ASSESMENT OF THE NUTRITIONAL VALUE, MICROBIAL QUALITY AND SENSORY ACCEPTANCE OF COOKIES ENRICHED WITH EDIBLE LONGHORN GRASSHOPER (*Ruspolia differens* Serville) FLOURS FROM DIFFERENT PROCESSING METHODS

ONYANGO BRIAN OCHIENG

A Thesis Submitted to the Graduate School in Partial Fulfillment of the Requirements for the Master of Science Degree in Food Science of Egerton University

EGERTON UNIVERSITY

JULY, 2023

DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been presented in this university or any other for the award of a degree

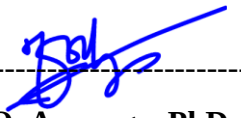
Signature: ----------  ----------Date: ----7th July, 2023----------

Brian Onyango Ochieng

KM16/14627/18

Recommendation

This thesis has been submitted with our approval as the university supervisors

Signature:  ----------Date: -11th July, 2023----------

Dr. Joseph O. Anyango, PhD

Department of Dairy and Food Science and Technology,

Egerton University, Njoro (Kenya).

Signature: ------  ----------Date: ---12th July 2023----------

Dr. John M. Nduko, PhD

Department of Dairy and Food Science and Technology,

Egerton University, Njoro, Nakuru, Kenya.

Signature: ----------  ---------- Date: --------7th July, 2023--------

Dr. Chrysantus M. Tanga, PhD

Head of Insect for Food, Feed and Other Uses

International Centre for Insect Physiology and Ecology (*icipe*)

Duduville Campus, Nairobi, Kenya.

COPYRIGHT

DEDICATION

This work is wholeheartedly dedicated to my parents; Mr. Edward Ochieng Odoo and Mrs. Annah Ochieng, my siblings; Fredrick Ochieng, Tonny Ochieng, Jackline Ochieng and Lovenah Ochieng, and my uncles; Benard Orimba, Gabriel Odoo and George Nyandare. Their words of inspiration, prayers and push for tenacity sustained my hard-working spirit through the course of this study.

ACKNOWLEDGEMENTS

Firstly, I thank the Almighty God for granting me life, good health and the intellectual capacity to undertake this study to its conclusion. Secondly, I wish to personally commend, Egerton University for granting me an opportunity for academic development, Centre of Excellence in Sustainable Agriculture and Agribusiness Management (CESAAM) and International Centre of Insect Physiology and Ecology (*icipe*) for financially supporting me and my research work. Thirdly, I also wish to express my deepest and profound gratitude to my university supervisor, Dr. Joseph Anyango for his relentless and indefatigable commitment to mentoring me and guiding my research work from its commencement to the end. I also thank Dr. John Nduko for his reliable and resourceful research advisory geared towards polishing my research work. I also gratefully acknowledge Prof. George Owuor of CESAAM, Egerton University, for enrolling me to the CESAAM family and providing the financial support to ensure that my M.Sc. course work and research are successfully completed. I wish to exceptionally appreciate Dr. Chrysantus Mbi Tanga for offering me a valuable opportunity at *icipe* and the necessary resources to conduct my experiments and publish the findings within the stipulated time. Special mention of Dr. Xavier Cheseto of *icipe* for his wise counsels and mentoring. I also treasure and cherish all the post-graduate students from the Department of Dairy and Food Science and Technology, Egerton University and *icipe* for their peer-to-peer discussions and deliberations that bolstered my skills and ultimately enriched my work. Finally, I wish to give special thanks to my parents, siblings and the family at large for their unending advice, financial and emotional support throughout my Masters programme.

ABSTRACT

Long-horned grasshopper (*Ruspolia differens*), an edible insect native to sub-Saharan Africa, is considered a delicacy by some communities in the region and contributes 5-10% of the protein intake. However, its full utilization as food across the different cultural constructs has been hampered by neophobia and disgust occasioned by uncommon food cultural practices, thereby limiting its potential to combat malnutrition in the region. Therefore, there is need to process and hide the insect into modern food products to improve its utilization. The current study investigated the effects of enriching cookies with differently processed *R. differens* flours on the nutritional composition, digestibility and volatile organic compounds profile, the microbial quality and the sensory acceptance on a 5-point hedonic scale. Blanched, boiled and toasted *R. differens*-based cookies demonstrated significantly (p<0.05) higher protein (10.90-11.09%) whereas deep-fried *R. differens*-based cookies exhibited higher fat (25.82%) and energy (514.69 kcal). The levels of essential amino acids; leucine (8.57-8.97 mg/g) and isoleucine (5.56-6.07 mg/g) were significantly (p<0.05) higher in blanched, boiled and toasted *R. differens*-based cookies than in control and deep-fried *R. differens*-based cookies. Omega-3 fatty acid, methyl (9*Z*,12*Z*,15*Z*)-octadecatrienoate (0.34-2.12µg/g) was only detected in the cookies integrated with the processed *R. differens* flours. Methyl hexadecanoate (5.02-10.58µg/g), methyl (9*Z*)-octadecenoate (11.55-17.20µg/g) and methyl (9*Z*,12*Z*)-octadecadienoate (0.19-12.10µg/g) were the dominant fatty acids in the different cookie types. Cookies supplemented with blanched and boiled *R. differens* exhibited significantly (p<0.05) higher levels of Fe (7.11-7.12 mg/100 g) and Zn (4.17-4.33mg/g). Pleasant aroma compounds; 2*E*,4*E*-dodecadienal, pentanal, and octanal, methyl pyrazine, furfurals, benzaldehyde, and 2-pentyl furan, were more pronounced in cookies prepared with boiled, toasted and deep-fried *R. differens*. The digestibility of *R. differens* based cookies and control cookies ranged between 71.23-80.41% and 88.22%, respectively. All the cookie types expressed permissible levels of total viable counts (>30 Log cfu/g), *Staphylococcus aureus*, *Salmonella spp* and yeast and moulds that are compliant with Kenya Bureau of Standards guidelines on insect-based products. Sensory evaluation suggested that panelists preferred the overall sensory characteristics of control (4.24) and deep-fried *R. differens*-based cookies (3.97) while lowly rating flavour of blanched *R. differens*-based cookies (3.17). Summarily, blanched and boiled *R. differens*-based cookies, expressed excellent nutritional profiles and digestibility. However, blanched *R. differens* requires masking of the objectionable flavours to enhance consumer preference.

Table of Contents

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND ACRONYMS

ANOSIM	Analysis of Similarities
AOAC	Association of Official Analytical Chemists
CBD	Central Business District
DCM	Dichloromethane
FAMEs	Fatty Acid Methyl Esters
FAO	Food and Agriculture Organization of the United Nations
GHGs	Green House Gases
icipe	International Centre for Insect Physiology and Ecology
LAB	Lactic Acid Bacteria
KEPHIS	Kenya Plant Health Inspectorate Services
MUFA	Monounsaturated Fatty Acids
PCA	Plate Count Agar
PDA	Potato Dextrose Agar
PUFAs	Polyunsaturated Fatty Acids
RDA	Recommended Dietary Allowance
RDI	Recommended Dietary Intake
SFA	Saturated Fatty Acids
SIMPER	Similarities of Percentages
TFC	Total Flavonoids Content
TVC	Total Viable Counts
VOCs	Volatile Organic Compounds
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Cookies are a popular snack among all the segments of a population including the young and old people (Bawa *et al*., 2020; Ho & Abdul-Latif, 2016; Noor-Aziah *et al*., 2012). They constitute the largest proportion of bakery products consumed as snacks globally. This is chiefly attributed to their ready-to-eat and grab-as-you-go nature, affordability due to low manufacturing cost and shelf stability due to their low water activity (Ho & Abdul-Latif, 2016). Recently, urbanization has reportedly led to their increased consumption (Noor-Aziah *et al*., 2012). Hence, they are the most appropriate food vehicles for conveyance of essential nutrients to target vulnerable populace (Awobuyusi *et al*., 2020). However, despite being popular snacks, cookies are energy dense foods because their endowment with high contents of fat and carbohydrate but low contents of protein and moisture (Florence *et al*., 2014). Therefore, their recipes can be adjusted to accommodate diverse ingredients to improve their nutritional profiles (Hrušková & Švec, 2015). Wheat flour is the largest component in cookie formulation ingredients. Being a cereal, wheat products have been reported to be low in protein content, vitamins, minerals and some essential amino acids like methionine, tryptophan, threonine and lysine (Lu *et al*., 2018; Okoye *et al*., 2016; Turfani *et al*., 2017). Efforts to revamp the nutritional content of wheat products using grains and pulses have not been successful since such plant-derived products are reportedly nutritionally incomplete with low essential amino acids profiles (Okoye *et al*., 2016) and are often associated with mycotoxin contamination (Belluco *et al*., 2013). Edible insects appear to be better candidates as alternative sustainable protein sources for modern food enrichment (Skotnicka *et al*., 2021).

Longhorn grasshopper (*Ruspolia differens*) locally known as *nsenene* in Luganda or *senene* in Kiswahili, is an edible insect of the family Tettigoniidae in the order Orthoptera and is native to the sub-Saharan Africa (Mmari *et al*., 2017; Ssepuuya *et al*., 2019). The insect is a common delicacy to the communities living in Lake Victoria region e.g. the Haya of Tanzania, Luo of Kenya and Baganda of Uganda, from ancient times (Mmari *et al*., 2017). It is a highly regarded delicacy consumed by locals as a snack or an accompaniment to a main meal (Mmari *et al*., 2017). Studies have shown that *R. differens* has about 43-48% protein content (Fombong *et al*., 2017;

Kinyuru *et al*., 2010). This protein content supersedes 10 g protein/100 g edible portion of a food required to regard a food as high protein according to Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) (Fombong *et al*., 2017), hence labelling it a good protein source. Moreover, the protein content of *R. differens* is much greater than that of meat (about 20-24%) (Ahmad *et al*., 2018) and eggs (12.5%) (Réhault-Godbert *et al*., 2019). It has also been shown that *R. differens* has some 46-48% fat content, approximately 3% mineral content and about 4-5% fibre content (Kinyuru *et al*, 2010). *Ruspolia differens* can therefore contribute to combating malnutrition in East Africa where it swarms in abundance (Mmari *et al*., 2017; Ssepuuya *et al*., 2019).

Africa being a tropical continent is home to numerous edible insects which have formed part of the food traditional heritage of the dwellers (Kelemu *et al*., 2015). Despite the abundance of diverse edible insects, the region still grapples with high prevalence of undernourishment (van Huis *et al* 2013) exacerbated by poverty, famine and food crises (Adeyeye *et al*., 2021; Kelemu *et al*., 2015). Poverty has been cited as the key drive to heightened chronic hunger in the region characterized by low energy intakes than the recommended 1800 calories per day (Adeyeye *et al*., 2017). Further, in the years 2014-2015, FAO established that one-nineth of hungry people in Africa suffered from chronic malnutrition (FAO *et al*., 2015), translating to 226 million starving starving people (Adeyeye *et al*., 2021). New scientific interventions have poised sustainable food sources like edible insects to potentially combat malnutrition (Mandal & Dutta, 2022).

Consumption of insects is, however, hampered by cultural beliefs disgust and food neophobia (Kröger *et al*., 2022). For instance, among the Haya community of Tanzania and the Baganda community of Uganda, pregnant women were prohibited from eating *R. differens* for fear of delivery children with cone-shaped (resembling the *R. differens*' head) (Kinyuru *et al*., 2018). Children were also not allowed to eat the insect as this was believed to render them dumb (Kinyuru *et al*., 2018). These cultural beliefs are thought to have been masterminded by men to selfishly enjoy the delicacy on their own (Mmari *et al*., 2017). Instead of pregnant women and children being the major nutritional beneficiaries of *R. differens*, such cultural beliefs have been a hindrance, encouraging child malnutrition in the region. Ironically, in Kagera region of Tanzania, where *R. differens* swarms in abundance and is considered a cultural delicacy, child stunting was reported to surpass the WHO upper ceiling for severe stunting (Mmari *et al*., 2017).

Neophobia has been another key factor contributing to the fear of eating edible insects (Kröger *et al*., 2022). Despite entomophagy being common habit in Africa, some communities like the Kikuyus from central Kenya still consider it as an uncivilized practice (Kinyuru *et al*., 2018). Furthermore, youngsters in some African counties i.e. Botswana, Zimbabwe and Nigeria have been reported to averse entomophagy (Melgar-Lalanne *et al*., 2019). In the Western countries, insect-based food neophobia had been in the rise due to the poor sensory qualities of the insects and therefore considered disgusting and 'barbaric' (Menozzi *et al*., 2017). However, consumer awareness on the nutritional, health and environmental benefits of edible insects has been linked to increasing insect acceptability in Europe (Menozzi *et al*., 2017). Further, previous studies have reported that inclusion of edible insects in ground or powdered form into conventional products with known flavours have improved consumer perception of edible insects in the Western European countries (Caparros-Megido *et al*., 2016). Earlier, edible insects have been ground and used to enrich bakery wheat products such as cookies, biscuits and bread (Adeboye *et al*., 2016; Awobusuyi *et al*., 2020; González *et al*., 2019; Ogunlakin *et al*., 2018; Ojinnaka *et al*., 2015; Osimani *et al*., 2018).

Wheat has been substituted with insect flours, fruit pulps, flours from cereal grains and pulses for cookie formulation to establish the effects on the nutritional value, dough forming properties, physicochemical properties, shelf-life and sensory properties of the product (Barreto-Ferreira *et al*., 2020; Ho & Abdul-Latif, 2016; Hrušková & Švec, 2015; Noor-Aziah *et al*., 2012). However, protein digestibility, proximate composition, aroma composition, microbial quality and sensory acceptability of cookies enriched with *R. differens* flour processed using different methods has never been done and compared.

1.2 Statement of the Problem

Protein malnutrition is reportedly rampant in East Africa. Conventional protein sources that are expected to cushion inhabitants of the region against the situation are either expensive and unsustainable with reference to the rising global population (animal sourced products) or are nutritionally imbalanced and contain antinutritive elements (plant-based products). Fortunately, the region seasonally receives swarms of edible grasshopper, *R. differens*, which as an edible insect, is nutrient dense, offers low ecological footprints and has high feed to biomass conversion, hence ideal sustainable replacement for the conventional protein sources. However, food neophobia and unpleasant sensory characteristics have triggered aversion among communities in

which *R. differens* consumption is uncustomary. Therefore, there is need to process the *R. differens* to transform the original distasteful sensory characteristics and incorporate it into cookies to encourage youthful millennials, who are more opposed to entomophagy. Cookies are widely consumed bakery product with unbalanced nutritional profile, emanating from wheat, the key ingredient. This study aimed to develop nutritious cookies with flours of *R. differens* from different processing methods as a sustainable and cheap alternative protein source for cookie formulations.

1.3 Objectives

1.3.1 General Objective

To contribute to food and nutrition security by characterizing their nutritional, microbial, and volatiles content and assessing the sensory acceptability of cookies enriched with *R. differens* flour from different processing methods.

1.3.2 Specific Objectives

i. To determine the effect of *R. differens* flour processing method on the nutritional composition, protein quality and volatile organic compounds of the insect-based cookies.
ii. To determine the effect of *R. differens* flour processing method on the microbial quality of the insect-based cookies.
iii. To determine the effect of *R. differens* flour processing method on the sensory acceptance of the insect-based cookies

1.4 Hypotheses

i. *Ruspolia differens* flour processing method has no significant effect on the nutritional composition, protein quality and volatile aromatic compounds of the insect-based cookies
ii. *Ruspolia differens* flour processing method has no significant effect on the microbial quality of the insect-based cookies
iii. *Ruspolia differens* flour processing method has no significant effect on the sensory acceptance of the insect-based cookies

1.5 Justification

The current world population is mostly over reliant on meat, eggs, cereals and pulses for protein source. Meat demand is still expected to rise by 76% from 2005 to 2050. However, livestock production already covers more than two-thirds of world agricultural land and

responsible for more than 14% of GHGs hence a threat to the environment. Crop production is also highly affected by the changing climatic conditions and the shrinking agricultural land as the population rises with most cereal grains having low protein content while taking time to attain edible maturity. These reasons justify the need for a dietary change to sustainable diets which are ecologically safe, contribute to food and nutrition security for the current and future generations.

Utilization of *R. differens* in making cookies will promote commercialization of edible insects as part of sustainable diet and as an alternative protein source to livestock and cereal sources. Consequently, this will contribute to reduced ecological pressure due to the environmental advantages coupled with insect rearing as opposed to livestock production (van Huis & Oonincx, 2017), re-enforcing the campaign towards dietary change to sustainable diet for the increasing global population.

This study developed nutritious insect-based cookies to curb the heightened malnutrition cases in East Africa and promote rearing of *R. differens* and other edible insects as part of sustainable agriculture. This study therefore concurs with Sustainable Development Goal (SDG) number 2 which strives to end hunger, achieve food security and improved nutrition, and promote sustainable agriculture (United Nations, 2017). It also fosters dietary diversification to minimize over-reliance on maize as a staple food Kenya. This would be in line with the policies instituted by the government of Kenyan to help realize the Big Four Agenda, Pillar 2 (Food security) (Government of the republic of Kenya, 2020).

The study will also help minimize insect food neophobia in communities and age-groups where the fear of eating insect is eminent. This would be achieved through the inclusion of *R. differens* into the cookies in ground form and determination of the disgusting aroma components which can be suppressed by addition of other artificial flavours.

CHAPTER TWO

LITERATURE REVIEW

2.1 Entomophagy

Entomophagy is the deliberate habit of eating insects (Gahukar, 2011; Ssepuuya *et al*., 2017; van Huis *et al*., 2013). The culture of eating insects all over the world has been practiced since the antique times as evidenced in the biblical citations (Evans *et al*., 2015; van Huis *et al*., 2013). Presently, approximately 2 billion people comprising 3071 ethnic groups in 130 countries practice entomophagy (Liu & Zhao, 2018).

Literature has documented over 1900 species of edible insects eaten by humans worldwide (van Huis *et al*., 2013). The number of insects consumed per region are as follows; 148 in Mexico, 12 in Central America and Caribbean Islands, 65 in South America, 83 in Southern Africa, 163 in Central and Eastern Africa, 25 in North and West Africa, 16 in Southwest Asia, 52 in South-Central Asia, 151 in South Eastern Asia, 66 in Eastern Asia, and 84 in Oceania (Jongema, 2017; van Huis *et al*., 2013). However, the Western society still trails behind with regards to adoption of entomophagy (Kröger *et al*., 2022). The high aversion has reportedly been triggered by disgust, since insects are not traditionally ingrained in their culture and neophobia, the avoidance of new food (Kröger *et al*., 2022). Strategies encompassing improving the taste, visbility and familiarity of the edible insects have proved to significantly counteract revulsion of entomophagy in Europe (Kröger *et al*., 2022; Mancini *et al*., 2019). As a result, the region presently experiences increased insect-based products in the market due to increased commercialization of edible insects (Caparros-Megido *et al*., 2017; Deroy *et al*., 2015; FAO, 2010; Vandeweyer *et al*., 2015).

Insect groups with a long history of consumption are beetles, caterpillars, bees, wasps, ants, grasshoppers, locusts, crickets, cicadas, leaf and plant hoppers, scale insects and true bugs, termites, dragonflies and flies (van Huis *et al*., 2013). The insects fall into different order and vary in terms of their global consumption (Table 2.1). Dominantly consumed insect orders in Africa include; Lepidoptera, Orthoptera and Coleoptera. *Ruspolia differens* from the order Orthoptera is the major edible insect in Eastern, Southern and Central Africa (Kelemu *et al*., 2015).

Table 2.1. Global ranking of most consumed insects

Order	Examples	% Share of world entomophagy
Coleoptera	Beetles	31
Lepidoptera	Caterpillars	18
Hymenoptera	Bees, wasps and ants	14
Orthoptera	Grasshoppers, locusts and crickets	13
Hemiptera	Cicadas, leafhoppers, planthoppers, scale insects and true bugs	10
Isoptera	Termites	3
Odonata	Dragonflies	3
Diptera	Flies	2
Others (Dictyoptera, Megaloptera and others)		6

Source: Kinyuru *et al*. (2015)

2.2 Harvesting and Consumption of *Ruspolia differens* in Africa

Long-horned grasshopper (*Ruspolia differens*), locally known as *Nsenene*, is a major delicacy in both southern and eastern parts of Africa (Lehtovaara *et al*., 2018). It is widely harvested and consumed as a traditional delicacy in Zambia, East African countries bordering Lake Victoria (Kenya, Tanzania and Uganda) and Democratic Republic of Congo where it is considered nutritious and to confer medicinal benefits (Mmari *et al*., 2017). In East Africa, *R. differens* swarms twice a year (Kinyuru 2021; Kinyuru *et al*., 2010). The insect has been reported to exist in three distinctive colour polymorphs, namely green, purple and brown morphs (Kinyuru, 2021) (Figure 2.1). The green *R. differens* signify a rich and plentiful swarming season, brown *R. differens* marks the end of the swarming season whereas the purple and purple-striped green are the rarest and the most nutritious of the morphs (Mmari *et al*., 2017). The culture of *R. differens* consumption is rapidly and widely hence offering a marketing and trading potential to the residents (Odongo *et al*., 2018).

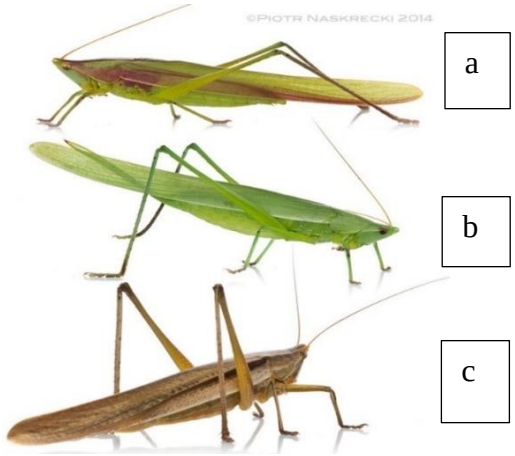


Figure 2.1. Purple striped green (a), green (b) and brown (c) *R. differens* polymorphs. (http://www.jkuat.ac.ke/would-you-consider-a-plate-of-grasshoppers-for-lunch-if-not-think-again/ruspolias/)

During the swarming season of *R. differens*, households equipped themselves with saucepans, baskets and polythene bags to trap this nutritious delicacy (Kinyuru *et al*., 2010). However, presently, commercialization has led to the advancement in the collection technology for the insect. The modern technology incorporates the use of large buckets with holes on the lid and rows of drums fitted with long corrugated iron sheets shimmering under powerful fluorescent bulbs (Figure 2.2) (Sengendo *et al*., 2021).

Figure 2.2. Modern *R. differens* trapping technology. Source: Mmari *et al*. (2017)

Ruspolia differens, just like most of the other insects, are poikilotherms hence are less active in the mornings when temperatures are relatively low (Lehtovaara *et al*., 2018) and are easily attracted to light in the evenings due to their nocturnal nature (Leonard *et al*., 2021; Matojo &

Yarro, 2010) simplifying their collection using stationary traps (Kinyuru *et al*., 2010). Upon collection, *R. differens* can either be consumed raw after removal of wings or processed before consumption (Mmari *et al*., 2017). Despite some locals preferring to eat raw *R. differens* due to their nutritious nature, cases of stomach aches have been reported by residents interviewed by Mmari *et al.* (2017), possibly due to the presence of pathogenic microorganisms, since the insects are collected from the wild and handled uncontrollably. Due to its seasonality, the locals in East Africa prefer to preserve the insects through sun drying and smoking methods to ensure availability throughout the year (Kinyuru *et al*., 2010; Mmari *et al*., 2017). Mmari *et al.* (2017) reported cleaning, boiling, smoking, toasting, deep frying and sun drying as the commonly adopted techniques for *R. differens* processing in Tanzania (Mmari *et al*., 2017). Deep-frying was reported as the most preferred technique because the of the appealing taste associated with the product and the its efficiency in decontaminating *R. differens* from pathogenic microorganisms (Ng'ang'a *et al*., 2019).

