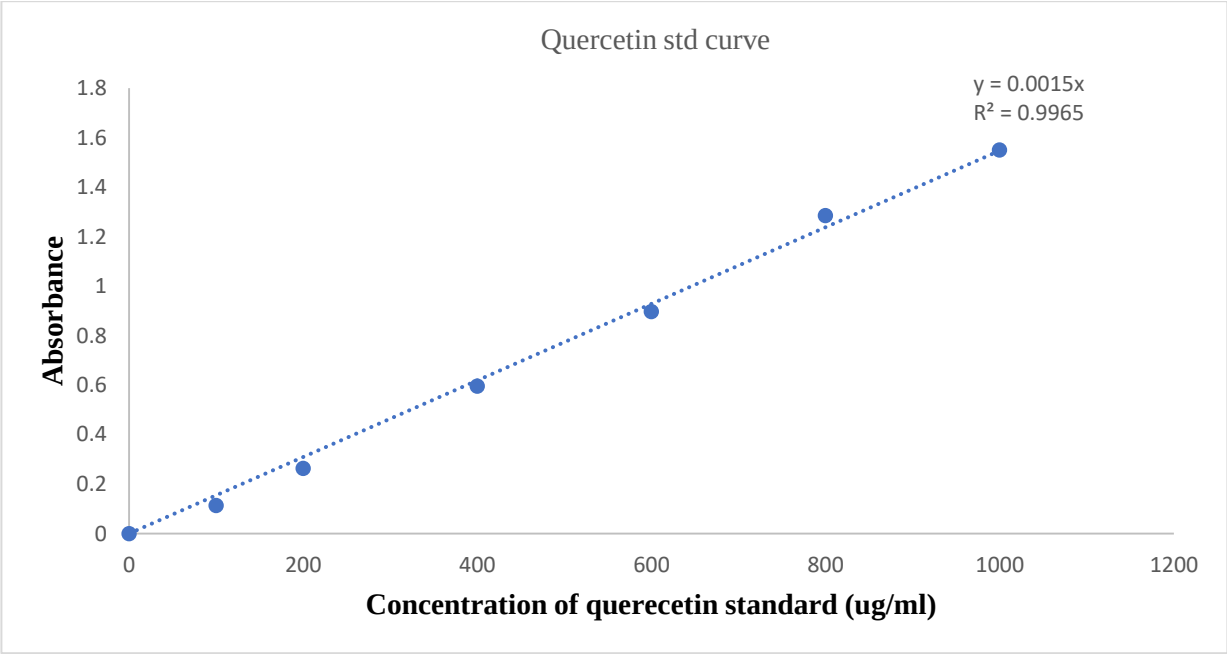
Appendix I: Study Area



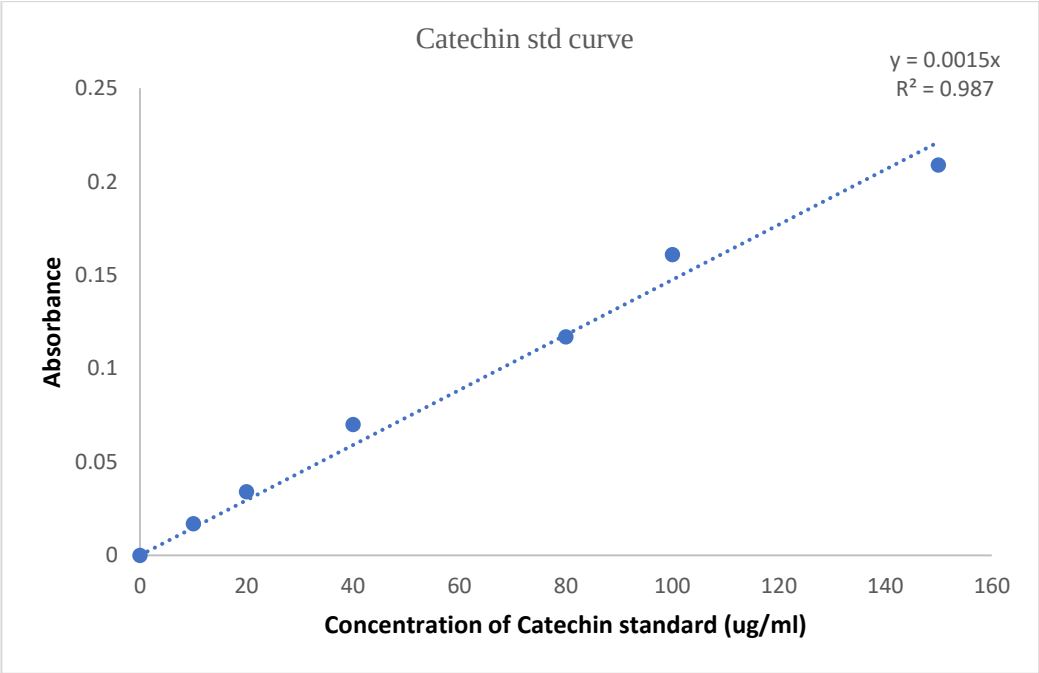
Appendix II: Gallic Acid Standard Curve



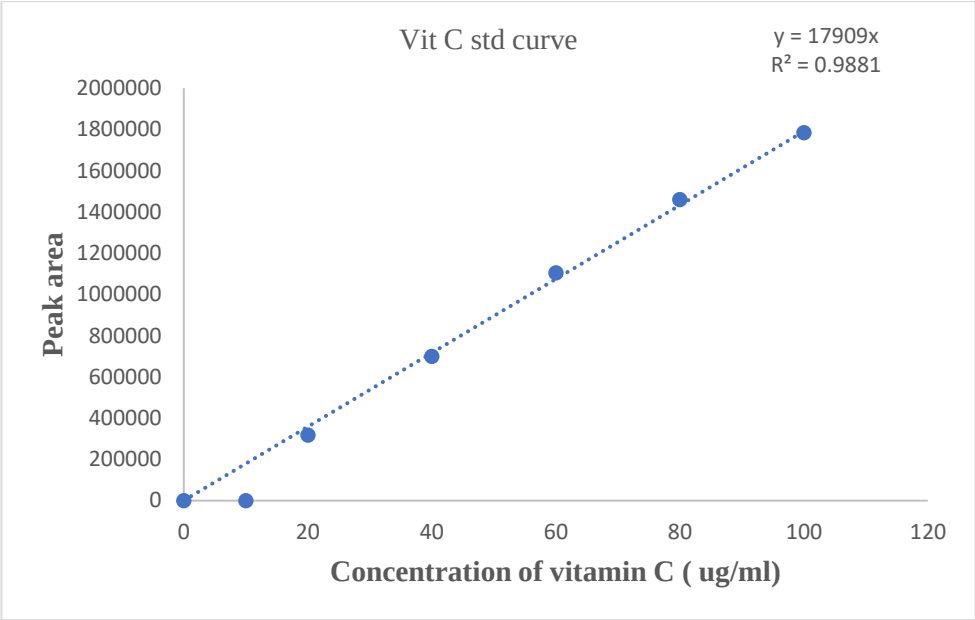
Appendix III: Quercetin Standard Curve



Appendix IV: Catechin Standard Curve



Appendix V: Vitamin C standard curve



Appendix VI: Published Papers

Akullo, J. O., Kiage, B., Nakimbugwe, D., & Kinyuru, J. (2022). Effect of aqueous and organic solvent extraction on in-vitro antimicrobial activity of two varieties of fresh ginger (Zingiber officinale) and garlic (Allium sativum). *Heliyon*, *8*(9), e10457. https://doi.org/10.1016/j.heliyon.2023.e18806

Akullo, J.O, Kiage-Mokua B, Nakimbugwe D, Ng'ang'a J, Kinyuru J. 2022. Potential Sources of Natural Antioxidants and Antimicrobials: Comparative Analysis of Turmeric (*Curcuma longa*) Extracts from Different Solvent Extraction Systems. *J Food Chem Nanotechnol* 8(4): 138-146. https://doi.org/10.17756/jfcn.2022-136

Akullo, **J. O**., Kiage-Mokua, B. N., Nakimbugwe, D., Ng'ang'a, J., & Kinyuru, J. (2023). Color, pH, microbiological, and sensory quality of crickets (Gryllus bimaculatus) flour preserved with ginger and garlic extracts. *Food Science & Nutrition*. https://doi.org/10.1002/fsn3.3262

Akullo, J. O., Kiage-Mokua, B. N., Nakimbugwe, D., & Kinyuru, J. (2023). Phytochemical profile and antioxidant activity of various solvent extracts of two varieties of ginger and garlic. *Heliyon*, *9*(8). https://doi.org/10.1016/j.heliyon.2023.e18806