2.3 Insect-based Foods Developed in the Past

Insects are rich in quality proteins, essential fatty acids, vitamins and minerals which are comparable to those from animal products (Gere, 2017; Kinyuru *et al*., 2015; Kouřimská & Adámková, 2016). Therefore incorporation of the insects into nutritionally imbalanced diets, especially of cereal origin, could increase their familiarity and acceptability thus advancing entomophagy (Kim *et al*., 2019; Mwangi *et al*., 2019). Most of the insect enriched products are bakery products especially cookies (Figure 2.3) and bread (Figure 2.4) since they are universally recognized and consumed by people across age groups (Adeboye *et al*., 2016; Awobusuyi *et al*., 2020; Bawa *et al*., 2020; González *et al*., 2019). In Kenya, finger millet composited with desert cricket and grasshoppers flours expressed increased protein and fat content suitable for weaning children (Mwangi *et al*., 2019). Additionally, termites and lake flies were baked, boiled and processed and subsequently utilized in the preparation of crackers, muffins and meat loaves to promote entomophagy (Ayieko *et al*., 2010). *Ruspolia differens* has also been successfully incorporated in soy bean-sweet potato complementary food for children (Mmari, 2017). All these approaches ultimately broaden the scope of entomophagy, valorizing and popularizing edible insects amongst a wider populace to consequently contribute to alleviation of malnutrition and undernutrition while minimizing livestock rearing for protein sources.

Figure 2.3. Cookies enriched with 5, 10 and 15% insect meal. Source- (Awobusuyi et al., 2020)

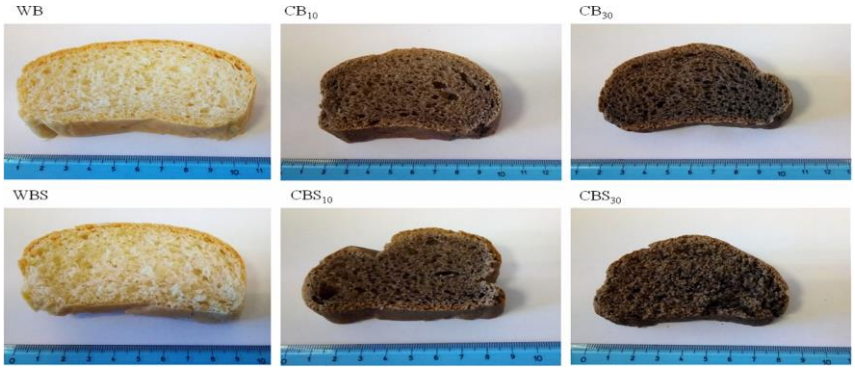


Figure 2.4. Bread enriched with 10% (CB10) and 30% (CB30) cricket powder. Source : Osimani *et al*. (2018)

2.4 Nutritional Value of *R. differens*

Several reports have attested to the nutritional quality of insects (Amadi & Kiin-Kabari, 2016; Hlongwane *et al*., 2020; Rumpold & Schlüter, 2013). They are rich sources of proteins, lipids, carbohydrates, certain vitamins and minerals such as calcium, iron and zinc (Zielińska *et al*., 2015). Their energy supply is averagely commensurate to that of animal sources with the exception of pork (FAO, 2010). Of great importance is that edible insects contain complete essential amino acids that meet human dietary needs and polyunsaturated fatty acids which are beneficial health-wise (Rumpold & Schlüter, 2013). *Ruspolia differens* being a popular delicatessen in Africa is no exception and is known to be highly nutritious by the local consumers (Fombong *et al*., 2017; Kinyuru *et al*., 2010). In fact, consumption of 100g of *R. differens* has been reported to meet the RDA retinol, α-tocopherol, niacin, riboflavin and folic acid as well as iron, zinc, magnesium, manganese and selenium (Fombong *et al*., 2017; Kinyuru *et al*., 2010).

Kinyuru (2014) reported two *R. differens* (brown and green polymorphs) in Siaya, Kenya, and nutritionally characterized them. The resulting nutritional contents coincided with those reported by Ssepuuya *et al*. (2019) and Kinyuru *et al*. (2010) (Table 2.2). *Ruspolia differens* has a high protein content of 34.2–45.8% that supersedes that of conventional sources of protein i.e. 23.2% for beef, 24.8% for veal and 21.5% for mutton (Ahmad *et al*., 2018). These protein levels qualifies *R. differens* as ideal novel food candidates suitable for counteracting malnutrition in developing nations (Mmari *et al*., 2017). Fat (46.2-54.6%) comprises the largest proximate component of *R. differens* contributing to its palatability and flavour (Kinyuru *et al*., 2010; Mmari *et al*., 2017).The insect oils are related to unpleasant flavours since such volatile organic compounds are liposoluble (Tzompa-Sosa *et al*., 2019) and may be a turn off factor to those not accustomed to eating insects. Moreover, this high fat translates to 519-539 Kcal/g of energy (Fombong *et al*., 2017) and also largely contributes to the shorter shelf-life of the insect to 24-48 h at room temperature (Mmari *et al*., 2017). *Ruspolia differens* also has a fibre content of 3.93–5.34% attributable chiefly to the exoskeleton chitin (Ssepuuya *et al*., 2019). Chitin serves as a prebiotic for the establishment of beneficial gut microorganisms which in turn boosts the immunity systems of the consumers (Mutungi *et al*., 2019).

The major minerals and microminerals in *R. differens* are potassium, phosphorus, calcium, iron and zinc (Table 2.2). Consumption of 100g of *R. differens* has been reported to supplement the daily trace mineral (sodium, potassium and calcium) requirements of both children and adults but meeting the phosphorous and magnesium requirements are of children only (Ssepuuya *et al*., 2019). The mineral levels have been found to be either greater or equal to the levels reported in pork and chicken meat (Ssepuuya *et al*., 2019). Ssepuuya *et al*. (2019) low calcium levels in *R. differens* attributable to their lack of bony skeleton which is richly mineralized in vertebrates. Nonetheless, the calcium levels are comparably greater than those reported in beef and other conventional meat sources (Kinyuru & Kenji., 2010; Ssepuuya *et al*., 2019). Similarly, the micro-elements; iron, zinc, copper and manganese contents were reportedly higher than the levels reported in meats such as pork and chicken meat (Ssepuuya *et al*., 2019). *Ruspolia differens* has been reported to contain sufficiently high levels of micro-elements compared to macro-elements hence deemed ideal food for combating micromineral deficiencies in Africa (Ssepuuya *et al*., 2019).

Table 2.2. Nutritional composition of *R. differens*

Nutrient	*Ruspolia differens*	Whole chicken Eggs
	Macronutrients (%)	
Protein	34.2-42.8	11.54-12.6
Fat	42.2-54.3	9.0
Ash	2.6-2.8	0.80-1.30
Fibre	3.9-5.34	-
	Minerals (mg/100g)	
Potassium	446.0-673.0	145
Phosphorous	429.0-627.0	179
Calcium	34.9-128.0	46
Iron	13.0-16.6	1.72
Zinc	12.4-17.3	1.12
	Vitamins (mg/100g)	
Retinol	0.21-0.28	12.6
α-tocopherol	15.2-20.1	1.29
Riboflavin	1.2-1.4	0.50
Niacin	2.1-2.4	0.05

Source : Kinyuru *et al*. (2010), Ogunwole *et al*. (2015), Roe *et al*. (2013), Ssepuuya *et al*. (2019)

The *R. differens* contains considerable amounts of vitamins (vitamin A, vitamin E, riboflavin and niacin) (Table 2.2) which are beneficial to humans. The vitamin A content has been reported to be 2.1 μg/g, 2.8 μg/g for green and brown grasshoppers, respectively (Kinyuru *et al*., 2010). This can partly contribute to the daily vitamin A requirements of infants, lactating mothers and adults. Appreciable levels of vitamin E (152.0-201.0μg/g) were also registered from this

grasshopper (Kinyuru *et al*., 2010). These values are comparable to those reported in red meat and could sufficiently contribute to the Recommended Dietary Intake (RDI) of vitamin E, a beneficial antioxidant (Kinyuru *et al*., 2010). Other vitamins reported to be in *R. differens* are niacin, riboflavin, ascorbic acid, folic acid, pyridoxine and thiamine, all of which are potentially beneficial to humans (Kinyuru *et al*., 2010).

2.5 Methods of Processing Edible Insects and their Effects on Nutritional Value

The indigenous methods for preparation of edible insects for human consumption encompasses thermal treatment (boiling, blanching, frying, toasting, roasting), drying (sun drying and oven drying) and size reduction (grinding and chopping) (Mutungi *et al*., 2019). Cooking enhance the flavour and aroma, colour and texture of edible insects (Melgar-Lalanne *et al*., 2019) which could improve the acceptability of insects and related products. Some of the processing methods are discussed below.

2.5.1 Degutting

This is accomplished by squeezing the gut contents of the insects through the anus. The gut contents basically comprises the plant materials that the insect feed on and therefore their removal proportionally enhances the levels of other nutrients in the insects due to the elimination of nutrient dilution effect (Kwiri *et al*., 2014). This phenomenon was observed when protein levels and *in vitro* true dry matter digestibility spiked upon degutting of mopane (Kwiri *et al*., 2014). On the other hand, degutting caterpillars resulted in the marked decrease in ash, fibre, condensed tannins, calcium and phosphorous possibly originating from the plant material (Mutungi *et al*., 2019). It is therefore important to consider the gut contents of insects, which could influence the general nutritional value of the edible insects. Degutting has also been practiced to improve the shelf-stability of caterpillars in Zambia (Hlongwane *et al*., 2020). Degutting enhances the shelf-life of the edible insects by reducing the spoilage enteric microorganisms (Grabowski & Klein, 2017a) as well as elimination of after tastes (Melgar-Lalanne *et al*., 2019).

2.5.2 Boiling/Blanching

Insects are placed in a boiling water for a short while with an aim of suppressing spoilage and pathogenic microorganisms, and degradative enzymes (Melgar-Lalanne *et al*., 2019). Boiling has been reported to increase the moisture content of yellow mealworm larvae, *Tenebrio molitor* (from 62.81% to 70.44% after 40 s) with a constant water activity of 0.96 (Vandeweyer *et al*., 2017) attributable to the absorption and entrapment of water just below the chitin (Melgar-Lalanne

et al., 2019). Boiling has been reported to dramatically reduce levels of ash, crude protein, insoluble nitrogen and *in vitro* true dry matter digestibility of Mopane caterpillar (Mutungi *et al*., 2019). Boiled Sudanese tree locusts (*Anacridium melanorhodon*) in Khartoum exhibited a 50% digestibility, high protein of 66.24%, ash content of 5.53% and a high oil content (Mutungi *et al*., 2019). Egan *et al*. (2014) reported that traditional boiling of *Hemijana variegata* caterpillars in South Africa lowered the energy level by 44% and protein by 15%.

2.5.3 Frying

This refers to oil cooking of the insects. Mmari *et al*. (2017) reported deep-frying to be the most prevalent traditional processing technique of *R. differens* in Tanzania. However, a lower digestibility of 41% has been revealed in fried Sudanese tree locusts from Khartoum with higher levels of potassium, lower levels of phosphorous and rather constant levels of calcium, sodium, iron magnesium, zinc and cobalt. Frying of *Rhynchophorus phoenicis* and *Oryctes monoceros* resulted to 6% and 10% lipid contents increment, respectively and a cholesterol level increment of 3%-10% (Mutungi *et al*., 2019).

2.5.4 Toasting

Insects are exposed to grill or pan heated to attain a brownish colour. It is considered one of the oldest methods of traditional processing of *R. differens* in Tanzania (Mmari *et al*., 2017). Presently, in urban areas, toasting is done using baking ovens (Mmari *et al*., 2017). Toasting has been reported to reduce the digestibility of both brown and green grasshoppers (*R. differens*) but not termites (*Macrotermes subhylanus*) (Kinyuru *et al*., 2010). On the other hand, toasting caused a dramatic reduction in vitamins (riboflavin, niacin, pyridoxine, retinol, ascorbic acid, folic acid and α-tocopherol) as well as fats by approximately 8% (Kinyuru *et al*., 2010).

2.5.5 Smoking

Smoking is an old traditional process of flavouring, browning, cooking, or preserving foods by exposing it to smoke from burning or smouldering material, most often wood. Mmari *et al*. (2017) reported smoked *R. differens* to be preferred by 21% of the consumers in Tanzania especially those aged between 51-60 years due to the aroma and antique of the smoked insect product. Smoking of *R. phoenicis* and *O. monoceros* has been reported to decrease their lipid contents by 9% and 19% and cholesterol contents by 41% and 80%, respectively (Mutungi

et al., 2019). Smoking results to a synergistic actions of heat enzymes resulting to changes in lipids and proteins hence stabilizing the lipids (Tiencheu *et al*., 2013).

2.5.6 Roasting

Roasting involves cooking the insect in an oven or over a fire whereby hot air envelops the food to cook evenly and develop flavour and aroma through caramelization and Maillard reaction. Roasting increased the mineral contents, neutral detergent fibre, acid detergent fibre, acid detergent lignin and crude protein of *mopane* caterpillars while decreasing acid detergent insoluble nitrogen and *in vitro* true dry matter digestibility (Mutungi *et al*., 2019). In another study, roasting did not influence the protein content of *Henicus whellani* (crickets) and protein digestibility of *Eulepida mashona* (beetles) but significantly reduced the protein digestibility of crickets. Roasting did not also influence the contents of a majority of minerals and their bioavailability in both the insects (Manditsera *et al*., 2019).

2.5.7 Drying

Drying is done to reduce microbial activity, chemical activity and allergenic hazards while at the same time retaining the nutritional value of the insects (Kröncke *et al*., 2019). Most commonly applied drying processes are solar or sun-drying, oven drying and freeze-drying while others are air convection drying, smoke drying, microwave drying, fluidized bed drying (Parniakov *et al*., 2022). Oven drying has been reported to increase mineral concentration of *Sternocera orissa* while doubling the concentration of essential and non-essential amino acids which is supposedly due to hydrolytic and chemical inter-conversions (Shadung *et al*., 2012). Oven drying of *H. variegata* caterpillars for 24-72h was reported to reduce the energy content by 9% without distorting the proximate composition (Egan *et al*., 2014). Comparatively, sun-dried maggot meal recorded a higher energy level than the oven-dried meal whereas the oven dried meal indicated a slightly higher protein content (Mutungi *et al*., 2019). Additionally, Aniebo and Owen (2010) reported oven-dried maggots to have higher protein content but lesser fat content than the sun-dried maggots. Kinyuru *et al*. (2010) also reported that solar-drying of grasshoppers and termites resulted in significant loss of riboflavin (29-46% loss), folic acid (47-66%), niacin (6-26%), pyridoxine (9-13%), retinol (30-56%), ascorbic acid (25-55%), and α- tocopherol (9-30%).

2.6 The Microbiology of Insects

2.6.1 Microflora of Edible Insects

The safety of edible insects is anchored on their taxonomic profiles, rearing and processing methods (Rumpold & Schlüter, 2013a; van Huis *et al*., 2013). Common safety risks associated with entomophagy have been reported to be chemical and microbial hazards (Belluco *et al*., 2013; Milanović *et al*., 2016; van der Spiegel *et al*., 2013). Microorganisms colonize both the internal and external surfaces of the insects including the rearing and processing environments and are often transmitted to humans through consumption (Ssepuuya *et al*., 2019).

Insects are nutrient dense, have adequate moisture content and favourable pH foods which are conditions conducive for rapid proliferation of microorganisms to degrade the quality of the fresh insects (Klunder *et al*., 2012; Ssepuuya *et al*., 2019). Klunder *et al*. (2012) reported 107 cfu/g, 104-106 cfu/g and 102-104 cfu/g total viable counts (TVC), *Enterobacteriaceae* and spore-forming bacteria, respectively, from fresh edible insects. Stoops *et al*. (2016) also reported 7.7—8.3 log cfu/g, and 7.8-8.6 log cfu/g TVC for edible mealworm larvae and grasshopper, respectively. Further, lactic acid bacteria (LAB) (7.0-8.5 log cfu/g) and *Enterobacteriaceae* (6.8-7.6 log cfu/g) have been reported in mealworm larvae and grasshopper, respectively (Stoops *et al*., 2016). However, lower numbers of spore-forming microorganisms and yeasts and moulds were discovered from both the insects (Stoops *et al*., 2016). Similarly, De Smet *et al*. (2018) and Ng'ang'a *et al*. (2019) affirmed that that insects can harbour different kinds of pathogenic bacteria and fungi, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus cereus* and *Aspergillus niger*.

In Uganda, *R. differens* is manually harvested, packaged and transported to trade points where the wings are plucked manually and presented for sale often at the road sides which could greatly influence microbial types and numbers (Ssepuuya *et al*., 2019). Cultural microbial analysis of fresh *R. differens* has revealed 8.38-9.41 log cfu/g for TVC, 6.89-7.83 log cfu/g for *Enterobacteriaceae*, 7.99-9.11 log cfu/g for lactic acid bacteria, 3.75-4.87 log cfu/g aerobic bacterial spores and 5.77-7.12 log cfu/g yeast and moulds in fresh *R. differens* (Ssepuuya *et al*., 2019). The dynamics in the number and types of the microorganisms were discovered to be influenced significantly by geographical area of source, swarming season, trading points and swarming (Ssepuuya *et al*., 2019).

2.6.2 Effects of Processing Techniques on the Microbial Load of Edible Insects

Edible insects may endanger the lives of consumers due to the pathogens they reportedly possess hence posing potential health risk (Schlüter *et al*., 2017). This can be curtailed through processing of insects prior to consumption for safety (Rumpold & Schlüter, 2013). Grabowski and Klein (2017b) reported dried and powdered insects to contain higher microbial counts than the deep-fried and cooked ones. The effectiveness of deep-frying in decontaminating edible insects has been reported elsewhere (Gatheru *et al*., 2019; Labu *et al*., 2021). It has also been established that *Bacilli spp, Staphylococci spp, Enterobacteriaceae,* yeast and moulds are the main groups of microorganisms implicated in both the processed products (Grabowski & Klein, 2017b). However, no salmonellae, *L. monocytogenes, E. coli* and *Staphylococcus aureus* were detected (Grabowski & Klein, 2017b).

Elsewhere, cooking of mealworm larvae (*Tenebrio molitor*) and house crickets (*Acheta domesticus*) proved to be adequate in the elimination of *Enterobacteriaceae* however ineffective against spore-forming microorganisms due to their marked thermal resistance (Klunder *et al*., 2012). Additionally, roasting coupled with blanching has also been found to be effective against the enteric microorganisms more than roasting of insects alone (Klunder *et al*., 2012). Surprisingly, fermentation of composite insect flours proved a promising processing technique to eliminate spoilage and pathogenic microorganisms to ensure safety of insect products (Klunder *et al*., 2012). Moreover, baked products enriched with edible insects subjected to pre-processing (drying and grinding) have reportedly expressed satisfactory microbial quality. This was evidenced by Ayensu *et al*. (2019) when biscuits were fortified with palm weevil and by Olamide *et al*. (2020) when energy dense-biscuits were enriched with silkworm pupae and locust.

2.7 Sensory Acceptability of Insect-based Products

Acceptability is an indirect measure of preference of a food product. Generally, entomophagy is an unconventional eating culture in the Western world and acceptability of insects as human food is still facing resistance amidst the incorporation of the insects as part of familiar food products to promote it (Kröger *et al*., 2022; Mancini *et al*., 2019). However, in developing countries in continents like Africa where entomophagy has long been practiced, insect-based foods are likely to be more easily accepted although more research needs to be done on the willingness-to-accept/consume. Preference is only based on the level of insect added to compose the new

product mostly by relying on the sensory organs (Adeboye *et al*., 2016; Awobusuyi *et al*., 2020; González *et al*., 2019; Ogunlakin *et al*., 2018; Ojinnaka *et al*., 2015; Osimani *et al*., 2018).

In Uganda, boiled and dried *R. nitidula* has been reported to be highly accepted than the deep-fried samples (Ssepuuya *et al*., 2017). In Kenya, wheat-termite buns formulated with 5% termite flour was highly accepted than those formulated with 10% and 20% termite flour. Researchers have established that there is a general trend of a negative association between increasing levels of insect incorporation into products and their acceptability (Caparros Megido *et al*., 2016; González *et al*., 2019) These observations are attributable to the change in size, texture, aroma, colour and taste which adversely deviates from the control or conventional products in the shelves as the substituting substrate increased. For instance, increasing the termite flour results in the reduction in the level of wheat gluten, disorienting the gluten network which is responsible for viscoelasticity in bakery products (Osimani *et al*., 2018) hence the reported low volume of the bakery products with increasing insect flour levels. Moreover, the insect ingredients introduce new proteins and amino acids that serve as reactants during Maillard reaction to yield dark brown objectionable colour, aroma and flavour (Ojinnaka *et al*., 2015).

Termites and lake flies have also been used to process crackers, muffins, sausages and meat loaf and their acceptability tested (Ayieko *et al*., 2010). The developed products were highly rated bases on their taste and flavour, smell, texture, and appearance(Ayieko *et al*., 2010) . However, the colour score for meat loaf fortified with lake flies was low due to the black colour of lake flies which is uncharacteristic of meat and meat products (Ayieko *et al*., 2010). Elsewhere, Hwang and Choi (2015), reported higher preference for the flavour , taste and overall acceptability of muffins incorporated with 1-8% *Tenebrio molitor* powder thereby, labelling the powder a useful ingredient in acceptability and functionality of muffins.

2.8 Research Gaps

Commercialization of insect-based products has suffered set-backs from the trivialization of the edible insects as food. This could only be amended through the processing of the insects into products that match the modern eating habits (Ayieko *et al*., 2010). However, little research has been done on the use of differently processed *R. differens* in the enrichment of bakery products and to compare the sensory acceptability of the enriched cookies. Mwangi *et al*. (2019) and Mmari (2017) recommended the exploitation of the nutritional potential of *R. differens* and incorporating it into cereal based products to help fight malnutrition in East Africa. To date, no research has been

done on the protein digestibility of the cookies enriched with *R. differens* flour processed using different methods as protein makes a key basis for food enrichment. Further, the aroma compounds, that majorly influence the acceptability of food has never been characterised in *R. differens*-based products such as cookies

CHAPTER THREE

MATERIALS AND METHODS

3.1 Description of Study Sites

Ruspolia differens samples were acquired from Kampala and Masaka, which represent the principal sourcing and harvesting centres in Uganda. Kampala is the capital city of Uganda. It is located in the southern region of the country (0.3476° N, 32.5825° E), approximately north of Lake Victoria, on a series of hills with an elevation of around 3,900 ft (1,190 m). Kampala is located close north of Mengo, the 19th-century capital of the kingdom of Buganda (Encyclopaedia Britannica, 2015). On the other hand, Masaka is a town in southern Uganda (0°20'28.0"S 31°44'10.0"E), about 80 miles (130 km) southwest of Kampala at a height of 4,300 ft (1,310 m) (Encyclopaedia Britannica, 2015). Experiments on nutritional value, digestibility, volatile aroma compounds and microbial analysis of the processed *R. differens* and the respective enriched cookies were conducted at the International Centre for Insect Physiology and Ecology (icipe), Duduville campus, Nairobi, while sesnory evaluation of the *R. differens*-based cookies was done at the Department of Dairy and Food Science and Technology, Egerton University, Njoro, Nakuru County. International Centre of Insect Physiology and Ecology (*icipe*) is located 13.2 km from the Nairobi central business district (CBD). It is situated in Kasarani sub-county, northeast of Nairobi's CBD (Coordinate: 01°13′44″S 36°54′16″E) . Egerton University, Njoro campus is located 25 km, southwest of Nakuru town. This is located approximately 182 km, by road, northwest of Nairobi. (Coordinate: 0°22'11.0"S, 35°55'58.0"E) .

3.2 Experimental Material

Samples of *R. differens* (20 kg) were purchased from commercial harvesters in Masaka and market sellers in Kampala, Uganda (Figure 3.1 A&B), during the October-December swarming season in the year 2019. The insects had their appendages, wings and ovipositor removed at the time of purchase. Generally, the *R. differens* from the two locations chiefly comprised of green and brown polymorphs while the purple polymorph was scarce. The samples were packed in polyethylene sterile zip lock bags (SC Johnson brand, Size 13 ×15"), transferred into cooler boxes, ice-packed (Figure 3.1C) and hermetically sealed.

Figure 3.1. *R. differens* from commercial harvesters in Masaka (**A**), market sellers in Kampala (**B**) and samples ice-packed in a cool box (**C**).

The samples were then transported to International Centre of Insect Physiology and Ecology (*icipe*) laboratory, Nairobi, Kenya with the facilitation of permits provided by the Kenya's Kenya Plant Health Inspectorate Services (KEPHIS), Ministry of Agriculture and Rural Development (Permit No.: KEPHIS/21591/2019) and Uganda's Ministry of Agriculture, Animal Industries and Fisheries Plant Quarantine and Inspection Services (License No.: UQIS4269/93/PC (E)). All the samples were concocted together to make up one lump. A portion of the samples (5 kg) were immediately processed within 24 h of collection and delivery to *icipe*, for microbial assessment. The other portion was transferred into a 24 × 24" 48 L polyethylene sterile sampling bag (Thomas scientific) then frozen-stored at -20°C (RZ41FARAEWW, Samsung, China). Wheat flour and other baking ingredients were acquired from a local supermarket, Kasarani, Nairobi.

3.3 Processing of *R. differens*

Samples (1 kg) intended for assessment of efficacy of processing methods on microbial loads were immediately subjected to processing upon arrival to *icipe* laboratories within 24 h. On the other hand, the frozen samples (2 kg) meant for nutritional, sterols, flavonoids contents and digestibility determinations were thawed for 12 h at 5℃ refrigeration temperature. Both the sets of samples followed a standard washing procedure in a portable water to remove dirt and debris then drained. Samples for microbial analysis were divided into four portions of 250 g each while those for nutritional, sterols, flavonoids and digestibility determinations were divided into four parts of about 500 g each. The processing techniques adopted during this study are shown in Figure 3.2. The first portion from each set was blanched at 100℃ for 5 min (Fombong *et al*., 2017) in a stainless steel pot on an electric coil cooker (Von, China) and regular turning with a wooden spoon every 1 min. The second portions were boiled at 100℃ for 15 min (Mmari *et al*., 2017) in a stainless steel pot on an electric coil cooker (Von, China) with a 3-min turning interval. The third portions were toasted at approximately 150℃ for 10 min in a stainless steel pan (Gatheru *et al*., 2019), on a heated electric coil cooker (Von, China) with a 2-min turning frequency. The fourth portions were deep fried in preheated vegetable cooking oil (Fresh Fri, Pwani Oil Ltd) at 175℃ (Bordin *et al*., 2013) turned every 2 min crunchy and dark brownish (Mmari *et al*., 2017).

Figure 3.2. Different processing methods for the different *R. differens* proportions

Blanched, boiled, toasted and deep-fried samples were drained using a metallic strainer of medium pore size 1.5875 mm. Samples for microbial analysis were transferred into sterile zip lock bags and immediately assessed for microbial loads. The other set of samples were uniformly spread on aluminium foils to cool for 5 min followed by drying in an oven (SDO-225, Wagtech

International, Thatcham, UK) at 60℃ for 24 h (Fombong *et al*., 2017). Processed samples were transferred into polyethylene sterile zip lock bags and frozen-stored at -20℃ awaiting chemical analyses at *icipe* and International Livestock Research Institute (ILRI) laboratories, Nairobi.

3.4 Experimental Design

Fresh *R. differens* were randomly collected from harvesters in Masaka and market sellers in Kampala, Uganda. The obtained batches of *R. differens* were pooled together and mixed thoroughly. Samples for processing (blanching, boiling, toasting and deep-frying) were randomly collected from the pooled consignment. The experiment employed a simple completely randomized design (CRD) in which cookies were formulated with eggs (control), blanched *R. differens* flour, boiled *R. differens* flour, toasted *R. differens* flour and deep-fried *R. differens* flour representing the factors. The statistical model for this design was according to the following equation: $Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$……..Equation 1

Where; Y_{ij} =the response variable of the i^{th} processed *R. differens* flour and j^{th} replication; μ =the overall mean; τ_i =effect of the i^{th} processed *R. differens* flour; ε_{ij} = the random error component.

3.5 Formulation and Baking of Cookies

Cookies were formulated according to Aziah *et al*. (2012) with a few modifications. The ingredients were weighed in proportions as indicated in Table 3.1 and baking followed the procedure outlined in the Figure 3.3.

Table 3.1. Ingredients for cookies formulation (g w/w)

Ingredients (g)	Control (Raw eggs)	Processed *R. differens*			
		Blanching	Boiling	Toasting	Deep frying
Flour	408.2	408.2	408.2	408.2	408.2
Sugar	172.2	172.2	172.2	172.2	172.2
Shortening	172.2	172.2	172.2	172.2	172.2
Eggs	84.0	-	-	-	-
Blanched *R. differens* flour	-	84.0	-	-	-
Boiled *R. differens* flour	-	-	84.0	-	-
Toasted *R. differens* flour	-	-	-	84.0	-
Deep-fried *R. differens* flour	-	-	-	-	84.0
Salt	3.4	3.4	3.4	3.4	3.4
Dough weight	840.0	840.0	840.0	840.0	840.0

Cookie enrichment with *R. differens* flours was done at 10% as based on the successful insect levels of 5% and 10% inclusion in bakery products such as cookies, biscuits and bread exhibiting appealing sensory attributes and high overall acceptability (Adeboye *et al*., 2016; Awobusuyi *et al*., 2020; González *et al*., 2019; Ogunlakin *et al*., 2018; Ojinnaka *et al*., 2015; Osimani *et al*., 2018). Creaming was achieved by mixing shortening, refined sugar and salt in a bakery mixer (BJY-BM10, Berjaya, Malaysia) for 15 min before adding whisked eggs (for control) or processed *R. differens* flours and mixing for another 10 min. The cream was again mixed with a fortified wheat flour (Exe, Unga Ltd, Kenya) and hand-kneaded to a consistent dough. The dough was rolled to 5 mm thickness, cut into 5 cm diameter sizes and baked in a pre-heated oven (BISTROT 665; BestFor®, Ferrara, Italy) at 180℃, 30% dryness for 15 min.

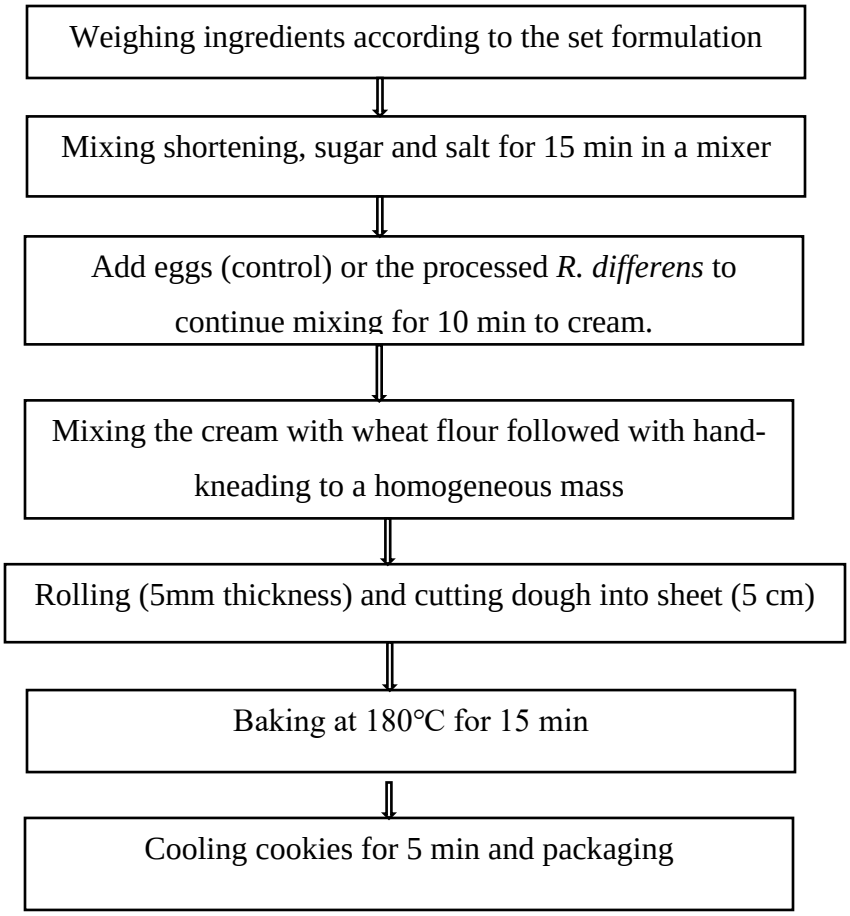


Figure 3.3. Flow diagram for preparation of cookies.
Adopted from Soni *et al*. (2018) and modified.

Baked cookies (Figure 3.4) were cooled for 5 min and then packed and stored depending on experiment to be conducted.

Control cookie

Blanched *R. differens*-based cookie

Boiled *R. differens*-based cookie

Toasted *R. differens*-based Cookie

Deep-fried *R. differens*-based cookie

Figure 3.4. Cookies made from differently processed *R. differens*.

3.6 Assessment of the Proximate Composition of Processed *R. differens* and Respective Developed Cookies

3.6.1 Determination of Moisture and Dry Matter content

Moisture and dry matter were determined according to AOAC (2000), Method 930.15. Samples (2 g) were weighed into clean, pre-dried and pre-weighed porcelain crucibles (W1). The joint weight of the crucible and the sample were recorded (W2) and the sample dried in a forced draft air oven (WTB binder, Tuttlingen, Germany) at 105℃ for 3 h. The dried samples in the crucibles were then cooled in a desiccator for 30 min then weighed (W3). Percentage moisture content was calculated according to the following equation:

$$MC\ (\%) = \frac{W2-W3}{W2-W1} \times 100 \text{.....Equation 2}$$

Where: MC= Moisture content; $W1$= weight of porcelain crucibles; $W2$=weight of crucible and wet sample; and $W3$= weight of crucible and dried sample.

3.6.2 Determination of Crude Protein Content

Crude protein was determined according to AOAC (2000), Method 955.04. Samples (1 g) were weighed into 250 mL digestion tubes with two tubes being reserved for blank runs (without samples). A catalyst mixture (15 g) in ratio of 9 K_2SO_4 to 1 $CuSO_4.5H_2O$ was added to each tube. Fifteen-millimetres of 98% concentrated sulphuric acid was then added into each tube. The mixtures in the tubes were subjected to a digestion block heater for 2 h at 420℃ until transparent liquids were observed. Cooled sample sets were then transferred for distillation and titration in an automatic Kjeldahl analyser (Velp UDK 159, Velp Scientifica, Europe) with a pre-set nitrogen-protein conversion factor of 6.25 (Finke, 2007). The Kjeldahl analyser was connected to 40% NaOH source to alkalize the digests and liberate ammonia. The liberated ammonia was harnessed by excess 4% boric acid premixed with indicators (10 mL of bromocresol green and methyl red solution) to yield ammonium borate. Ammonium borate distillate was then titrated against 0.2 M HCl until a pink colour change was auto-detected by the analyser. The nitrogen and protein contents were calculated according to the following equations:

$$\%\, Nitrogen = \frac{100\times(VT-VB)\times NA\times 14}{WS\times 1000} \quad \text{.....Equation 3}$$

Where: VT; volume (mL) of standard acid used in titration; VB =volume (mL) of standard acid used in blank; NA= normality of acid (HCl); WS =Sample weight.

$$\%\, Crude\ protein = \%\, Nitrogen \times 6.25 \quad \text{.....Equation 4}$$

3.6.3 Determination of Crude Fat Content

Crude fat was assessed following AOAC (2000), Method 920.39. Extraction cups were cleaned, dried in an oven at 105℃ min and weighed (W_0). Samples (5 g) were weighed (W_s) into extraction thimbles and covered with cotton wools. Soxhlet extractor (Velp SER 148, Velp Scientifica, Europe) was then preheated before the thimbles were attached to the extraction columns. Petroleum ether (70 mL), serving as an extractant, was added into each extraction cup, sufficient enough to cover samples in the thimbles during boiling. Thimbles were subsequently immersed into the solvents and boiled for 30 min. The thimbles were then raised to allow washing for 60 min, followed by solvent recovery from the cups to dryness. The cups containing the extracted oils were transferred to fume hood to evaporate any residual solvent followed by further drying in an oven at 105℃ for 30 min to eliminate moisture and traces of solvents. The cups were

then cooled and weighed (W_f). The percentage fat was calculated according to the following equation:

$$\%Crudefat = \frac{Wf-W0}{Ws} \times 100$$.....Equation 5

Where: Wf= Weight of recovered fat and the extraction cup; $W0$= Weight of empty extraction cup; Ws=Weight of sample

3.6.4 Determination of Crude Fibre Content

Crude fibre was analysed following procedures in AOAC (2000), Method 985.29. Defatted samples (2 g) were transferred into a 500 Ml round bottomed flask. Boiling 1.25 % H_2SO_4 (200 Ml) was then added to each sample and boiled for 30 min under continuous condensation. The flask contents were filtered using a glass wool then thoroughly washed with hot water thrice to remove traces of acid. The residue was then boiled in 200 Ml 1.25% NaOH for 30 min, filtered again using a glass wool and washed with hot water thrice to remove traces of the base. The residues were transferred into a Gooch crucible and the adhering residues washed off into the crucible using 15 Ml 10% HCl and filtered. The crucibles and their contents were weighed and dried in an oven (WTB binder, Tuttlingen, Germany) overnight at 105℃, cooled in desiccator and reweighed. They were then incinerated in a muffle furnace (Heraeus-Kundendienst, Düsseldorf, Germany) at 550℃ for 3 h. The crucibles were cooled in a desiccator and reweighed. The difference in the weight of crucible and its content before and after ashing was expressed as the percentage crude fibre as indicated in the following equation:

$$\%\ Crude\ Fibre = \frac{A-B}{C} \times 100$$.....Equation 6

Where: A is =weight of crucible and dried sample before ashing, B = the weight of the crucible and sample after ashing, C =weight of the original sample.

3.6.5 Determination of Crude Ash Content

Crude ash was assayed according to AOAC (2000), Method 923.03. Samples (3 g) were transferred into pre-weighed porcelain crucibles. The samples were charred in an oven for 3 h then ashed in a muffle furnace (Heraeus-Kundendienst, Düsseldorf, Germany) at 550℃ for 24 h. Samples were then cooled in a desiccator and weighed. The difference in weight was expressed as the percentage total ash of the sample as described in the following equation:

$\% \, Crude \, Ash = \frac{W3-W1}{W2-W1} \times 100$……..Equation 7

Where: $W1$ = weight of the crucible; $W2$ = weight of crucible and raw sample; and $W3$ =weight of crucible and dried sample.

3.6.6 Determination of Carbohydrate Content

The total carbohydrate content will be computed by subtracting the sum of % moisture, % crude protein, % crude fat, % crude fibre and % ash content from 100% according to Ayensu *et al*. (2019) as indicated in the following equation:

$\% \, Total \, carbohydrate \, content = 100\% - (\%moisture + \%protein + \%fat + \%ash + \%crude \, protein)$……..Equation 8

3.6.7 Determination of Total Energy

The energy content of the cookies was also calculated by Atwater's method (FAO, 2003) as indicated in the following equation:

$Total \, Energy \, (kcal/100g) = (4 \times \%Protein) + (4 \times \%Carbohydrate) + (9 \times \%Fat)$……..Equation 9

3.7 Amino Acid Analysis of Cookies

The amino acid composition was determined as previously described by Musundire *et al*. (2016). Cookie samples (10 mg) were accurately weighed on an analytical scale and then transferred into 5 mL micro-reaction vials (Supelco®). Subsequently, 1.5 mL of 6N HCl, nitrogen introduced and then capped. Samples were transferred into GC-oven programmed to a temperature rising rate of 10℃/ 5 min to a targeted temperature of 110℃ maintained for 24 h to ensure complete hydrolysis. The hydrolysates were then vacuum-evaporated to dryness. The hydrolysed samples were then reconstituted in 1 mL 0.01% formic acid-acetonitrile (95:5) mix, vortex-shaken for 30 s, sonicated at 50 kHZ for 30 min, and then centrifuged (Eppendorf AG, 22331 Hamburg, Germany) at 14000 rpm (18624 × *g*, 20°C). The supernatant (0.2 µL) was analysed using UPLC-MS/MS. Chromatographic separation was performed on a ACQUITY UPLC I-class system (Waters Corp., Milford, MA) fitted with an ACQUITY UPLC BEH C18 column (2.1 mm × 150 mm, 1.7 µm particle size; Waters Corp., Wexford, Ireland, oven temp 45°C). The autosampler tray was cooled to 5°C. The mobile phase comprised of (A) water and (B) methanol (solvent B) both

acidified with 0.01% formic acid. The gradient system used was 0–2 min, 5% B, 2–4 min, 40% B, 4–7 min, 40% B, 7–8.5 min 60% B, 8.5–10 min 60% B, 10–15 min, 80% B, 15–19 80% B, 19–20.5 min, 100% B, 20.5–23 min, 100% B, 23– 24 min 95% B, 24–26 min, 95% B. The flow rate was held constant at 0.2 mL/min. The UPLC was interfaced with an electrospray ionization (ESI) Waters Xevo TQ-S operated in full scan MS in positive ionization mode. The m/z range 40–2,000 with a capillary voltage of 0.5 kV, sampling cone voltage of 30 V, source temperature 150°C desolvation temperature of 120°C. The nitrogen desolvation flow rate was 800 L/h. Data was acquired using MassLynx version 4.1 SCN 712 (Waters). Mass spectrometric data, retention time, and co-injection of the hydrolysate with an authentic standard amino acid mixture were used to identify the amino acids. Amino acid standard solution (AAS 18) obtained from Sigma-Aldrich (Chemie GmbH, Munich, Germany) was used for external quantification of the amounts of each amino acid present. This was repeated three times using different batch of samples.

3.8 Determination of Fatty Acid Profiles of *R. differens* and Cookies, and Sterols Content of *R. differens*

3.8.1 Folch Oil Extraction from the Processed *R. differens*

Oil extraction was achieved following a modified previous method (Igiehon *et al*., 2021). In 50 mL falcon tubes, each sample (1 g) was combined with 10 mL dichloromethane (DCM) and methanol (MeOH) (2:1) mix. The mixtures were vortexed for 1 min, then sonicated for 10 min at 50 kHz and centrifuged (Eppendorf AG, 22331 Hamburg, Germany) for 10 min at 4200 rpm (2500 ×*g*, 20°C). Filter papers (Whatman, Grade 1, diameter 90 mm, pore size 2.5 µm) were used to carefully filter the supernatants into clean 250 mL round bottomed flasks and solvent evaporated under vacuum to yield approximately 400 mg of oil.

3.8.2 Analysis of Fatty Acids and Sterols

The fatty acid (FA) profiles and sterols of processed *R. differens* derived from the recovered lipids (300 mg each), and the FA profiles of the cookies (100 mg each), were determined as fatty acid methyl esters (FAMEs) and sterols according to modified previously adopted procedures (Cheseto *et al*., 2020, 2015). One-millilitre of sodium methoxide solution (100 mg/mL), prepared by dissolving 2000 mg of sodium methoxide in 20 mL of dry methanol, was added to each sample in micro-reaction vials. The vials and the contents were vortexed (Assistant Reamix 2789, Vortex Mixer) for a min, sonicated (Branson Ultrasonic, Dunbury, USA) at 40 kHz for 10 min and

incubated in a water bath (mgw, Lauda, Koningshoten, Germany) at 70℃ for 1 h. Deionized water (100 µL) was added to quench the reaction then vortexed for another 1 min. One-millilitre of gas chromatography (GC)-grade hexane (Sigma–Aldrich, St. Louis, MO, USA) was added to extract the resulting FAMEs followed by centrifugation (Eppendorf AG, 22331 Hamburg, Germany) at 14,000 rpm (2500 ×*g*, 20°C) for 20 min. The supernatant was carefully dried over anhydrous sodium sulphate, filtered and analysed (1.0 µL) by GC-MS on a 7890A gas chromatograph linked to a 5975 C mass selective detector (Agilent Technologies Inc.,Santa Clara, CA, USA). The GC was fitted with a (5%-phenyl)-methylpolysiloxane (HP5 MS) low bleed capillary column (30 m _ 0.25 mm i.d., 0.25_µm; J&W, Folsom, CA, USA). The carrier gas was helium at a flow rate of 1.25 mL/min. The oven temperature, programmed from 35℃ to 285℃ with a rising rate of 10℃/min had the initial and final temperatures set to hold for 5 min and 20.4 min, respectively. The ion source and quadrupole mass selective detector temperatures were maintained at 230℃ and 180℃, respectively. Acquisition of spectral masses from electron impact (EI) were at acceleration energy of 70 eV. Fragment ions were analysed over 40–550 m/z mass range in the full scan mode. The filament delay time was set at 3.3 min. Authentic standard methyl octadecanoate (0.2–125 ng/µL) prepared from octadecanoic acid (≥ 95 % purity) (Sigma-Aldrich, St. Louis, MO) and serially diluted, was analysed by GC-MS in full scan mode to generate a linear calibration curve (peak area vs. concentration) with the following equation:

$$[y = 5E + 07x + 2E + 07; R^2 = 0.9997] \quad \text{.....Equation 10}$$

Where; the gradient = 5E + 07x, y-intercept = 2E + 07 and R^2 = coefficient of determination

This regression equation was used to externally quantify different fatty acids and sterols from the samples. Integration parameters; 3 for initial threshold, 0.010 for initial peak width, 1 for initial area reject and 'on' for shoulder detection were set for the generation of peak spectral masses using ChemStation B.02.02. acquisition software installed in a Hewlett-Packard (HP Z220 SFF intel xeon) workstation. Mass spectrum data and retention times were compared to authentic standards and reference spectra published in library–MS databases: NIST 05, 08, and 11. Determination of the FAMES and sterols in all the processed samples were made in triplicates.

3.9 Mineral Analysis

Mineral analysis of the processed *R. differens* flours and their respective developed cookies were conducted in consonance with official analytical methods (AOAC, 2000) Methods 9.1.09 and 50.1.14. Ground samples (0.5 g) were weighed into digestion tubes into which 8.0 mL conc. HNO_3 (67–69% w/v, VWR Chemicals, Fontenay-sous-Bois, France) and 2 mL 30% H_2O_2 (w/w) (Sigma-Aldrich, USA) were introduced, mixed and left to stand overnight in a fume hood. The samples were then transferred to microwave digestion system (Multiwave Go Plus, Anton Paar, VA, US) and subjected to a programmed temperature-time digestion of 75℃/30 min, 120℃/20 min, 180℃/20 min and 200 ℃/10 min for the insect flours and 100℃/10 min and 180℃/10 min for the cookies. Clear solutions, indicative of completely digested samples was cooled, transferred into 25 mL falcon tubes and adjusted to the mark with 2% HNO_3. The contents of minerals under study from the samples and standard solution were analysed on an inductively coupled plasma optical emission spectrometry (ICP-OES) measurements (Optima 2100™DV ICP-OES, Perkin Elmer Massachusetts, USA). The working parameters of the ICP-OES were as follows: 1450 W for radio frequency power; 15 L min^{-1} for plasma gas flow rate; 0.2 L min^{-1} for auxiliary gas flow rate; 0.8 L min^{-1} for nebulizer gas flow rate; 1.5 L min^{-1} for sample flow rate; axial mode for view mode; the read was peak area; Source equilibration time was 10 s; Read delay was 10 s; Replicates was set at 1; Background correction was 2-point (manual point correction); Spray chamber was Scott type; Nebulizer cross was Flow GemTip Nebulizer (HF resistant); The detector was CCD; Purge gas was nitrogen; Shear gas was air; Plasma gas was nitrogen. The characteristic elemental spectrum emitted by individual minerals were measured at the following wavelengths; Mg-285.213 nm, Fe-259.939 nm, Mn-257.61 nm, Ca-317.933 nm, P-213.617 nm, K-766.49 nm, Al-396.153 nm, Cu-224.7 nm, Co- 228.616 nm and Zn- 213.857 nm. ICP-OES mix standard CatNo.43843 (Sigma-Aldrich, USA) prepared through serial dilution with 2% nitric acid to generate calibration standards of 400, 800, 2000 and 4000 µg/L were also assessed by the ICP-OES to yield linear calibration curves with elemental correlation coefficient of R^2= 0.999 for all the minerals under examination. External standard calibration and data acquisition were achieved on a Perkin Elmer Winlab 32 software (Perkin Elmer, USA). The data obtained was used to quantify all the mineral elements. The analysis was done in triplicate.

3.10 Determination of Total Flavonoid Content of *R. differens*

The total flavonoid content of the *R. differens* flours were determined following methods by Dewanto *et al*. (2002). Samples (0.5 g) were transferred into propylene tubes and mixed with 10 mL of 80% methanol. The contents of the propylene tubes were shaken on a mechanical shaker at 25℃ for 24 h then centrifuged at 4000 rpm ((2500 ×*g*, 20°C)) for 10 min. The supernatant 20 µL or standard solution of catechin (10, 20, 40, 60, 80 and 100 µg/mL) was conveyed into microtiter tubes then mixed with 80 µL of deionized water. 5% Sodium nitrite (10 mL) was added to each tube, gently mixed and left to stand for 5 min. Ten-microlitres of 10 % $AlCl_3$ were then added, allowed to stand for 5 min then followed by addition of 80 µL of 2M NaOH and gentle mixing. The reaction was then incubated at room temperature for 30 min. The absorbance was read against a reagent blank (80% methanol) in a plate reader spectrophotometer (Bio Tek Instruments, Winooski VT, USA) at 510 nm wavelength in comparison with a standard calibration plot (0.01-0.02 – 0.04 - 0.06 – 0.08 – 0.1 mg/mL) curve of catechin in 80 % methanol.

3.11 Analysis of Volatile Organic Compounds of Cookies

The volatile compounds were determined according to (Cheseto *et al*., 2020; Mekonnen *et al*., 2021). Briefly, cookie samples, shortly kept wrapped in aluminium foils in a deep freezer at ₋70℃ were ground within 12 h of preparation using pestle and mortar. The ground samples (10 g), were accurately weighed into 250 mL quick fit chamber Agricultural Research Service (ARS), (Gainesville, FL, USA). A push-pull Gast pump (Gast Manufacturing Inc., Benton Harbor, MI, USA), was used to pump air filtered through activated charcoal and humidified over the samples at 340 mL/min flowrate. Meanwhile, the volatiles adhered on GC grade dichloromethane (DCM) precleaned Super-Q traps (30 mg, Analytical Research System, Gainesville, FL, USA) at 170 mL/min flow rate facilitated by Vacuubrand CVC2 vacuum pump (Vacuubrand, Wertheim, Germany) for 24 h. Trapped volatiles were then eluted with 200 µL of GC-grade DCM into 250 µL conical point glass inserts (Supelco, Bellefonte, PA, USA) fitted into 2 mL glass vials and immediately queued for GC-MS analysis.

The volatiles were identified by a GC-MS on an HP 7890A series gas chromatograph (Agilent Technologies, Wilmington, NC, USA) attached to an HP 5975C mass spectrometer (Agilent Technologies, Wilmington, NC, USA) operated in electron ionization mode of 70 eV. A non-polar HP-5MS capillary column (30 m 0.25 mm i.d.; 0.25 m film thickness; J & W Scientific,

Folsom, CA, USA) was fitted to the instrument. Helium was employed as the carrier gas at a rate of 1.2 mL min^{-1}. One microliter of each sample was injected in a splitless mode at 35°C for 5 min, then increased to 280°C at 10 °C min^{-1}. The injector and detector were held isothermal at 280°C for 35 min. The temperature of the ion source was 230°C. Electron ionization mass spectra were recorded at 70 eV spanning a mass range of 38–550 Daltons over a scan period of 0.73 scans s^{-1} (Da). Authentic standard hexanal was run in the GC-MS in full scan mode to generate a linear calibration curve (peak area vs. concentration) with the following equation;

$[y = 203482x - 451578;\ R^2 = 0.9997]$……..Equation 11

Where; the gradient = 203482x, y-intercept = -451578 and R^2 = coefficient of determination

To identify volatile compounds, their retention periods and mass fragmentation spectra were compared to authentic standards (those available). Others were tentatively identified using Adams, Chemoecol, and the National Institute of Standards and Technology mass spectrum library matching (NIST) (MSD Chemstation E.02.00.493, MS HP, USA). All assays were done in triplicates.

3.12 Determination of *in vitro* Protein Digestibility of the Cookies

Determination of *in vitro* protein digestibility of the processed *R. differens* and their respective cookies were conducted according to Chavan *et al*. (2001) and modified by Wang *et al*. (2010). Samples (1 g) were weighed into 50 mL centrifuge tubes and 20 mL of 0.10M HCl added. Similarly, 50 mg of pepsin (from porcine gastric mucosa, ≥250 units/mg solid, Sigma-Aldrich) in 1 mL of 0.01M HCl were added and mixed. The mixture was shaken gently under incubation in a water bath at 37°C for 3 h. A mixture of 10 mL distilled water and 10 mL of 0.1 M phosphate buffer (pH 8.0) containing 5 mg of trypsin (porcine pancreas, lyophilized powder, BioReagent, 1,000-2,000 BAEE units/mg solid, Sigma-Aldrich). The mixture was again subjected to incubation in a water bath at 37°C for 3 h with continuous shaking. The enzymes were inactivated by adding 10 mL trichloroacetic acid (TCA) followed by centrifugation at 14000 rpm (18624 ×*g*, 20°C) for 10 min. The supernatant was discarded and the residue dried in an oven at 105°C for 3 h. Dried residue (0.5 g) were assayed for nitrogen content using the Kjeldahl method. The protein digestibility was calculated by the difference between the total amount of protein in the cookie and the residual protein after enzyme digestion divided by the total protein from the samples.

3.13 Microbial Analysis

3.13.1 Serial Dilutions

Samples, 5 g each, were blended with 45 mL of sterile peptone physiological salt solution (0.85% (wt/vol) NaCl, 0.1% (wt/vol) peptone (OXOID LP0034) and 8.5 g/L NaCl) in a sterile filter stomacher bag (Bagmixer 400W, Interscience, St. Nom, France) and homogenized (Seward, 400 circulator, West Sussex, UK) at normal speed for 1 min. From the homogenate, a 10-fold serial dilution with 1 mL was then made.

3.13.2 Enumeration of Total Viable Counts (TVC)

Enumeration of TVC adhered to the procedure by Klunder *et al*. (2012). From the serial dilutions, 0.1 mL of the homogenates were each aseptically aliquoted and inoculated onto a pair of triplicate Petri dishes containing sterile solidified 20 mL Plate Count Agar (Oxoid CM0463). A sterile glass rod was gently used to gently spread the inoculums uniformly onto the media. The media were incubated at 30℃ for 48 h.

3.13.3 Enumeration of Lactose Positive *Enterobacteriaceae*

Assessment of *Enterobacteriaceae* followed the procedure delineated by Nyangena *et al*. (2020). Serial dilutions 10^{-3}, 10^{-5} and 10^{-6} were considered for inoculation of 0.1 mL of the homogenates on freshly prepared sterile MacConkey agar (Oxoid CM0007) in a pair of triplicate Petri dishes. The inoculums were evenly spread on the media using sterile glass rod and the plates incubated at 37℃ for 24 h.

3.13.4 Detection of *Staphylococcus aureus*

Detection *Staphylococcus aureus* was conducted according to previous procedure (Ramashia *et al*., 2020). Aliquots (0.1 mL) were aseptically inoculated onto sterile Baird Parker agar (Oxoid CM1127,) enriched with 5% Egg Yolk Tellurite Emulsion (Oxoid CM0276) in a pair of triplicate Petri dishes. The inoculums were uniformly spread on the media using sterile glass rod. The plates were incubated at 35℃ for 48 h. Colonies of characteristic circular (2–3 mm diameter), smooth, convex, moist appearance were enumerated.

3.13.5 Detection of *Salmonellae*

*Salmonella*e detection was done according to Nyangena *et al*. (2020). Sample (25 g) were first enriched in 225 mL of nutrient broth containing 5 g peptone, 5 g NaCl, 1 g Lab-Lemco beef

extract, and 2 g yeast extract per 1L of water, pH 7.4 (Oxoid CM0067) and incubated at 35℃ for 24 h. The homogenate (25 mL) was further selectively enriched by transferring into 225 mL of Rappaport-Vassiliadis broth (Oxoid CM0669) and incubated at 37℃ for 24 h. A sterile wire loop was used to collect Rappaport-Vassiliadis broth culture and streaked on Salmonella- Shigella Agar (Oxoid CM0099) and plates incubated at 37℃ for 24 h. Typical salmonella colonies which are colourless with black centres were monitored of *Salmonella* presence.

3.13.6 Enumeration of Yeast and Moulds

Yeast and moulds were cultured on Potato Dextrose Agar (PDA) (Oxoid Ltd., United Kingdom) according to Ramashia *et al*. (2020). Approximately 20 mL of sterile molten PDA will be aseptically poured into three plates in duplicate then left to solidify. One millilitre each of the decimal dilutions 10^{-3} ,10^{-4} and 10^{-5} was aseptically transferred onto the solid media in each of the plates and gently spread with a sterile glass rod. The plates were then incubated at 25°C for 5 days after which colonies were examined and counted using a magnifying lens.

3.14 Sensory Evaluation of the Cookies

Sensory attributes of the developed cookies in relation to colour, flavour, mouthfeel, texture and overall acceptability were evaluated using a 5-point hedonic scale (where; 5 denoted like extremely, 4 denoted like, 3 denoted neither like or dislike, 2 denoted dislike and 1 denoted dislike extremely) (Lawless & Heymann, 2013), the ranking test evaluated differences in intensity of the sensory properties among samples using comparable intervals between the categories (See sensory evaluation questionnaire in the appendix section). The experiment randomly enrolled a team of 145 semi-trained panellists of age ranging 18-50 years. The team comprised of 72 males and 73 females achieving an excellent gender balance. They were chosen for their experience in describing food products and knowledge of cookies, despite having no previous experience in insect consumption. As part of the assessments, individual booths equipped with pens and questionnaires for data collection and processing were set up in a sensory laboratory room that nearly met the ISO standards (ISO, 2008). The coded cookie samples were served to the panellists at room temperature. Alongside the samples, the panellists were given a cup of room-temperature spring water for palate cleansing before commencement of the test and between every tasting done. They were instructed to consent to the study, carefully read the instructions and focus on the texture and colour of the cookies first before proceeding to taste. Panellists were required to score the

samples against attributes provided in the evaluation forms. The scores were compiled and analysed.

3.15 Statistical Analysis

R Studio software version 1.3.1093-1 (R Core Team, 2020) was used to perform statistical analyses for all the descriptive and quantitative data. Shapiro–Wilk test and Bartlett's test ($p > 0.05$) confirmed that the data sets were normally distributed and had homogeneous variances respectively. Analysis of variance (ANOVA) was used to determine the effects of processing on nutritional, sterol, and flavonoid contents of *R. differens* and cookies with differently processed *R. differens* flour on the nutritional composition, aroma compounds & protein digestibility, microbial quality and sensory acceptability of the cookies. The means were separated using Tukey's multiple comparison tests at $p < 0.05$. The results were expressed as mean ± standard deviation. Principal component analysis (PCA) was applied to assess the difference in fatty acid of the differently processed *R. differens* and the difference in sensory scores of the developed cookies. To analyse the chemical profiles of different enriched cookie volatiles, one-way analysis of similarities (ANOSIM) utilizing the Bray–Curtis dissimilarity matrix was used. The relative contribution of different compounds to the dissimilarity between volatiles from different cookies was calculated and visualized using the non-metric multidimensional scaling approach based on the similarity percentages (SIMPER) analysis. A Pearson's correlation coefficient was adopted to compare the correlations between the sensory scores of colour, flavour, mouthfeel and texture with that of overall acceptability of the different insect-based cookies.

CHAPTER FOUR

RESULTS

4.1 Nutritional Composition of the Processed *R. differens*

4.1.1 Proximate Composition

The proximate components of *R. differens* differed significantly ($p<0.05$) across the processed *R. differens* samples (Table 4.1). Blanched *R. differens*, boiled *R. differens* and toasted *R. differens* had significantly higher protein contents than the deep-fried samples. Deep-fried *R. differens* indicated the highest levels of dry matter and fat whilst boiled *R. differens* had the lowest dry matter and fat content. Deep-fried *R. differens*, on the other hand, exhibited the lowest levels of protein, ash, and fibre.

Table 4.1. Proximate composition (% dry matter) of *R. differens* processed using different methods.

Processing method	**Moisture**	**Dry Matter**	**Protein**	**Fat**	**Ash**	**Fibre**
Blanching	1.8 ± 0.05^{b}	98.2 ± 0.05^{b}	40.1 ± 1.33^{b}	43.8 ± 0.41^{b}	2.2 ± 0.00^{b}	11.2 ± 0.01^{b}
Boiling	14.5 ± 0.10^{c}	85.6 ± 0.10^{a}	43.1 ± 1.60^{bc}	36.3 ± 1.06^{a}	2.3 ± 0.09^{b}	10.9 ± 0.19^{b}
Toasting	1.6 ± 0.06^{b}	98.4 ± 0.06^{b}	44.7 ± 1.03^{c}	46.0 ± 0.82^{b}	2.4 ± 0.17^{b}	9.0 ± 0.74^{a}
Deep-frying	0.8 ± 0.03^{a}	99.2 ± 0.03^{c}	7.8 ± 0.59^{a}	83.0 ± 1.54^{c}	1.2 ± 0.16^{a}	8.7 ± 0.39^{a}
F_{df}	$F_{(3,8)}$	$F_{(3,8)}$	$F_{(3,8)}$	$F_{(3,8)}$	$F_{(3,8)}$	$F_{(3,8)}$
P-value	0.001	0.001	0.001	0.001	0.001	0.001

Same small superscript letters within columns indicate no significant differences of proximate composition at $p<0.05$. All values are presented as mean ± SD.

4.1.2 Fatty Acids Profile of the Processed *R. differens*

Table 4.2. show the fatty acid content of processed *R. differens* oils. All of the samples comprised a total of 32 fatty acids (FAs). Saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs) accounted for 19, 10, and 3 % of the total FAs, respectively. The most abundant FAs recorded were methyl hexadecanoate and methyl octadecenoate of the SFA, methyl (9*Z*)-hexadecenoate and methyl (11*Z*)-eicosenoate of the MUFAs and methyl (9*Z*,12*Z*)-octadecadienoate of the PUFAs. Deep-frying significantly increased levels of methyl (9*E*)-octadecenoate (oleic acid) ($F_{(3,8)}$ = 371.4, p<0.001), toasting significantly increased levels of methyl (9*Z*)-hexadecenoate (palmitoleic acid) ($F_{(3,8)}$ = 66.0, p<0.001), and blanching significantly increased levels of methyl (10*Z*)-nonadecenoate ($F_{(3,8)}$ = 58.3, p<0.001), methyl (10*Z*)-heptadecenoate ($F_{(3,8)}$ = 152.1, p<0.001) and methyl (9*E*)- tetradecenoate (myristoleic acid) ($F_{(3,8)}$ = 41.9, p<0.001). Of the PUFAs, methyl (9*Z*,12*Z*,15*Z*)-octadecatrienoate (α-linolenic acid) was the only detected omega 3 whereas methyl (9*Z*,12*Z*)-octadecadienoate (Linoleic acid) and methyl (5*Z*,8*Z*,11*Z*,14*Z*)-eicosatetraenoate (Arachidonic acid) represented the detected omega 6. Significantly higher methyl (9*Z*,12*Z*)-octadecadienoate ($F_{(3,8)}$ = 423.1, p<0.001), methyl (5*Z*,8*Z*,11*Z*,14*Z*)-eicosatetraenoate ($F_{(3,8)}$ = 89.9, p<0.001), methyl (9*Z*,12*Z*,15*Z*)-octadecatrienoate ($F_{(3,8)}$ = 49.3, p<0.001), total PUFAs ($F_{(3,8)}$ = 424.1, p<0.001), total MUFAs ($F_{(3,8)}$ = 68.1, p<0.001) and PUFAs/MUFAs ($F_{(3,8)}$ = 135.3, p<0.001) were realized in blanched *R. differens* compared with the boiled, toasted and deep-fried ones.

Based on the influence of four processing methods on the levels of detected FAs (Figure 4.1A) and grouping the FAs based on their differences in concentration from the four processing methods (Figure 4.1B), a two-dimensional Principal Component Analysis (PCA) explained 68.3% of the variation. The 1st and 2nd PCs, respectively, accounted for 42.6% and 36.1% of the overall variance. In the first quadrant, the concentrations of methyl 15-methylhexadecanoate, methyl (5*Z*)-dodecanaote, methyl pentadecanoate, methyl (10*Z*) heptadecanoate, methyl (10*Z*)-nonadecanoate, methyl tridecanoate, methyl (5*Z*,8*Z*,11*Z*,14*Z*)-eicosatetraenoate, methyl (9*Z*)-tetradecenoate, methyl (9*Z*,12*Z*)-octadecadienoate, methyl (11*Z*)-eicosenoate, methyl (9*Z*)-heptadecenoate and methyl dodecanoate positively correlated with blanching. In the second quadrant, the concentrations of methyl 3-methoxyoctadecanoate, methyl (9*Z*)-hexadecenoate, methyl undecanoate, methyl tetracosanoate, methyl 3-methyltridecanoate, methyl 12-methyltridecanoate, methyl nonadecanoate, methyl decanoate, methyl tetradecanoate, methyl hexadecanoate, methyl

docosanoate, methyl (13*Z*)-docosenoate and methyl (11*Z*)-octadecenoate positively correlated with toasting In the third quadrant, the levels of methyl 20-methylhexacosanoate, methyl octadecenoate, methyl tricosanoate, methyl eicosanoate and methyl (9*E*) octadecenoate positively correlated with boiling and deep-frying

Table 4.2. Fatty acid composition (µg /g dry matter) of oil extracted from *Ruspolia differens* processed differently

Peak No.	tR (min)	Compound name	Corresponding fatty acids	ω-n(Δn)	Blanching	Boiling	Toasting	Deep-frying
1	16.36	Methyl decanoate	Capric acid	C10:0	0.1 ± 0.05^{a}	0.3 ± 0.03^{a}	1.3 ± 0.30^{b}	0.2 ± 0.02^{a}
2	17.70	Methyl undecanoate	Undecyclic acid	C11:0	0.05 ± 0.02^{a}	0.06 ± 0.02^{a}	0.06 ± 0.008^{a}	0.04 ± 0.007^{a}
3	18.96	Methyl dodecanoate	Lauric acid	C12:0	17.2 ± 1.08^{b}	10.05 ± 2.69^{a}	15.8 ± 2.56^{b}	14.0 ± 1.89^{ab}
4	20.12	Methyl tridecanoate	Tridecyclic acid	C13:0	0.2 ± 0.10^{b}	0.05 ± 0.01^{a}	0.09 ± 0.01^{a}	0.03 ± 0.002^{a}
5	20.61	Methyl 3-methyltridecanoate	Tridecyclic acid	iso-methyl-C13:0	0.09 ± 0.03^{a}	0.09 ± 0.01^{a}	0.8 ± 0.24^{b}	0.006 ± 0.0005^{a}
6	20.86	Methyl 12-methyltridecanoate	Tridecyclic acdi	so-methyl-C13:0	0.09 ± 0.02^{b}	0.01 ± 0.005^{a}	1.75 ± 0.04^{c}	0.008 ± 0.001^{a}

7	21.29	Methyl tetradecanoate	Myristic acid	C14:0	73.2 ± 20.30[a]	77.1 ± 4.03[a]	162.3 ± 18.59[b]	92.6 ± 24.16[a]
8	22.34	Methyl pentadecanoate	Pentadecyclic acid	C15:0	4.2 ± 1.98[b]	0.9 ± 0.005[a]	0.1 ± 0.02[a]	0.1 ± 0.02[a]
9	23.23	Methyl hexadecanoate	Palmitic caid	C16:0	1361.4 ± 107.90[a]	1354.9 ± 235.84[a]	1652.9 ± 80.27[a]	1404.5 ± 97.18[a]
10	24.35	Methyl 15-methylhexadecanoate	Palmitic acid	iso-methyl-C16:0	17.6 ± 3.51[b]	48.6 ± 10.76[c]	1.8 ± 0.23[a]	0.4 ± 0.04[a]
11	25.54	Methyl octadecanoate	Stearic acid	C18:0	271.1 ± 39.70[a]	311.6 ± 10.52[ab]	341.1 ± 30.28[b]	350.9 ± 15.41[b]
12	26.21	Methyl nonadecanoate	Nonadecyclic acid	C19:0	5.9 ± 0.51[b]	3.6 ± 0.19[a]	17.9 ± 1.03[c]	5.6 ±0.22[b]
13	26.35	Methyl 3-methoxyoctadecanoate	Stearic acid	iso-methoxy-C18:0	3.6 ± 0.82[b]	4.4 ± 0.64[b]	4.9 ± 0.85[b]	0.5 ± 0.9[a]
14	27.11	Methyl eicosanoate	Arachidic acid	C20:0	54.3 ± 8.70[a]	49.8 ± 0.55[a]	57.8 ± 2.75[a]	64.5 ± 7.66[a]

15	27.87	Methyl heneicosanoate	Heneicosylic acid	C21:0	3.9 ± 1.06[b]	0.2 ± 0.03[a]	0.5 ± 0.15[a]	2.9 ± 0.86[b]
16	28.65	Methyl docosanoate	Behenic acid	C22:0	3.5 ± 1.42[a]	11.0 ± 0.44[a]	28.8 ± 7.80[b]	12.9 ± 1.49[a]
17	29.42	Methyl tricosanoate	Trycosylic	C23:0	3.8 ± 0.99[a]	5.08 ± 0.46[ab]	5.6 ± 0.16[b]	6.3+ 0.02[b]
18	30.20	Methyl tetracosanoate	Lignoceric acid	C24:0	4.5 ± 0.31[a]	8.3 ± 1.47[b]	8.7 ± 2.26[b]	2.6 ± 0.52[a]
19	33.40	Methyl 20-methylhexacosanoate	Cerotic acid	iso-methyl-C26:0	2.5 ± 0.28[a]	3.9 ± 0.57[b]	3.1 ± 0.25[ab]	3.0 ± 0.57[ab]
		∑ SFA			1827.3 ± 92.24[a]	1889.9 ± 242.93[a]	2305.4 ± 89.85[b]	1961.1 ± 114.16[ab]
20	21.13	Methyl (9*Z*)-tetradecenoate	Myristoleic acid	14:1 (n-5)	2.3 ± 0.35[c]	1.4 ± 0.16[b]	1.4 ± 0.33[b]	0.01 ± 0.002[a]
21	22.18	Methyl (5*Z*)-dodecenoate		C10:1 (n-5)	1.7 ± 0.43[b]	1.7 ± 0.21[b]	0.05 ± 0.007[a]	0.03 ± 0.01[a]

22	23.21	Methyl (9*Z*)-hexadecenoate	Palmitoleic acid	C16:1 (n-7)	130.3 ± 7.61^{b}	107.6 ± 5.54^{a}	159.9 ± 3.68^{c}	94.4 ± 6.90^{a}
23	24.15	Methyl (10*Z*)-heptadecenoate		C17:1(n-7)	41.5 ± 4.11^{c}	6.6 ± 0.45^{a}	14.3 ± 1.33^{b}	10.3 ± 0.98^{ab}
24	24.24	Methyl (9*Z*)-heptadecenoate		C17:1(n-9)	13.9 ± 0.99^{b}	11.9 ± 3.46^{b}	15.3 ± 1.74^{b}	2.4 ± 0.27^{a}
25	24.56	Methyl (11*Z*)-octadecenoate	Vaccenic acid	C18:1(n-11)	1.4 ± 0.07^{a}	2.7 ± 0.12^{ab}	4.7 ± 1.06^{c}	3.7 ± 0.45^{bc}
26	24.85	Methyl (9*E*)-octadecenoate	Oleic acid	C18:1(n-9)	8.4 ± 0.79^{b}	1.8 ± 0.21^{a}	3.1 ± 0.59^{a}	42.1 ± 3.27^{c}
27	26.01	Methyl (10*Z*)-nonadecenoate		C19:1 (n-9)	43.3 ± 6.11^{c}	14.9 ± 2.21^{ab}	20.9 ± 1.76^{b}	9.4 ± 0.49^{a}
28	26.59	Methyl (11*Z*)-eicosenoate		C20:1(n-9)	50.7 ± 13.71^{a}	30.5 ± 4.00^{a}	53.5 ± 9.76^{a}	31.9 ± 10.27^{a}
29	28.48	Methyl (13*Z*)-docosenoate	Erucic acid	C22:1(n-9)	0.6 ± 0.19^{a}	5.0 ± 0.86^{b}	5.7 ± 0.53^{b}	2.4 ± 1.07^{a}

		∑ MUFA			294.0 ± 16.87[b]	184.1 ± 5.52[a]	278.9 ± 14.24[b]	196.8 ± 5.95[a]
30	25.25	Methyl (9Z,12Z)-octadecadienoate	Linoleic acid	C18:2(n-6)	2177.6 ± 42.67[d]	1542.7 ± 33.59[b]	1723.3 ± 80.41[c]	678.4 ± 41.97[a]
31	26.50	Methyl (9Z,12Z,15Z)-octadecatrienoate	Linolenic acid	C18:3(n-3)	4.3 ± 0.33[c]	2.5 ± 0.14[b]	1.7 ± 0.27[a]	2.7 ± 0.30[b]
32	26.59	Methyl (5Z,8Z,11Z,14Z)-eicosatetraenoate	Arachidonic acid	C20:4(n-6)	4.0 ± 0.22[c]	2.1 ± 0.03[a]	2.9 ± 0.08[b]	1.7 ± 0.30[a]
		∑ PUFA			2185.9 ± 43.18[d]	1547.3 ± 33.44[c]	1727.9 ± 80.65[b]	682.8 ± 41.37[a]
		∑ n-6 PUFA			2181.6 ± 42.89[d]	1544.8 ± 33.56[b]	1726.2 ± 80.46[c]	680.1 ± 41.67[a]
		∑ n-3 PUFA			4.3 ± 0.33[c]	2.5 ± 0.14[b]	1.7 ± 0.27[a]	2.7 ± 0.30[b]
		∑n-6/n-3			507.3	617.9	1015.3	251.9
		∑PUFA/SFA			1.2 ± 0.04[c]	0.8 ± 0.09[b]	0.7 ± 0.03[b]	0.3 ± 0.005[a]

Fatty acids presented as a Mean ± SD (standard deviation) of triplicate determinations). tR Retention time, SFA saturated fatty acids, MUFA monounsaturated fatty acids, PUFA polyunsaturated fatty acids.

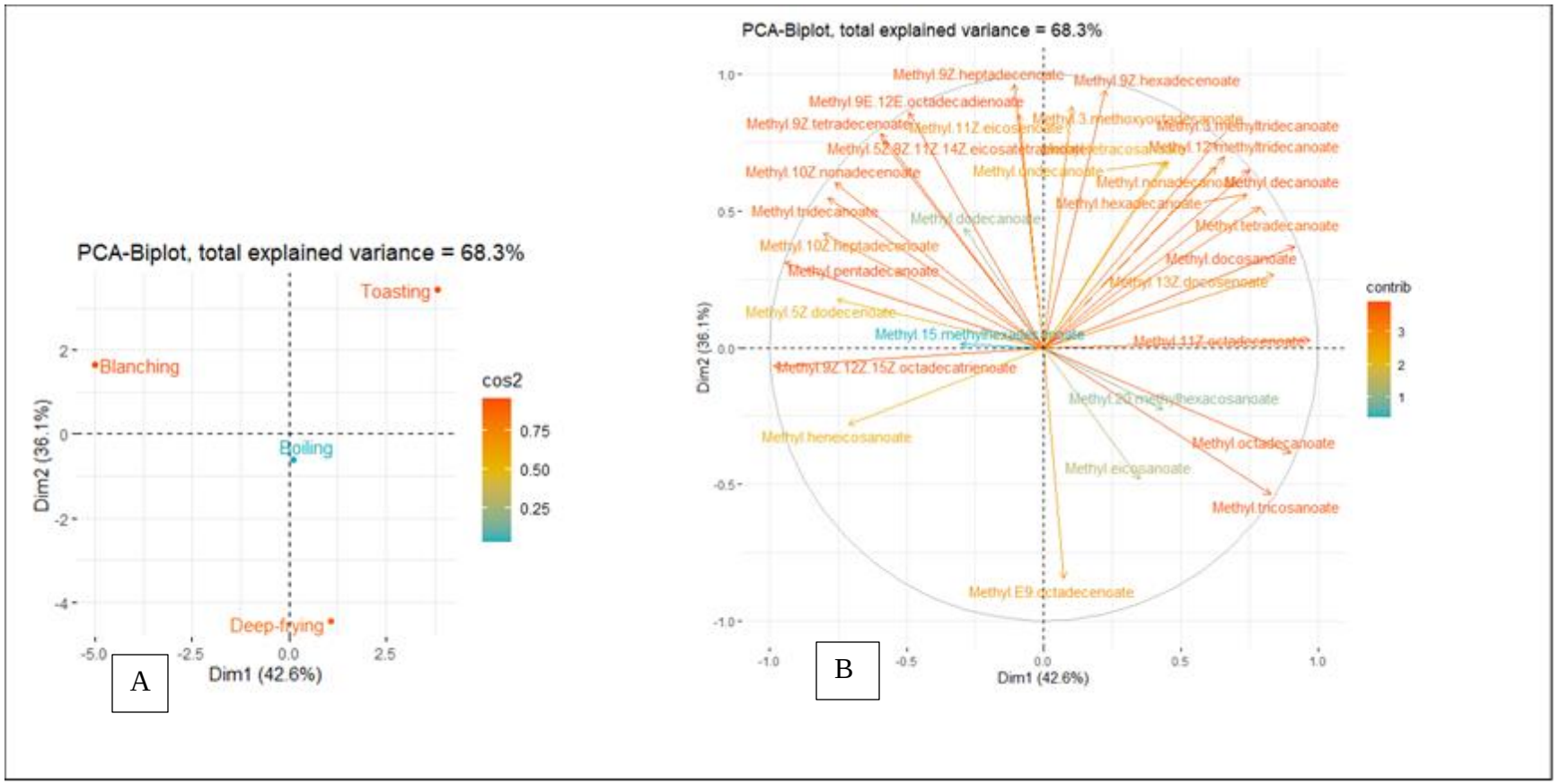


Figure 4.1. A. Principal Component Analysis (PCA) showing the variation of effects of processing methods on the concentration of FAs; B. Principal component analysis (PCA) biplot showing the variation of fatty acids among the oils from processed *R. differens*

4.1.3 Sterols Composition of Processed *R. differens*

The proportions of (22Z)-27-norergosta-5,22-dien-3-ol ($F_{(3,8)}$ = 71.3, p<0.001), cholesterol ($F_{(3,8)}$ = 34.8, p<0.001), campesterol ($F_{(3,8)}$ = 17.1, p<0.001), cholest-4-ene-3-one ($F_{(3,8)}$ = 14.4, p<0.01), and sitosterol ($F_{(3,8)}$ = 37.6, p<0.001) displayed significant variations in the processed samples. When compared to other processing methods, blanching induced considerably higher amounts of (22Z)-27-Norergosta-5, 22-dien-3-ol, cholesterol, campesterol, cholest-4-en-3-one, and -sitosterol (Table 4.3).

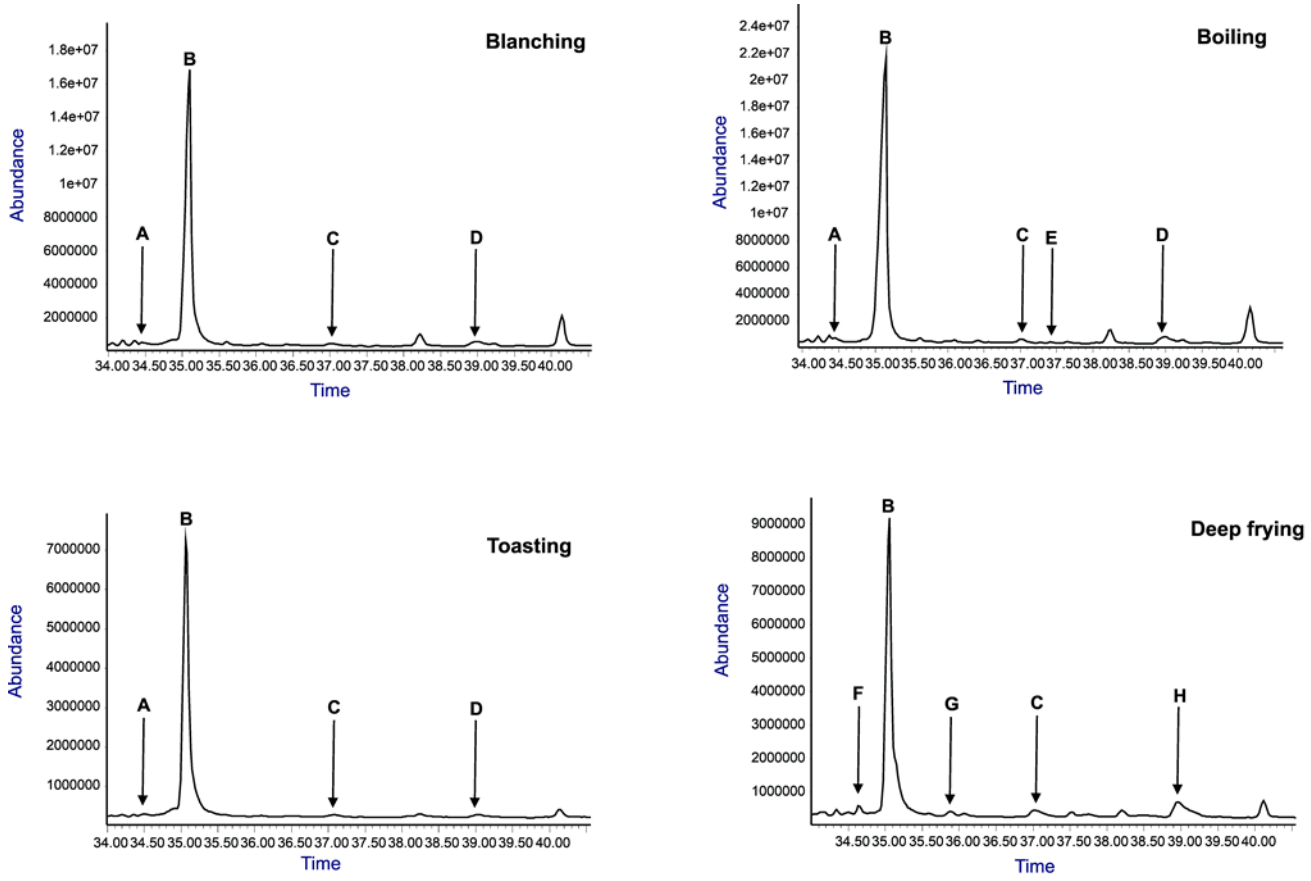
Table 4.3. Sterols content (µg/g dry matter) of oil extracted from *R. differens* processed

Compound	Blanching	Boiling	Toasting	Deep-frying
(22Z)-27-Norergosta-5,22-dien-3β-ol	2.3 ± 0.05^{b}	2.3 ± 0.14^{b}	2.6 ± 0.19^{b}	1.2 ± 0.09^{a}
Ergosta-4,6,22-trien-3-β-ol	-	-	-	1.9 ± 0.04
Cholesterol	82.2 ± 6.04^{c}	63.2 ± 5.79^{b}	43.4 ± 6.61^{a}	41.8 ± 3.24^{a}
Stigmasta-4,6,22-triene-3-β -ol	-	-	-	2.5 ± 0.22
Campesterol	4.5 ± 0.37^{c}	4.4 ± 0.25b^{c}	2.8 ± 0.37^{a}	3.6 ± 0.34^{ab}
*Cholest-4-en-3-one	1.0 ± 0.16^{b}	1.1 ± 0.03^{b}	0.9 ± 0.06^{a}	0.7 ± 0.08^{a}
β Sitosterol	7.6 ± 0.59^{b}	4.2 ± 0.40^{a}	3.8 ± 0.79^{a}	-
γ Sitosterol	-	-	-	3.5 ± 0.13

Results are presented as mean ± standard deviation. Same small letters within rows indicate no significant differences of the minerals at p<0.05. *Not a sterol.

Figure 4.2. shows the total ion chromatograms of the sterols and their thermally changed derivatives from the four processed *R. differens*. Cholest-4-en-3-one levels were extremely low, and only boiled samples revealed a noticeable peak. (22Z)-27-Norergosta-5,22-dien-3-ol was converted to ergosta-4,6,22-triene-3-ol during deep-frying, and β sitosterol was replaced by γ

sitosterol isomer. In comparison to the other processing methods, however, Stigmasta-4,6,22-triene-3-ol was discovered in this sample for the first time.



A -(22Z)-27-Norergosta-5,22-dien-3β-ol, **B** - Cholesterol, **C** - Campesterol, **D** - β-Sitosterol, **E** -Cholest-4-en-3-one, **F** - Ergosta-4,6,22-triene-3-β-ol
G - Stigmasta-4,6,22-triene-3-β-ol, **H** - γ-Sitosterol

Figure 4.2. Representative total ion Chromatograms of sterols from *R. differens* after subjecting to four different processing methods.

4.1.4 Mineral Profile of Processed *R. differens*

Except for Copper (Cu) and Cobalt (Co), calcium (Ca), phosphorus (P), magnesium (Mg), iron (Fe), manganese (Mn), and zinc (Zn) varied significantly throughout the treated *R. differens* (Table 4.4). P, Ca, and Mg were the most common macro minerals found in all processed *R. differens*, while Fe, Zn, and Mn were the most common trace minerals. Mineral levels were considerably reduced (p<0.05) in deep-fried *R. differens* in all treated samples.

Table 4.4: Mineral profiles (dry matter) of *R. differens* processed using different methods.

	Processing Method					
Minerals	**Blanching**	**Boiling**	**Toasting**	**Deep-frying**	***F_{df}***	***P-value***
Ca (mg/100g)	47.9 ± 1.06^{b}	54.5 ± 1.92^{b}	55.0 ± 5.05^{b}	35.8 ± 0.47^{a}	$F_{(3,8)}$	0.001
P (mg/100g)	427.8 ± 1.80^{b}	427.7 ± 14.36^{b}	496.8 ± 27.60^{c}	305.4 ± 18.02^{a}	$F_{(3,8))}$	0.001
Mg (mg/100)	52.8 ± 0.54^{b}	56.5 ± 1.57^{b}	53.2 ± 7.10^{b}	33.3 ± 1.15^{a}	$F_{(3,8)}$	0.001
Fe (mg/100g)	140.9 ± 8.59^{b}	179.1 ± 14.18^{c}	22.2 ± 0.27^{a}	12.9 ± 2.48^{a}	$F_{(3,8)}$	0.001
Cu (mg/100g)	1.9 ± 0.05^{b}	2.3 ± 0.18^{b}	2.2 ± 0.22^{b}	1.6 ± 0.04^{a}	$F_{(3,8))}$	0.01
Mn (mg/100g)	5.3 ± 0.04^{c}	4.1 ± 0.0003^{b}	4.0 ± 0.0^{8b}	2.9 ± 0.70^{a}	$F_{(3,8)}$	0.001
Zn (mg/100g)	16.8 ± 0.97^{c}	18.4 ± 0.08^{c}	13.2 ± 1.82^{b}	8.5 ± 0.15^{a}	$F_{(3,8)}$	0.001
Co (µg/100g)	25.6 ± 4.49^{a}	33.1 ± 0.005^{a}	28.1 ± 5.71^{a}	29.9 ± 1.00^{a}	$F_{(3,8)}$	ns

Results are presented as mean ± standard deviation. Same small superscript letters within rows indicate no significant differences of the minerals at $p<0.05$.

4.2 Total flavonoid content of Processed *R. differens*

The flavonoid composition of the variously processed *R. differens* pastes is shown in Figure 4.3. The flavonoid content of processed *R. differens* differed considerably ($F_{(3,8)} = 248.6$; $p<0.001$) Blanched (529.59 mg/100 g) > boiling (248.02 mg/100 g) > toasted (231.35 mg/100 g) > deep-fried (169.68 mg/100 g) were the highest in total flavonoid content

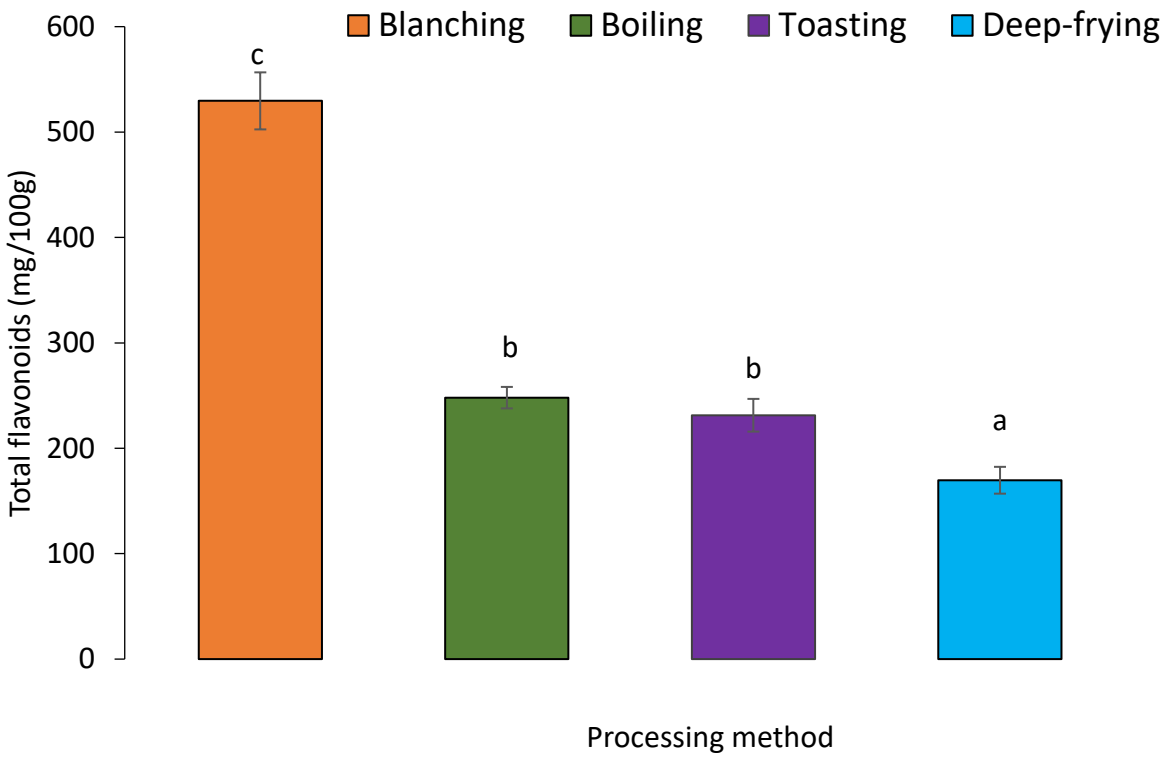


Figure 4.3: Bar chart displaying the variability of total flavonoids (mg/100 g DM) in the different processed *R. differens* samples. Error bars indicate standard deviation of the means. For every processing method, the bars carrying the same small letters correspond to total flavonoid values that are not significantly different ($p<0.05$).

4.3 *In vitro* Protein Digestibility of the Processed *R. differens*

The % *in vitro* protein digestibility of differently processed *R. differens* are presented in Figure 4.4**.** below. Protein digestibility was significantly higher ($p<0.05$) in both blanched and boiled *R. differens* as compared with toasted and deep-fried samples.

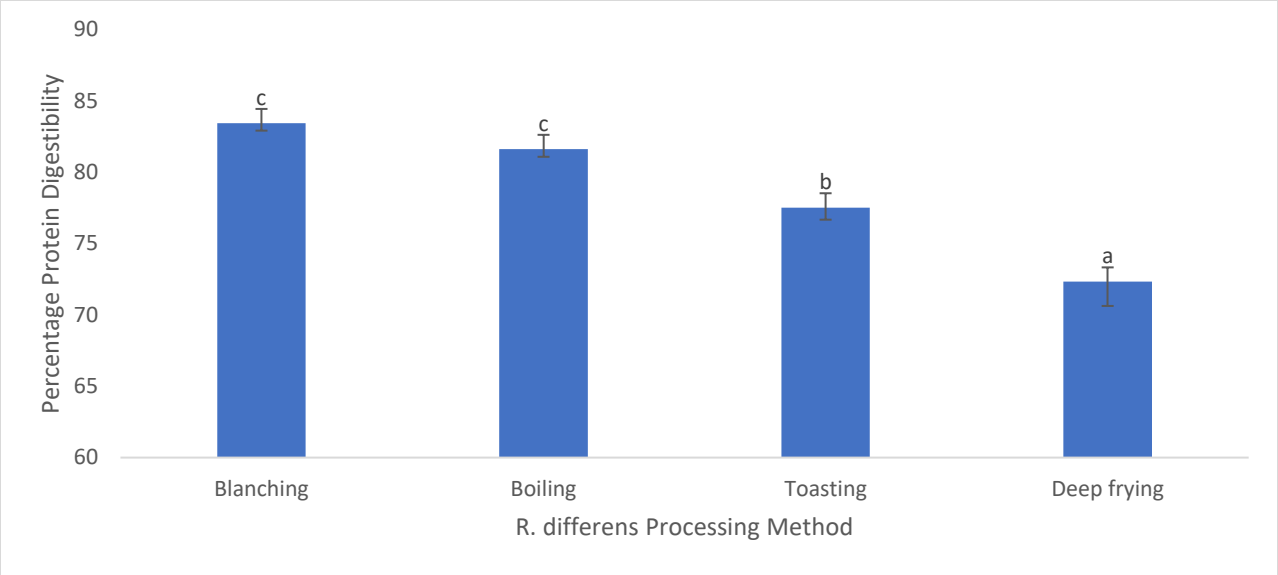


Figure 4.4: Bar chart showing the variation of *in vitro* protein digestibility (%) of differently processed *R. differens*. Error bars indicate standard deviation of the mean. Bars with same small letter are not significantly different.

4.4 Microbial Loads of the Processed *R. differens*

The microbial assays of the different processed *R. differens* are shown in Table 4.5. Blanched *R. differens* exhibited significantly higher (p<0.05) microbial counts of total viable counts (TVC), *Enterobacteriaceae*, *S. aureus* and yeast & moulds. The microbial counts of TVC and *S. aureus* depreciated in the order blanching>boiling>toasting>deep-frying. No *Salmonellae* was detected with no yeast & mould counts in all the processed samples.

Table 4.5: Effect of different processing methods on the microbial levels (cfu/g) of *R. differens*

Processing method	**TVC**	**Lac + *Enterobacteriaceae***	***Staphylococcus aureus***	***Salmonellae***	**Yeast & Moulds**
Blanching	4.54 ± 0.11^{c}	3.04 ± 0.22^{c}	4.83 ± 0.02^{c}	–ve	0.0
Boiling	2.57 ± 0.05^{a}	0.0 ± 0.0^{a}	2.61 ± 0.17^{b}	–ve	0.0
Toasting	3.38 ± 0.10^{b}	1.04 ± 0.03^{b}	3.70 ± 0.33^{b}	–ve	0.0
Deep-frying	2.42 ± 0.33^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	–ve	0.0

Means on the same column followed by the same small letters are not significantly different ($p < 0.05$; $n = 3$). *-ve = Not detected. Lac+= Lactose positive

4.5 Nutritional Composition of the Cookies Enriched with Differently Processed *R. differens*

4.5.1 Proximate Composition of the Enriched Cookies

The proximate composition of the cookies enriched processed *R. differens* flours is presented in Table 4.6. Moisture and dry matter levels were not significantly different ($p<0.05$). Significantly higher ($p<0.05$) protein levels were recorded in blanched, boiled and toasted *R. differens*-based cookies. Deep-fried *R. differens*-based cookies exhibited the highest level of fat and energy followed closely by blanched, boiled and toasted *R. differens*-based cookies. Generally, Control cookie displayed the lowest average levels of key proximate components.

Table 4.6: Proximate composition (%) and gross energy (kcal/100g) of processed *R. differens*-based cookies

Enriched cookies	Moisture	Dry matter	Crude protein	Crude fat	Crude ash	Crude fibre	CHO	Energy
CTRC	6.17 ± 1.26^{b}	93.83 ± 1.26^{a}	7.74 ± 0.40^{a}	17.76 ± 0.41^{a}	2.64 ± 0.02^{b}	0.06 ± 0.02^{a}	65.63 ± 1.12^{b}	453.33 ± 6.70^{a}
BCRC	2.83 ± 0.29^{a}	97.17 ± 0.29^{b}	11.09 ± 0.65^{b}	19.61 ± 1.08^{ab}	2.06 ± 0.01^{a}	0.17 ± 0.14^{a}	64.23 ± 1.45^{ab}	477.81 ± 4.10^{b}
BLRC	3.0 ± 0.87^{a}	97.0 ± 0.87^{b}	10.90 ± 0.08^{b}	20.68 ± 0.20^{b}	2.23 ± 0.29^{ab}	0.22 ± 0.33^{a}	62.97 ± 0.86^{a}	481.59 ± 1.28^{b}
TSRC	2.33 ± 0.29^{a}	97.67 ± 0.29^{b}	10.99 ± 0.24^{b}	19.84 ± 0.53^{b}	2.56 ± 0.01^{b}	0.07 ± 0.03^{a}	64.21 ± 0.20^{ab}	479.37 ± 3.70^{b}
DFRC	1.33 ± 0.58^{a}	98.67 ± 0.58^{b}	6.78 ± 0.14^{a}	25.82 ± 0.89^{c}	2.20 ± 0.29^{ab}	0.08 ± 0.02^{a}	63.79 ± 0.43^{ab}	514.69 ± 6.52^{c}

Values are presented as mean ± standard deviation of triplicate determinations. Means in the same column followed by different small superscript letters are significantly different at $p<0.05$. kJ=Kilojoules; CHO= Carbohydrate; CTRC=Control cookies; BCRC=Blanched *R. differens*-based cookies; BLRC=Boiled *R. differens*-based cookies; TSRC=Toasted *R. differens*-based cookies; DFRC=Deep-fried *R. differens*-based cookies.

4.5.2 Amino Acid Profiles of the Cookies

A total of 11 amino acids were detected (5 essential fatty acids and 6 non-essential amino acids) (Table 4.7). Significant variations ($p<0.05$) could be discerned in the levels of amino acids across the enriched cookies. Deep-fried *R. differens*-based cookies showed relatively lower levels of both the essential and non-essential amino acids. Leucine and Isoleucine for the essential amino acids were more abundant in the blanched, boiled and toasted *R. differens*-based cookies.

Table 4.7: Amino acid profiles (mg/g) detected in cookies enriched with different processed *R. differens*-based cookies

Amino acid	Control cookies	BCRC	BLRC	TSRC	DFRC
Essential Amino Acids					
Phenylalanine	4.41 ± 0.28^{a}	4.91 ± 0.42^{a}	4.08 ± 0.40^{a}	4.53 ± 0.50^{a}	3.93 ± 0.54^{a}
Isoleucine	4.00 ± 0.84^{a}	5.62 ± 0.82^{b}	6.07 ± 0.33^{b}	5.56 ± 0.34^{b}	3.39 ± 0.16^{a}
Leucine	7.93 ± 0.68^{ab}	8.97 ± 0.42^{b}	8.60 ± 0.22^{b}	8.57 ± 0.22^{b}	7.32 ± 0.28^{a}
Methionine	2.49 ± 0.16^{a}	3.08 ± 0.33^{a}	2.82 ± 0.07^{a}	2.65 ± 0.42^{a}	2.72 ± 0.18^{a}
Valine	20.37 ± 0.72^{a}	22.41 ± 2.46^{a}	19.40 ± 1.82^{a}	19.77 ± 4.93^{a}	16.38 ± 1.45^{a}
Non-Essential Amino acids					
Tyrosine	3.41 ± 0.06^{a}	4.18 ± 0.12^{b}	4.17 ± 0.12^{b}	4.08 ± 0.17^{b}	3.57 ± 0.11^{a}
Arginine	11.88 ± 0.48^{b}	13.73 ± 0.25^{c}	11.27 ± 0.91^{b}	12.68 ± 0.80^{bc}	9.51 ± 0.50^{a}
Cystine	4.54 ± 0.21^{a}	8.75 ± 1.44^{bc}	9.96 ± 0.32^{c}	6.72 ± 1.32^{ab}	5.83 ± 0.49^{a}
Proline	4.14 ± 1.09^{a}	7.53 ± 0.55^{b}	5.25 ± 0.24^{a}	6.17 ± 0.85^{ab}	5.48 ± 0.82^{ab}
Glutamic acid	18.00 ± `1.88^{a}	21.54 ± 0.68^{a}	19.58 ± 0.48^{a}	20.69 ± 2.66^{a}	18.67 ± 0.20^{a}
Alanine	21.63 ± 0.37^{b}	24.13 ± 0.76^{b}	23.21 ± 1.37^{b}	23.14 ± 2.47^{b}	14.75 ± 0.75^{a}

Values are presented as mean ± standard deviation of triplicate determinations. Means in the same row followed by same small superscript letters are not significantly different at $p<0.05$. CTRC=Control cookies; BCRC=Blanched *R. differens*-based cookies; BLRC=Boiled *R. differens*-based cookies; TSRC=Toasted *R. differens*-based cookies; DFRC=Deep fried *R. differens*-based cookies.

4.5.3 Fatty Acids Profiles of the Cookies

Table 4.8 below and Figure 4.5 represents the fatty acid spectra and the categories of total fatty acids respectively of the enriched cookies. Methyl hexadecanoate of the SFA, methyl (*Z*)-9-octadecenoate of the MUFA and the methyl (9*Z*,12*Z*)-octadecadienoate of the PUFAs were discovered to be the most dominant fatty acids compositionally. Methyl (12,15)-octadecadienoate, methyl (9*Z*,12*Z*)-octadecadienoate, methyl (9*Z*,11*E*,13*E*)-octadecatrienoate, Methyl (5*Z*,8*Z*,11*Z*,14*Z*)-Eicosatetraenoate and Methyl (5*Z*,8Z,11*Z*,14*Z*,17Z)-eicosapentaenoate were the key PUFAs detected. Composition-wise, SFAs ranged 24.6-52.8% MUFAs 36.3-48.9% and PUFAs 0.8-30.0% in all the cookies. Blanched *R. differens*-based cookies exhibited the highest total PUFAs

Table 4.8: Fatty acid composition (µg/mg of cookies) made from different processed *R. differens* analysed by Gas Chromatography coupled to Mass Spectrometry (GC-MS).

Peak No.	tR (min)	Compound	Corresponding Fatty acid	ω-n(Δn)	CTRC	BCRC	BLRC	TSRC	DFRC
1	14.81	Methyl octanoate	Capric acid	C8:0	-	0.004 ± 0.00	0.004 ± 0.00	-	0.004 ± 0.00
2	16.85	Methyl decanoate		C10:0	0.005 ± 0.00	0.01 ± 0.00	-	0.007 ± 0.00	0.01 ± 0.01
3	14.88	Methyl nonanoate		C9:0	0.001 ± 0.00	-	-	-	-
4	17.36	Methyl butanoate	Lauric acid	C4:0	0.001 ± 0.00	0.001 ± 0.00	0.003 ± 0.00	0.002 ± 0.00	0.001 ± 0.00
5	18.95	Methyl dodecanoate		C12:0	0.11 ± 0.01	0.22 ± 0.06	0.18 ± 0.05	0.17 ± 0.11	0.36 ± 0.21
6	20.83	Methyl 12-methyltridecanoate	Tridecyclic acid	iso-methyl-C13:0	-	0.003 ± 0.00	-	-	-

Peak No.	tR (min)	Compound	Corresponding Fatty acid	ω-n(Δn)	CTRC	BCRC	BLRC	TSRC	DFRC
7	21.24	Methyl tetradecanoate	Myristic acid	C14:0	0.62 ± 0.10	0.69 ± 0.55	0.59 ± 0.48	0.64 ± 0.20	0.98 ± 0.22
8	21.8	Methyl 4,8-dimethylnonanoate		iso-dimethyl-C9:0	-	0.006 ± 0.00	-	-	-
9	23.52	Methyl hexadecanoate	Palmitic acid	C16:0	10.58 ± 1.33	5.02 ± 8.32	9.99 ± 2.85	10.37 ± 2.53	12.73 ± 0.59
10	25.49	Methyl octadecanoate	Stearic acid	C18:0	2.43 ± 0.18	3.07 ± 0.20	2.84 ± 0.35	2.60 ± 0.47	2.84 ± 0.13
11	26.3	Methyl 3-methoxyoctadecanoate	Stearic acid	iso-methoxy-C18:0	-	-	-	0.02 ± 0.00	-
12	26.99	Methyl eicosanoate	Arachidic acid	C20:0	-	-	0.59 ± 0.02	-	-
13	26.99	Methyl 18-methylnonadecanoate		iso-methyl-C19:0	-	0.78 ± 0.02	-	-	-

Peak No.	tR (min)	Compound	Corresponding Fatty acid	ω-n(Δn)	CTRC	BCRC	BLRC	TSRC	DFRC
14	28.61	Methyl docosanoate	Behenic acid	C22:0	0.11 ± 0.01	-	-	-	-
15	29.37	Methyl tricosanoate	Trycosylic acid	C23:0	0.06 ± 0.02	-	0.04 ± 0.01	-	0.08 ± 0.03
16	30.13	Methyl tetracosanoate	Lignoceric acid	C24:0	0.15 ± 0.00	0.16 ± 0.01	0.13 ± 0.02	0.08 ± 0.01	0.14 ± 0.04
17	32.08	Methyl hexacosanoate	Cerotic acid	C26:0	0.07 ± 0.01	0.06 ± 0.00	0.05 ± 0.02	0.01 ± 0.00	0.06 ± 0.02
		∑ SFA			14.14	10.02	14.42	13.9	17.21
18	20.97	Methyl (Z)-11-tetradecenoate	Vaccenic acid	C14:1 (n-3)	0.01 ± 0.00	-	-	-	-
19	20.97	Methyl (Z)-4-Octenoate		C8:1(n-4)	-	-	0.006 ± 0.00	-	-
20	22.09	Methyl (Z)-5-Dodecenoate		C12:1(n-7)	-	-	-	0.01 ± 0.00	0.01 ± 0.01

Peak No.	tR (min)	Compound	Corresponding Fatty acid	ω-n(Δn)	CTRC	BCRC	BLRC	TSRC	DFRC
21	22.15	Methyl 13-Methyltetradec-9-enoate	Myristoleic	iso-methyl-C14:1(n-5)	-	-	-	-	0.005 ± 0.00
22	23.12	Methyl hexadec-9-enoate	Palmitoleic acid	C16:1(n-7)	0.26 ± 0.03	0.77 ± 0.15	0.73 ± 0.17	0.62 ± 0.23	0.56 ± 0.03
23	24.1	Methyl (Z)-10-Heptadecenoate		C17:1 (n-7)	0.10 ± 0.01	0.12 ± 0.02	0.09 ± 0.03	0.04 ± 0.00	0.11 ± 0.03
24	25.22	Methyl (Z)-9-Octadecenoate	Oleic acid	C18:1(n-9)	11.55 ± 0.37	17.20 ± 2.24	13.16 ± 3.05	12.08 ± 2.17	16.19 ± 4.01
25	26.79	Methyl (Z)-11-Eicosenoate		C20:1(n-9)	0.32 ± 0.05	0.38 ± 0.04	0.32 ± 0.09	0.14 ± 0.01	0.37 ± 0.08
26	28.43	Methyl (Z)-11-docosenoate		C22:1(n-11)	-	0.04 ± 0.00	-	0.05 ± 0.05	-
27	28.43	Methyl (Z)-13-Docosenoate	Erucic acid	C22:1(n-9)	-	-	0.05 ± 0.00	-	0.04 ± 0.005
28	29.95	Methyl (Z) -15-Tetracosenoate		C24:1(n-9)	0.03 ± 0.00	-	0.02 ± 0.00	-	0.05 ± 0.00

Peak No.	tR (min)	Compound	Corresponding Fatty acid	ω-n(Δn)	CTRC	BCRC	BLRC	TSRC	DFRC
29	49.35	Methyl (E)-8-Octadecenoate		C18:1(n-10)	-	0.02 ± 0.00	-	-	-
		∑ MUFA			12.27	18.53	14.38	12.94	17.34
30	24.69	Methyl (12,15)-Octadecadienoate		C18:2(n-3)	-	0.04 ± 0.00	-	0.01 ± 0.00	-
31	25.18	Methyl (9Z,12Z)-Octadecadienoate	Linoleic acid	C18:2(n-6)	0.19 ± 0.05	12.10 ± 10.31	10.71 ± 9.16	5.46 ± 9.25	0.24 ± 0.05
31	26.3	Methyl (9Z,12Z,15Z)-octadecatrienoate	α Linolenic acid	C18:3(n-3)	-	2.12 ± 0.97	1.55 ± 0.21	0.49 ± 0.01	0.34 ± 0.00
32	26.45	Methyl (5Z,8Z,11Z,14Z)-Eicosatetraenoate	Arachidonic acid	C20:4(n-6)	0.14 ± 0.09	-	-	-	-
		∑ PUFA			0.33	14.26	12.26	5.96	0.58
		∑ n-6 PUFA			0.33	12.10	10.71	5.46	0.24
		∑ n-3 PUFA			-	2.16	1.44	0.50	0.34
		∑ n-6/n-3			-	5.60	7.44	10.92	0.71

Peak No.	tR (min)	Compound	Corresponding Fatty acid	ω-n(Δn)	CTRC	BCRC	BLRC	TSRC	DFRC
		∑PUFA/SFA			0.02	1.42	0.85	0.43	0.03

Values are presented as Mean ± SD (standard deviation) of triplicate determinations. tR= Retention time, SFA =saturated fatty acids, MUFA =monounsaturated fatty acids, PUFA= polyunsaturated fatty acids, CTRC=Control cookies; BCRC=Blanched *R. differens*-based cookies; BLRC=Boiled *R. differens*-based cookies; TSRC=Toasted *R. differens*-based cookies; DFRC=Deep fried *R. differens*-based cookies.

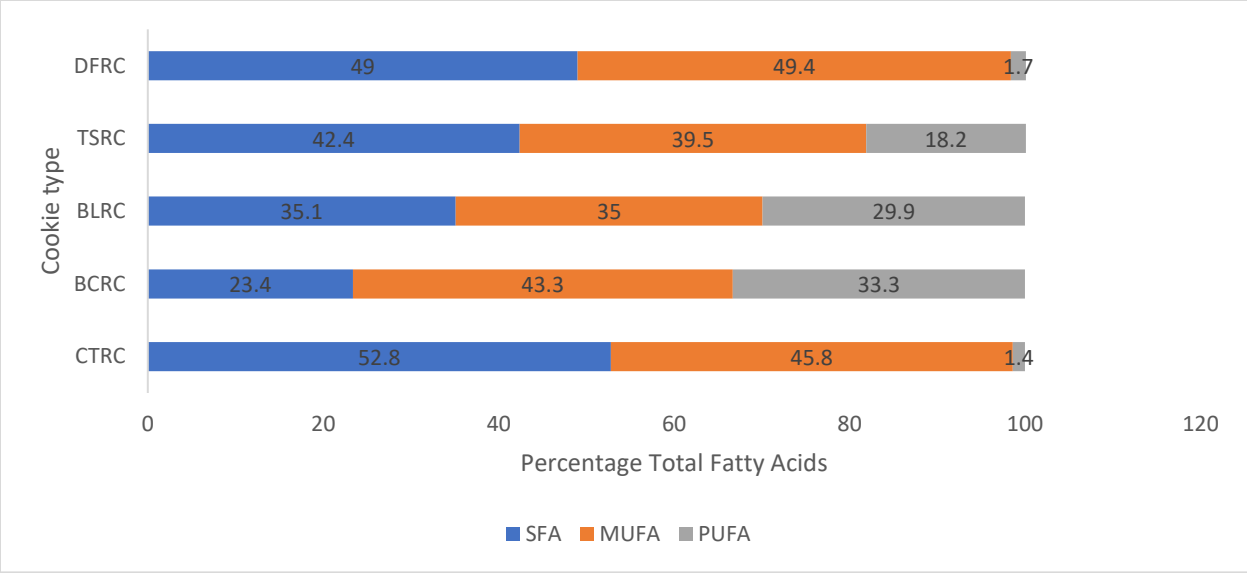


Figure 4.5: Categories of the total fatty acids (%) detected in the processed *R. differens* based cookies. CTRC=Control cookies; BCRC=Blanched *R. differens*-based cookies; BLRC=Boiled *R. differens*-based cookies; TSRC=Toasted *R. differens*-based cookies; DFRC=Deep fried *R. differens*-based cookies.

4.5.4 Mineral Profiles of the Cookies

Significant variations ($p<0.05$) were witnessed in the mean mineral concentrations of all the minerals in the cookies except for P (Table 4.9). The dominant minerals recorder were P, Na and K for the macro minerals and Mn and Cu for the trace elements. Ca was significantly abundant in control cookies whilst Fe and Zn were higher in blanched and boiled *R. differens*-based cookies.

Table 4.9: Mineral profiles (dry matter) of processed *R. differens*-based cookies

Enriched Cookies	CTRC	BCRC	BLRC	TSRC	DFRC
Macro minerals					
Mg (mg/100g)	24.55 ± 0.59^{b}	25.15 ± 0.01^{b}	24.12 ± 0.20^{ab}	23.53 ± 0.47^{ab}	22.34 ± 0.40^{a}
Ca (mg/100g)	20.17 ± 0.52^{d}	17.78 ± 0.11^{c}	16.64 ± 0.85^{bc}	15.09 ± 0.76^{a}	15.30 ± 0.02^{ab}
P (mg/100g)	290.80 ± 3.52^{a}	298.12 ± 19.80^{a}	289.33 ± 21.64^{a}	281.50 ± 4.14^{a}	275.80 ± 5.49^{a}
Na (mg/100g)	905.63 ± 8.92^{b}	922.32 ± 28.50^{b}	852.89 ± 14.03^{a}	886.05 ± 3.89^{ab}	917.27 ± 8.57^{b}
K (mg/100g)	145.22 ± 3.87^{a}	145.87 ± 6.66^{a}	151.88 ± 4.42^{ab}	160.59 ± 2.69^{b}	147.30 ± 7.30^{ab}
Trace Elements					
Fe (mg/100g)	4.19 ± 0.01^{a}	7.11 ± 0.10^{c}	7.12 ± 0.37^{c}	5.09 ± 0.02^{b}	5.67 ± 0.43^{b}
Cu (µg/100g)	128.83 ± 5.38^{a}	301.30 ± 7.65^{bc}	346.79 ± 21.74^{d}	331.08 ± 21.74^{cd}	273.30 ± 8.50^{b}
Mn (mg/100g)	681.28 ± 20.80^{a}	808.71 ± 45.14^{c}	760.99 ± 16.25^{c}	748.75 ± 17.33^{bc}	692.95 ± 0.92^{ab}
Zn (mg/100g)	3.73 ± 0.12^{a}	4.33 ± 0.08^{b}	4.17 ± 0.03^{b}	3.86 ± 0.16^{a}	3.79 ± 0.002^{a}
Al (mg/100g)	0.75 ± 0.03^{a}	2.52 ± 0.01^{d}	1.94 ± 0.03^{c}	1.92 ± 0.11^{c}	1.45 ± 0.04^{b}

Results are presented as mean ± standard deviation. Same small superscript letters within rows indicate no significant differences of the minerals at $p<0.05$. CTRC=Control cookies; BCRC=Blanched *R. differens*-based cookies; BLRC=Boiled *R. differens*-based cookies; TSRC=Toasted *R. differens*-based cookies; DFRC=Deep fried *R. differens*-based cookies.

4.6 Volatile Organic Compounds of the Cookies Headspace

Chemical characterization of the aroma in the enriched cookies yielded 79 different volatile organic compounds (VOCs) (Table 4.10). The VOCs dominantly comprised of hydrocarbons, aldehydes, monoterpenes and ketones. The Non-metric multidimensional scaling (NMDS) ordination (Figure 4.6A) generated a stress value of <0.1 (0.081) indicative of a great representation of the dissimilarities and proper clustering of the VOCs from the cookies enriched with differently processed *R. differens* (Figure 4.6B). The VOCs; nonanal, octanal, methyl-pyrazine, hexanal, tridecane, 2-pentylfuran, 2-heptanone, (*E*)-2-octenal, 2*E*-heptenal and dodecane are the key compounds which led to the differentiation of the cookies enriched with differently processed *R. differens* (Figure 4.6C).

Table 4.10: Volatile organic compounds (µg/g/h) of the cookies enriched with differently processed *R. differens* analysed by GC-MS.

RT	Compound	Compound class	CTRC	BCRC	BLRC	TSRC	DFRC	Odour description
3.27	3-methyl-Butanal	Aldehyde	0.23 ± 0.04				2.69 ± 0.16	
3.9	Pentanal	Aldehyde	0.45 ± 0.12	0.58 ± 0.11	0.75 ± 0.33	0.70 ± 0.10	3.59 ± 4.39	nutty, sweet
4.18	Acetoin	Ketone		0.12 ± 0.01	0.66 ± 0.21	0.25 ± 0.03	0.32 ± 0.04	
4.34	3-Hydroxy-2-butanone	Ketone	0.06 ± 0.003					
4.67	2,2,3-trimethyl-Butane	Hydrocarbon			0.05 ± 0.001			
4.78	Pyrazine	Nitrogen compound		0.09 ± 0.02	0.11 ± 0.02	0.12 ± 0.01	0.20 ± 0.03	
4.99	2-Octen-4-one	Ketone					0.65 ± 0.24	

RT	Compound	Compound class	CTRC	BCRC	BLRC	TSRC	DFRC	Odour description
5.00	(*E*)-3-Penten-2-one	Ketone		0.06 ± 0.01	0.31 ± 0.17	0.62 ± 0.03	0.69 ± 0.03	
5.34	Formic acid pentyl ester	Ester				0.07 ± 0.01	0.09 ± 0.01	
5.48	(2*E*)-Penten-1-al	Aldehyde			0.08 ± 0.01			
5.68	Phenyl ethyl alcohol	Alcohol				0.41 ± 0.04		
5.68	Toluene	Benzenoid	0.12 ± 0.11	0.16 ± 0.14	0.09 ± 0.04	0.09 ± 0.01	0.15 ± 0.09	
5.77	Pentanol	Alcohol	0.75 ± 0.18	0.66 ± 0.28	0.86 ± 0.35	0.98 ± 0.25	1.64 ± 0.17	plastic
6.17	2,3-Butanediol	Alcohol		0.61 ± 0.44	0.60 ± 0.36	0.73 ± 0.04	0.59 ± 0.21	

RT	Compound	Compound class	CTRC	BCRC	BLRC	TSRC	DFRC	Odour description
6.37	1-Octene	Hydrocarbon	0.12 ± 0.04					
6.59	Hexanal	Aldehyde	2.60 ± 0.42	3.78 ± 1.12	5.51 ± 3.43	4.82 ± 0.84	5.87 ± 3.30	green apple, grassy
6.95	2,3,5-trimethyl-Hexane	Hydrocarbon		0.08 ± 0.01	0.15 ± 0.03	0.13 ± 0.002	0.20 ± 0.01	
7.18	2,4-dimethyl-Heptane	Hydrocarbon	0.14 ± 0.02	0.12 ± 0.003	0.41 ± 0.24	0.13 ± 0.002		
7.18	Methyl-Pyrazine	Nitrogen compound		2.61 ± 0.20	1.80 ± 2.66	3.20 ± 1.00	5.84 ± 1.33	nutty, cocoa, roasted meat
7.56	Furfural	Aldehyde		0.72 ± 0.13	0.90 ± 0.30	1.00 ± 0.13	2.48 ± 0.15	almond-like, sweet
7.69	2,4-Dimethyl-1-heptene	Hydrocarbon		0.21 ± 0.03	0.42 ± 0.25	0.33 ± 0.04	0.82 ± 0.23	
8.07	(2*E*)-Hexenal	Aldehyde		0.38 ± 0.23		0.37 ± 0.10	0.48 ± 0.10	green, apple-like

RT	Compound	Compound class	CTRC	BCRC	BLRC	TSRC	DFRC	Odour description
8.07	2,3-dimethyl-Heptane	Hydrocarbon		0.11 ± 0.02	0.19 ± 0.08	0.44 ± 0.02	0.72 ± 0.04	
8.16	2-Furanmethanol	Furanoid			0.96 ± 0.07	0.52 ± 0.03	1.63 ± 0.31	
8.21	Ethylbenzene	Benzenoid	0.48 ± 0.10	0.11 ± 0.02	0.58 ± 0.14	0.42 ± 0.03	0.53 ± 0.05	
8.25	4-methyl-Octane	Hydrocarbon	0.42 ± 0.16	0.73 ± 0.69	1.08 ± 0.52	1.92 ± 0.17	1.64 ± 0.35	
8.39	*p*-Xylene	Benzenoid	0.31 ± 0.14	0.23 ± 0.07		0.43 ± 0.12	0.51 ± 0.26	
8.38	1,3-dimethyl-Benzene	Benzenoid		0.68 ± 0.12	0.44 ± 0.23			
8.47	1-Hexanol	Alcohol	0.54 ± 0.18			0.38 ± 0.11		Fruity, green
8.5	o-Xylene	Benzenoid			0.41 ± 0.12			

RT	Compound	Compound class	CTRC	BCRC	BLRC	TSRC	DFRC	Odour description
8.75	2-methyl-Butanoic acid	Carboxylic acid			0.58 ± 0.24			
8.9	2-Heptanone	Ketone		1.74 ± 0.77	2.16 ± 2.52	1.29 ± 0.30	1.69 ± 0.51	fruity, sweet
9.24	Methional	Aldehyde			0.73 ± 0.19	0.38 ± 0.02	0.94 ± 0.06	
9.81	*β*-Phellandrene	Monoterpene				0.30 ± 0.03		
9.82	*α*-Pinene	Monoterpene	0.59 ± 0.06	0.80 ± 0.22		0.90 ± 0.02		woody, green, pine-like
9.82	*γ*-Terpinene	Monoterpene			0.64 ± 0.28		1.51 ± 0.29	
10.08	3,4-Diethyl hexane	Hydrocarbon				0.35 ± 0.03		
10.11	Camphene	Monoterpene		1.10 ± 0.08	1.75 ± 0.21		1.71 ± 0.22	

RT	Compound	Compound class	CTRC	BCRC	BLRC	TSRC	DFRC	Odour description
10.11	4-methyl-Heptane,	Hydrocarbon	0.20 ± 0.03				0.20 ± 0.03	
10.21	2,3,4-trimethyl-Decane	Hydrocarbon			0.44 ± 0.08		0.56 ± 0.04	
10.35	(2*E*)-Heptenal	Aldehyde	0.51 ± 0.15	1.30 ± 0.10	2.73 ± 0.68			
10.45	Benzaldehyde	Aldehyde		0.67 ± 0.07	1.96 ± 0.43	1.57 ± 0.15	2.10 ± 0.07	almond
10.67	1-Heptanol	Alcohol			0.88 ± 013			green
10.71	*β*-Pinene	Monoterpene	0.59 ± 0.20	0.31 ± 0.05	0.72 ± 0.21		0.93 ± 0.02	woody, green, pine-like
10.71	Sabinene	Monoterpene		0.61 ± 0.08				
11.01	2,2,4,6,6-pentamethyl-Heptane	Hydrocarbon	0.53 ± 0.19		0.74 ± 0.24	0.39 ± 0.02		

RT	Compound	Compound class	CTRC	BCRC	BLRC	TSRC	DFRC	Odour description
11.03	2-Pentyl furan	Furanoid	1.36 ± 0.18	1.96 ± 1.01	4.56 ± 4.48	1.96 ± 0.50	2.94 ± 0.32	sweet, woody, almond-like, baked bread
11.32	Octanal	Aldehyde	2.07 ± 1.45	0.59 ± 0.09	5.22 ± 4.29	3.61 ± 0.12	4.89 ± 0.18	fruity
11.3	*δ*-3-Carene	Monoterpene	0.50 ± 0.16		1.92 ± 0.37	1.94 ± 0.19	1.62 ± 0.30	
11.62	2-ethenyl-6-methyl-Pyrazine	Nitrogen compound			1.12 ± 0.20			
11.79	Limonene	Monoterpene	1.01 ± 0.16	0.62 ± 0.16	0.91 ± 0.29	0.63 ± 0.19	0.97 ± 0.11	citrus-like
11.82	3-ethyl-2-methyl-1,3-Hexadiene	Hydrocarbon	1.32 ± 0.28	1.07 ± 0.08	1.75 ± 0.34	1.36 ± 0.12	1.57 ± 0.32	nutty
12.13	3-Octen-2-one	Ketone				0.21 ± 0.02		rose

RT	Compound	Compound class	CTRC	BCRC	BLRC	TSRC	DFRC	Odour description
12.33	Tricosane	Hydrocarbon	0.87 ± 0.10		1.80 ± 0.61			
12.53	Isoamyl isobutyrate	Ester				0.68 ± 0.11		green apple
12.55	(*E*)-2-Octenal	Aldehyde		0.91 ± 0.09		2.89 ± 0.18		nutty, cooked flour
13.11	Nonanal	Aldehyde	7.21 ± 0.57	2.08 ± 2.56	3.60 ± 1.77	4.78 ± 1.68	7.44 ± 0.64	fatty, waxy, pungent
13.77	trans-3-Nonen-2-one	Ketone		0.51 ± 0.28	0.84 ± 0.05		2.54 ± 0.29	
14.08	(2*Z*)-Nonen-1-al	Aldehyde			2.21 ± 0.41	0.45 ± 0.03		beany, cucumber
14.56	Ethyl octanoate	Ester			1.64 ± 0.45			
14.77	Dodecane	Hydrocarbon	1.79 ± 0.15	0.37 ± 0.10	0.90 ± 0.20	2.55 ± 3.23	1.00 ± 0.11	

RT	Compound	Compound class	CTRC	BCRC	BLRC	TSRC	DFRC	Odour description
15.66	(2*E*)-Decenal	Aldehyde		0.39 ± 0.03		0.57 ± 0.01	1.63 ± 0.31	fatty
15.69	4,6-dimethyl-Dodecane	Hydrocarbon			0.32 ± 0.02	0.22 ± 0.01		
15.71	2,6-dimethyl-Octane	Hydrocarbon			0.62 ± 0.26			
15.73	2,3,7-trimethyl-Octane	Hydrocarbon					0.28 ± 0.004	
15.84	Tridecane	Hydrocarbon	1.75 ± 0.23	2.00 ± 0.20	1.40 ± 0.36	0.73 ± 0.01	0.53 ± 0.37	
16.22	2-ethyl-1-Decanol	Alcohol				0.39 ± 0.03		
16.4	Eicosane (C20)	Hydrocarbon		0.19 ± 0.05	2.00 ± 0.17	0.78 ± 0.07	0.40 ± 0.04	
16.52	(2*E*,4*Z*)-Decadienal	Aldehyde		0.64 ± 0.30	0.80 ± 0.22	1.82 ± 0.76	1.23 ± 0.25	fatty

RT	Compound	Compound class	CTRC	BCRC	BLRC	TSRC	DFRC	Odour description
16.94	(*E*)-Calamenene	Sesquiterpene					2.36 ± 0.18	
17.34	Propyl butanoate	Ester			0.61 ± 0.30	1.28 ± 0.14		
17.5	Tetradecane	Hydrocarbon	3.01 ± 0.38	1.66 ± 0.21	5.14 ± 2.15	1.28 ± 0.35	1.38 ± 0.96	
17.62	Longifolene	Sesquiterpene	0.18 ± 0.03			0.28 ± 0.02		
17.73	α-Cedrene	Sesquiterpene				0.89 ± 1.14	0.35 ± 0.02	
18.98	Butylated hydroxytoluene	Phenylpropane	0.54 ± 0.02	0.44 ± 0.03	0.59 ± 0.33	0.36 ± 0.16	1.34 ± 0.21	
20.05	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	Ester		0.41 ± 0.05				
26.86	Docosane	Hydrocarbon				0.09 ± 0.004		

RT	Compound	Compound class	CTRC	BCRC	BLRC	TSRC	DFRC	Odour description
26.98	Octadecane	Hydrocarbon		0.06 ± 0.003		0.06 ± 0.001	0.93 ± 0.04	
27.65	Tetracosane	Hydrocarbon				0.13 ± 0.01		

Values are presented as Mean ± SD (standard deviation) of triplicate determinations. RT: Retention time; CTRC=Control cookies; BCRC=Blanched *R. differens*-based cookies; BLRC=Boiled *R. differens*-based cookies; TSRC=Toasted *R. differens*-based cookies; DFRC=Deep fried *R. differens*-based cookies. The odour descriptions are retrieved from literature (Guclu *et al*., 2016; Niu *et al*., 2020; Starowicz, 2021; Yang *et al*., 2008).

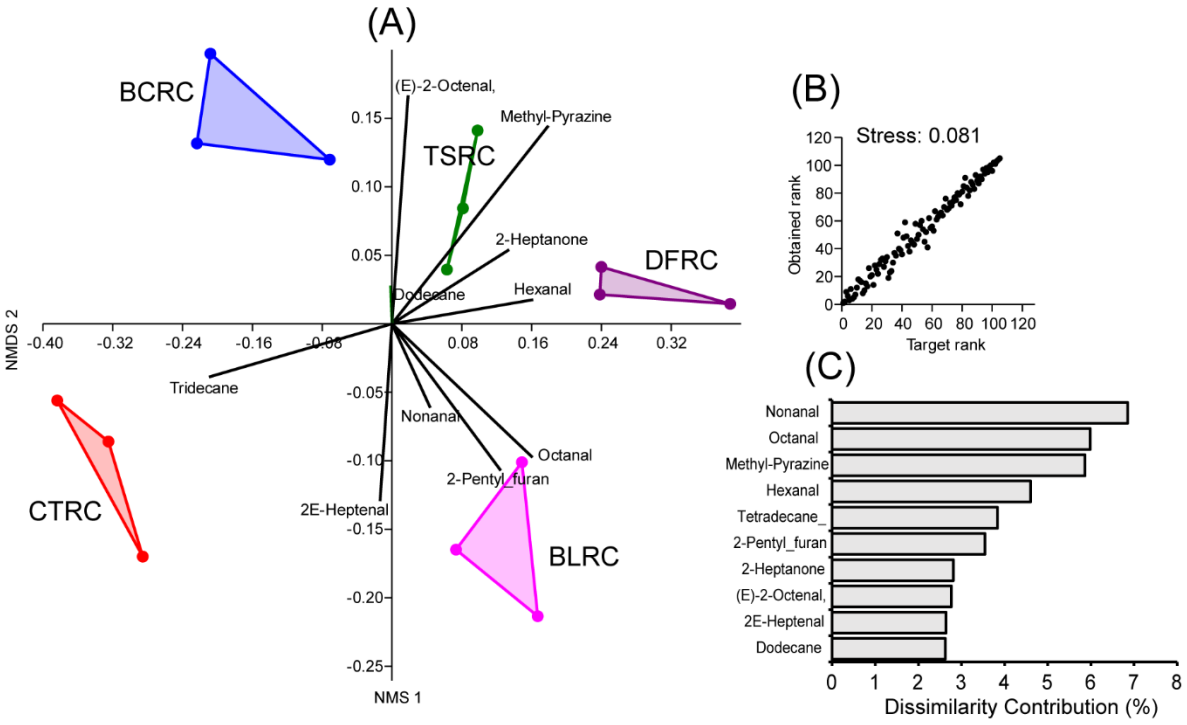


Figure 4.6: (A) Non-metric multidimensional scaling plot (NMDS) clustering the different *R. differens*-based cookies based on the type of volatile they emit, analysis of similarities (ANOSIM); CTRC=Control cookies with eggs; BCRC=Blanched *R. differens*-based cookies; BLRC=Boiled *R. differens*-based cookies; TSRC=Toasted *R. differens*-based cookies; DFRC=Deep fried *R. differens*-based cookies. (B) Shepard plot showing the great ordination of the NMDS analysis (stress value <0.1) (C) Histogram displaying the contribution of the 10 most important volatiles to the differentiation of all the different enriched cookies.

4.7 *In vitro* Protein Digestibility of the Cookies

Figure 4.7 illustrates the influence of differently processed insect flours on the *in vitro* digestibility of respective cookies. Control cookies manifested significantly higher (p<0.05) digestibility with deep-fried *R. differens*-based cookies showing the least. However, no significant difference in digestibility was noted in blanched and boiled *R. differens*-based cookies.

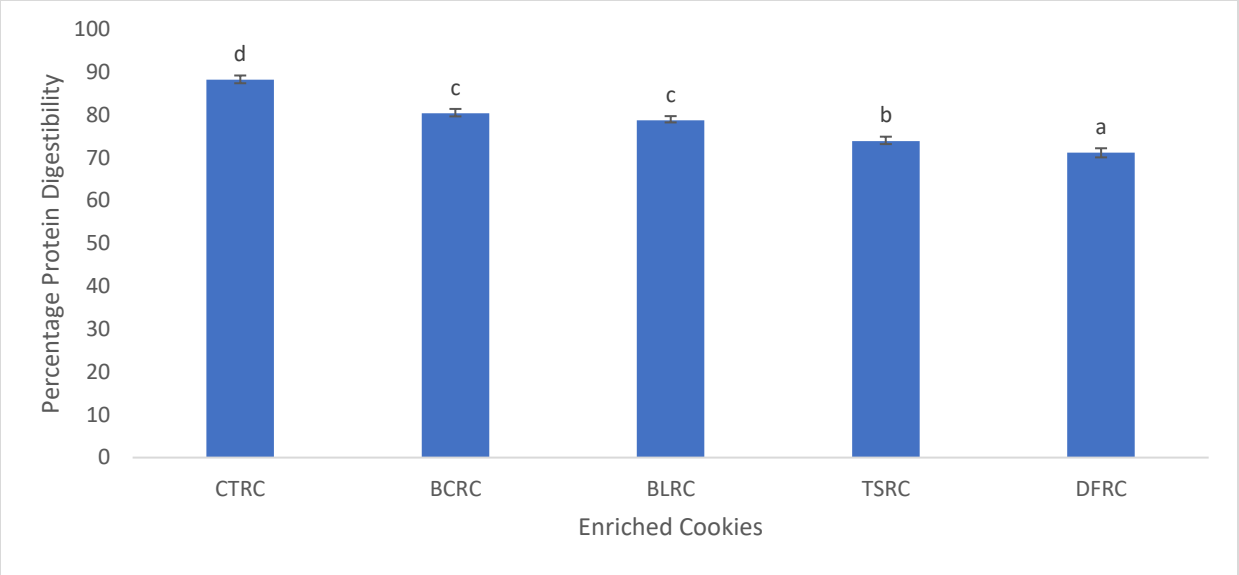


Figure 4.7: *In vitro* protein digestibility (%) of the processed *R. differens* based cookies. Bars with same small letters following each other are not significantly different (p<0.05). CTRC=Control cookies; BCRC=Blanched *R. differens*-based cookies; BLRC=Boiled *R. differens*-based cookies; TSRC=Toasted *R. differens*-based cookies; DFRC=Deep fried *R. differens*-based cookies.

4.8 Microbial Levels in the Enriched Cookies

The levels of microorganisms based on enumeration and detection are indicated in the Table 4.11**.** Microbial levels were extremely dismal encompassing non detection of *Staphylococcus aureus* and *Salmonella spp.*, low counts of TVC (<30) and the non-observation of *Enterobacteriaceae* and yeast & moulds.

Table 4.11: Microbial levels in cookies enriched with differently processed *R. differens* flours

Cookies	TVC (counts)	Lac +ve Enterics (Log_{10} cfu/g)	*Staphylococcus aureus* (Log_{10} cfu/g)	*Salmonella spp.*	YMC (Log_{10} cfu/g)
CTRC	<30	0.0	0.0	-ve	0.0
BCRC	<30	0.0	0.0	-ve	0.0
BLRC	<30	0.0	0.0	-ve	0.0
TSRC	<30	0.0	0.0	-ve	0.0
DFRC	<30	0.0	0.0	-ve	0.0

*TVC=Total viable counts; YMC=Yeast & moulds; Lac +ve=Lactose positive; CTRC=Control cookies; BCRC=Blanched *R. differens*-based cookies; BLRC=Boiled *R. differens*-based cookies; TSRC=Toasted *R. differens*-based cookies; DFRC=Deep fried *R. differens*-based cookies.

4.9 Sensory Acceptance of the Cookies Enriched with Differently Processed *R. differens* Flours.

The mean scores for each of the sensory attributes are presented in Table 4.12. Control cookies scores were significantly higher ($p<0.05$) in all the attributes under consideration. No significant difference in colour scores was observed between control and deep-fried *R. differens*-based cookies. Further, blanched *R. differens*-based cookies scored the lowest on flavour and mouthfeel compared with other cookies. A two-dimensional Principal Component Analysis (PCA) explained 97.6% of the variation in sensory scores of the differently enriched cookies (Figure 4.8B). Mouthfeel correlated strongly with the overall acceptability. Correlation analysis confirmed this by revealing a strong correlation between mouthfeel and overall acceptability of 0.99 whereas a weak correlation of 0.80 was recorded between flavour and the overall acceptability (Figure 4.9).

Table 4.12: Mean Sensory scores of the cookies enriched with differently processed *R. differens* flours

Cookies	Colour	Flavour	Mouthfeel	Texture	Overall acceptance
CTRC	4.47 ± 0.92^{c}	3.95 ± 1.04^{b}	4.22 ± 0.98^{c}	4.07 ± 0.94^{c}	4.24 ± 0.97^{d}
BCRC	3.83 ± 1.03^{ab}	3.17 ± 1.29^{a}	3.68 ± 1.12^{a}	3.55 ± 1.07^{ab}	3.58 ± 0.95^{ab}
BLRC	3.68 ± 1.11^{a}	3.83 ± 1.02^{b}	3.80 ± 1.02^{ab}	3.64 ± 0.91^{ab}	3.75 ± 0.95^{bc}
TSRC	3.92 ± 0.86^{ab}	3.72 ± 1.02^{b}	3.91 ± 1.07^{abc}	3.66 ± 1.01^{ab}	3.85 ± 0.94^{bc}
DFRC	4.15 ± 0.89^{bc}	3.81 ± 0.93^{b}	4.04 ± 0.94^{bc}	3.69 ± 1.05^{b}	3.97 ± 0.86^{cd}

Values are presented as means ± SD of triplicate determinations. Means in the same raw followed by same small superscript letters are not significantly different at $p<0.05$. CTRC=Control cookies; BCRC=Blanched *R. differens*-based cookies; BLRC=Boiled *R. differens*-based cookies; TSRC=Toasted *R. differens*-based cookies; DFRC=Deep fried *R. differens*-based cookies.

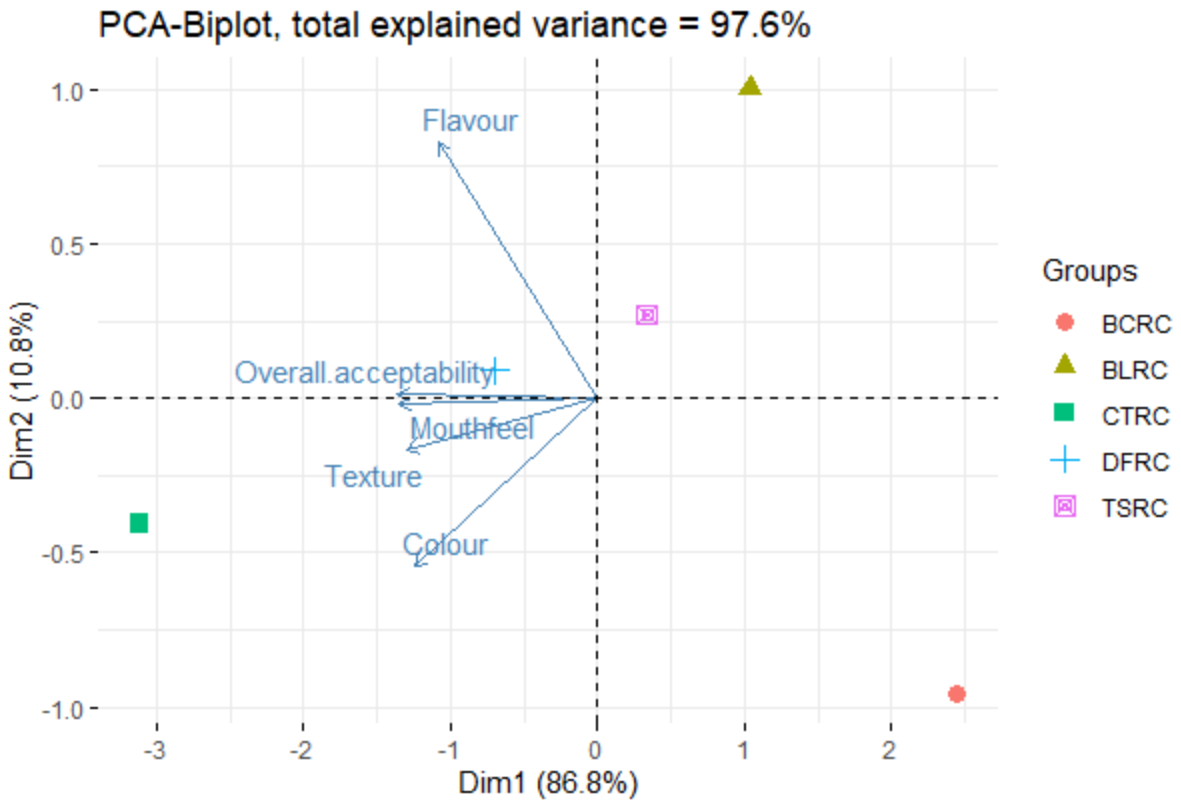


Figure 4.8: Principal component analysis (PCA) biplot showing the distribution of cookie types based on their sensory scores and the variation of sensory attribute scores among the among cookies enriched with differently processed *R. differens*. CTRC-Control cookies; BCRC-Blanched *R. differens*-based cookies; BLRC-Boiled *R. differens*-based cookies; TSRC-Toasted *R. differens*-based cookies; DFRC-Deep-fried *R. differens*-based cookies.

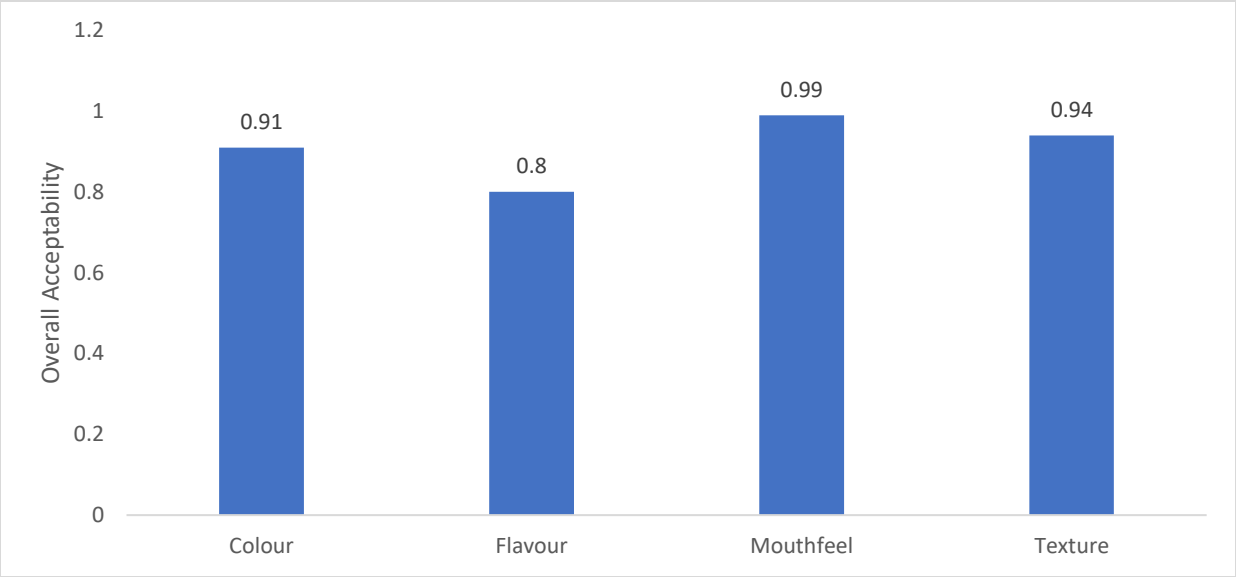


Figure 4.9: Bar charts indicating the correlation of mean scores of colour, flavour, mouthfeel and texture with overall acceptability

CHAPTER FIVE

DISCUSSION

5.1 Nutritional Composition of the Processed *R. differens*

5.1.1 Proximate Compositions

Higher dry matter and lower moisture content were noticed in deep fried samples compared with other samples. The immiscibility of oil and water coupled with the high deep-frying temperatures of 175°C rendered a dehydrative effect onto the samples. As a result, the oil medium into which the samples were suspended replenished the voids left in the food matrices (Bordin *et al*., 2013; Sainan & Kapute, 2017) culminating into higher dry matter and fat but, lower moisture in the deep-fried samples. Contrastingly, the lower dry matter and fat exhibited by the boiled *R. differens* is indicative of a continuous loss of fat into the boiling water. Toasted, boiled and blanched *R. differens* samples displayed higher protein content than the deep-fried one. A similar trend manifested when adult house crickets (*Acheta domesticus*), black soldier fly (*Hermetia illucens*), African cotton leafworm (*Spodoptera littoralis*), and grasshoppers (*Ruspolia differens*) were boiled and toasted (Nyangena *et al*., 2020). However, this may have been contributed by the masking effects of the significant loss of essential dry matter constituents, particularly fat, in boiling, blanched, and roasted *R. differens* (Nyangena *et al*., 2020; Ssepuuya *et al*., 2020) as the thermal processes have been reported to degrade proteins. Manditsera *et al*. (2019) found significant protein loss in boiled beetles and crickets, but no loss when they were toasted. Thermal hydrolytic breakdown of connective tissues, solubilization of soluble proteins in boiling water, nitrogen loss due to amine and amide loss, and complexing with reactants in the food media, notably lipid oxidation products, have all been connected to these losses (Nyangena *et al*., 2020). The low protein content in deep-fried samples may be due to the known biochemical reactions enhanced by the deep drying process which include denaturation of proteins, destruction of amino acids, Maillard reaction recruiting amino acids, peptides and proteins as reactants yielding flavour compounds (Bordin *et al*., 2013). The comparable ash contents of blanched, boiled and toasted samples are contrary to reported decrease and increase in ash contents of boiled and toasted insects, respectively (Nyangena *et al*., 2020; Ssepuuya *et al*., 2020). The loss of ash during boiling may be due to leaching loss but, in this study, the proportional loss during boiling might have overshadowed these effects. Higher fibre levels in the blanched and boiled samples compared with

toasted and deep-fried samples is evidence of high degree of thermal decomposition of fibrous materials at high temperature processing. MarkManuel and Godwin (2020) alluded to thermal degradation of fibre components such as cellulose, hemicellulose, pectin, gums and lignin during processing as the key contributors to fibre loss.

5.1.2 Fatty Acid Profiles of the Differently Processed *R. differens*

The most abundant SFAs, MUFAs and PUFAs discerned in the processed *R. differens* reinforces the pattern of dominant FAs that have been reported from house flies, Turkestan cockroaches, mealworms, super worms, waxworms, crickets, *tebo* worms, locusts (Mohamed, 2015) and *R. differens* oil (Cheseto *et al*., 2020). The proportion of SFA (42.4-69.0%), MUFAs (5.1-6.9%) and PUFAs (24.0-50.8)% in all the processed *R. differens* were reflective of the levels of 45% SFA, 20% MUFA and 34% PUFA previously reported by Cheseto *et al*. (2020) in *R. differens* oil. An interesting revelation of higher concentration of the PUFAs methyl (9*Z*,12*Z*,15*Z*)-octadecatrienoate, methyl (9*Z*,12*Z*)-octadecadienoate and methyl (5*Z*,8*Z*,11*Z*,14*Z*)-eicosatetraenoate in the blanched sample and their subsequent reductions from blanching>boiling>toasting>deep-frying with proportional rise in SFAs affirm the sensitivities of the FAs to extreme thermal temperatures. This pattern was spotted in high temperature treatment of coffee oils (Raba *et al*., 2018) and also observed by Ali *et al*. (2013) where upon heating of sunflower oil at frying temperatures of 185℃, the proportions of palmitic, stearic, and oleic acids increased with a proportionate drop in linoleic acid. The vulnerability of PUFAs to thermal decomposition through enhancement of double bonds attack by free radicals explain this characteristic phenomenon (Ali *et al*., 2013). Comparably, the ephemeral nature of blanching did not promote substantial loss of the PUFAs. The proportion of methyl (9*Z*,12*Z*)-octadecadienoate was outstanding in the processed *R. differens* occasioning a cumulative high PUFAs and ∑n-6/n-3 ratios. The n-6/n-3 is an index that evaluates the nutritional value of dietary fats and a ratio of 5:1 has since been recommended by WHO. The ratios reported in the current study exceeded the recommended ratio which may be attributed to the application of a single omega 3 (methyl (9Z,12Z,15Z)-octadecatrienoate (ALA)) despite the earlier detection of four (methyl (9*Z*,15*Z*)-octadecadienoate, methyl (9*Z*,11*E*,13*E*)-octadecatrienoate (α eleostearic acid), methyl (5*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-eicosapentaenoate (EPA) and methyl (4*Z*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-docosahexaenoate (DHA)) by Cheseto *et al*. (2020). The variations in the fatty acid profiles of insects even of the same species may be related to differences in diet and other physiological

parameters (Oonincx & Finke, 2020). Nevertheless, the abundance of methyl (9*Z*,12*Z*)-octadecadienoate in *R. differens* has been previously reiterated by some authors (Fombong *et al*., 2017; Kinyuru *et al*., 2010; Ssepuuya *et al*., 2019). On the other hand, the abundance of methyl (9Z)-octadecenoate in the deep-fried samples compared to others may be due to the used vegetable oil (palm oil) processed from refined palm olein (Montoya *et al*., 2014; Vispute & Dabhade, 2018). Another index of fats critical to health is PUFA/SFA ratio. This ratio remained higher than 0.4 in blanched, boiled and toasted *R. differens* but lower in deep fried. Ratios of <0.4 promote atherosclerotic disorders while those >0.4 are linked to suppression of cholesterol levels. The low ratio in deep-fried samples maybe due to extreme decomposition of PUFAs.

5.1.3 Mineral Profiles of the Processed *R. differens*

The minerals P, Ca, Mg, Zn, Fe and Co were the key elements detected in the processed *R. differens* and correspond to those previously recorded in *R. differens* (Fombong *et al*., 2017; Ssepuuya *et al*., 2020). The low levels of P in blanched, boiled and deep-fried samples may be due to solubilization in the process medium (Ssepuuya *et al*., 2020) as opposed to toasting which employed dry heating. This assertion, however, requires sufficient scientific support in the future. Nevertheless, the P values from blanched, boiled and toasted were slightly greater than values reported in boiled and roasted *R. differens* obtained from Masaka, Kampala and Fort portal except for deep-fried values which contrasted favourably (Ssepuuya *et al*., 2020). The P values of processed *R. differens* can therefore contribute largely to the RDA of 700 mg/ day in adults (Lewis, 2019). Higher levels of Ca, Mg and Zn in boiled, blanched and toasted *R. differens* confirm findings by researchers who conducted similar studies (Karimian-Khosroshahi *et al*., 2016; Mutungi *et al*., 2019; Ssepuuya *et al*., 2020). Ca, Mg and Zn are strongly bonded to proteins and chitin and therefore processes that influence proteins and chitin levels equally influence their levels (Ssepuuya, *et al*., 2020). The current study indicates low levels of Ca in the deep-fried samples and so do protein levels (Table 4.4&4.1) which further confirms this theory.

5.2 Sterol Levels of the Processed *R. differens*

This study presented four sterols and an intermediary product of steroid transformation were detected in the processed *R. differens*. Previously, sterols have been reported in insects such as desert locust (*Schistocerca gregaria*) (Cheseto *et al*., 2015) and crickets, mealworms and super worms (Finke, 2015). Metabolically, they are not equipped to synthesize 7-Dehydrocholesterol *de*

novo for vitamin D3 and steroid hormone synthesis despite its indispensable need. For their survival, they derive these phytosterols from plants for the synthesis of 7-Dehydrocholesterol (Sabolová *et al*., 2016). Blanched *R. differens* showed the highest levels of detected sterols; (22*Z*)-27-Norergosta-5,22-dien-3*β*-ol, cholesterol, campesterol, cholest-4-ene-3-one and *β* sitosterol while deep frying and toasting recorded the lowest levels. This pattern corroborates the findings by Dias-Martins *et al*. (2021) which expressed that phytosterols decomposition is initiated at 60℃ and further increase in temperature yields proportional quantities of oxysterols, sterol fragments, volatiles and oligomers as secondary products. Other studies with related findings also reported 50-60% phytosterol loss in milk heated at 90℃ (Menéndez-Carreño *et al*., 2008) and 200℃ heated oils (Thanh *et al*., 2006). Nonetheless, ergosta-4,6,22-triene-3-*β*-ol, *γ*-sitosterol and stigmasta-4,6,22-triene-3-*β*-ol (Figure 4.2) were transformational products detected in deep fried samples but not detected in the other profiles. These compounds are speculated to be derivatives of (22*Z*)-27-Norergosta-5,22-dien-3*β*-ol, *β*-sitosterol and stigmasterol upon undergoing hydrolysis, oxidation, polymerization and isomerization reactions reportedly common in deep-frying (Kmiecik *et al*., 2021), however, this hypothesis requires further research. Since it has been identified in palm oil, stigmasta-4,6,22-triene-3-ol could have resulted from a chemical change of stigmasterol present in the oil used (Hassanien, 2013). The toasting and deep-frying were done at temperatures >150℃ defining the low sterol values while blanching was conducted at 100℃ but for a shorter period than boiling, hence the record high levels of sterols. Phytosterols inhibit intestinal cholesterol absorption, resulting in a decrease in plasma low density lipoprotein levels i.e. their consumption in amounts of 1.5-1.8g/day result to 30-40% reduction in cholesterol absorption (Ogbe *et al*., 2015). On the other hand, cholesterol is essential in steroid hormones and bile acid synthesis (Sabolová *et al*., 2016) however, its consumption has been recommended to be a daily intake of 300 mg (Dinh *et al*., 2011). Cholest-4-ene-3-one has been demonstrated to be beneficial in the treatment of liver illness, obesity, and keratinization (Wu *et al*., 2015).

5.3 Total Flavonoids Content of the Processed *R. differens*

The higher and lower total flavonoids content (TFC) witnessed in blanched and deep-fried *R. differens*, respectively, validates the substantial influence of TFCs of foods during processing. Elsewhere, boiling and pressure cooking caused loss of appreciable levels of TFCs as compared with steaming and microwaving which offered minimal degradative effects (Yadav *et al*., 2018). Cooking at high temperatures softens and disintegrates cell walls, allowing flavonoid chemicals to

seep into the cooking medium. Furthermore, because these bioactive chemicals are heat-labile, severe thermal processes have a significant impact on their availability (Yadav *et al*., 2018). Because of the high temperature (175°C) used in the current investigation, deep-frying could be classified as a harsh heat treatment, which could explain the low levels of TFC in deep-fried samples. Processed foods have a wide range of TFC. This is dependent on the length and intensity of heat treatment, pH, phytochemical structure, and oxygen availability. The impact of the processing conditions on the various flavonoid components, whose contents after processing directly influence the TFC, causes these variations. As a result, food processing conditions may facilitate or hinder the extraction or degradation of phenolic chemicals (Irina & Mohame, 2012). Flavonoids are polyphenolic chemicals with pharmacological advantages in humans, including anti-inflammatory, antibacterial, antiviral, anti-allergenic, vasodilatory, and anti-cancer activities (Cheseto *et al*., 2020).

5.4 *In vitro* Protein Digestibility of the Processed *R. differens*

Protein digestibility is a measure of the bio-accessibility of proteins or its structural sub units (peptides and amino acids) in the gut relative to that consumed. The *in vitro* protein digestibility of the processed insects ranged 72.3-83.4% which corresponds to the values of 76.0-96.0% previously reported in edible insects (Stull, 2021). The digestibility of edible insect proteins has been postulated to be slightly lesser than that of eggs (95.0%) and beef (98.0%) but, greater than that of plant proteins (Kouřimská & Adámková, 2016). The protein digestibility of processed edible insects however, vary haphazardly, subject to a variety of factors. The digestibility of the processed *R. differens* assumed a decreasing trend from blanching>boiling>toasting>deep-frying exposing their impactful extents. Kinyuru *et al*. (2010) similarly reported a decline in protein digestibly upon toasting and drying of *R. differens*. Heat processing may diminish or improve protein digestibility, depending on the processing circumstances. By unfolding the polypeptide chain and making the protein more accessible to digestive enzymes, denaturation temperatures can improve native protein digestibility (Kinyuru *et al*., 2010). The low protein digestibility exhibited in toasted and deep-fried samples may be attributed to exposure of proteins to dry heat thereby promoting the development of disulphide bonds. Further, the underlying process conditions (low moisture and high temperatures) predisposed the samples to intense Maillard reactions utilizing available proteins and amino acids, limiting the amount of digestible proteins (Akullo *et al*., 2018). Other factors such as antinutrients (tannins and phytates), chitin and experimental methodology

applied in terms of enzymes used may come to play in justifying the variations discovered in the digestibility of edible insect proteins (Manditsera *et al*., 2019). There is still information paucity on the anti-nutrient content of *R. differens*, however, analysis of its closely related species, *S. prasiniferum* and *C. trachypterus* confirmed that their levels were within acceptable limits (Das & Mandal, 2013). *R. differens* has low chitinous material owing to its soft bodied nature, hence, may have had negligible influence on the digestibility (Traksele *et al*., 2021).

5.5 Microbial Levels of Processed *R. differens*

R. differens like other insects have a lot of moisture, favourable pH and are nutrient dense, therefore they provide a good environment for microbiological organisms to thrive and survive (Klunder *et al*., 2012; Ssepuuya *et al*., 2019). Moreover, the high microbial counts in harvested insects may further be attributable to wild collection under uncontrolled environment, unsanitary handling settings and inadequately cleansed hands, or handling equipment (Gatheru *et al*., 2019; Ng'ang'a *et al*., 2019). Therefore, consumption of edible insects may compromise the health of consumers by posing serious health risks (Belluco *et al*., 2013). Suitable processing, handling, and storage techniques have been recommended to reduce these health hazards (Rumpold & Schlüter, 2013). The levels of microorganisms were dramatically influenced by the processing techniques adopted in the current study. Higher counts of TVC, *Staphylococcus aureus* and lactose positive *Enterobacteriaceae* were revealed in blanched *R. differens* which demonstrates the inadequacy of the process in suppressing a variety of microorganisms. The levels of TVC in blanched *R. differens* (log_{10} 4.54 cfu/g) is comparable to those reported in 10 s blanched mealworm larvae (Vandeweyer *et al*., 2017), 4 min blanched house cricket, 1 min blanched termites, 5 min blanched Bingula caterpillar and 1 min blanched mealworm larvae (Caparros-Megido *et al*., 2017). Despite their higher levels in blanched *R. differens*, the TVC were within the acceptable levels of below Log_{10} 7 cfu/g which is a measure of the hygienic condition of food (Nyangena *et al*., 2020; Ramashia *et al*., 2020). This hygiene criterion has been borrowed from the advisory by Superior Health Council (SHC) and the Federal Agency for the Safety of the Food Chain (FASFC), which recommended process hygiene criteria for minced meat described in EU Regulation (EC) No. 1441/2007 to be used for edible insects (Ssepuuya *et al*., 2019). Lower TVC levels in boiled and deep-fried samples compared with the toasted samples were recorded. This is due to better heat diffusion through the tissues during boiling and deep-frying (Nyangena *et al*., 2020) than in toasting. Deep-frying in this study also registered low counts of *Staphylococcus aureus*, *Enterobacteriaceae* and yeast &

moulds and totally subdued *Salmonellae*. The effectiveness of deep-frying in decontaminating edible insects has been reported by other authors (Gatheru *et al*., 2019; Labu *et al*., 2021). *Enterobacteriaceae* counts were completely eliminated during boiling and deep-frying due to their high sensitivities to heat (Ng'ang'a *et al*., 2019) however, their presence in toasted samples depicts inefficient heat transfer to the core (guts) during this process (Klunder *et al*., 2012). The non-detection of *Salmonellae spp.* in all the samples justifies their sensitivities to heat, culminating into products compliant with microbiological guidelines on food safety (KEBS, 2020). Low yeast and mould counts in all the processed samples indicates that they were drastically suppressed during processing to yield counts below recommended maximal limits of 5 $\log_{10}$ CFU/g (Ramashia *et al*., 2020; Vandeweyer *et al*., 2017).

5.6 Proximate Composition of the Cookies

The control cookies showed higher moisture which translated to lower dry matter content compared to the other cookies. The control cookies samples had whole eggs inclusion during formulation. Eggs have relatively higher moisture content of 78.0% (Ogunwole *et al*., 2015) compared with the processed *R. differens* flours (Table 4.1) and this may have caused the high moisture in the control cookies. The moisture content of the insect-based cookies ranged 1.33-3.0% which agrees with values recorded by Ayensu *et al*. (2019) in biscuits fortified with palm weevil flours. These values suggest insect enriched cookies have lower water activity and reduced susceptibility to microbial growth hence longer shelf-life. Statistically, lower protein levels were discovered in the control and deep-fried *R. differens*-based cookies. Whole eggs used in control cookies formulation has been reported to contain protein levels of 11.5-11.6 % (Ogunwole *et al*., 2015) compared to 40.1-44.7% in blanched, boiled and toasted *R. differens* (Table 4.1) and may account for this huge difference in the respective cookies. On the other hand, deep fried *R. differens* demonstrated low protein content occasioned by a series of biochemical reactions resulting into their remarkable degradation (Bordin *et al*., 2013). Since they were directly utilized in dough formulation, their low levels were reflected in the baked cookies. The protein levels in all the insect enriched cookies failed to meet the Kenya Bureau of Standards (KEBS) specifications (KEBS, 2020). The protein levels can be improved by defatting *R. differens* (Lee *et al*., 2020) before inclusion into products, since fats are compositionally the largest followed by proteins (Ssepuuya *et al*., 2019). The insect-based cookies indicated higher fat and energy content than the control cookies. This was expected because, *R. differens* has a fat content of 42.2-54.3% (Ssepuuya *et al*.,

2019) which may have been reflected in the cookies. Similar results were reported in cookies enriched with sorghum-termite blends (Awobusuyi *et al*., 2020). These fats, however, are rich in PUFAs and MUFAs which may further limit the shelf-life of their respective cookies when compared to the control cookies. These fat levels may have played critical role in the variation of the energy levels of the cookies (Omoba & Omogbemile, 2013) since the carbohydrate levels were comparable (Table 4.1). Apparently, the control cookies had the lowest energy compared to the insect cookies. These patterns corroborate those reported by Awobusuyi *et al*. (2020).

5.7 Amino Acid Profiles of the Cookies

Amino acids profiles of the control cookies compared well with the insect-based cookies. *R. differens* has been reported to possess abundant levels of alanine, aspartic acid, glutamic acid and essential amino acids; leucine, lysine and valine (Ssepuuya *et al*., 2019). This is clearly evident in Table 4.1 where the levels of alanine, glutamic acid and valine were the dominant profiles especially in cookies enriched with blanched, boiled and toasted *R. differens* featuring higher mean protein levels. Similarly, eggs represents a standard and valuable source of all essential amino acids (Attia *et al*., 2020). The protein sources are therefore ideal for the enrichment of cereal-based flours like wheat which has been known to be deficient in essential amino acids lysine and threonine (Ayensu *et al*., 2019). Despite the noted abundance of lysine in both eggs and *R. differens*, their lack of detection in the cookies may be attributed to processing related loss (Hussein *et al*., 1979). Specifically, the *R. differens* underwent a two-stage cooking (initial blanching, boiling, toasting and deep-frying then baking) which may have rendered the amino acids unavailable in the baked goods. This assertion may apply suitably to DFCR which showed relatively low levels of majority of amino acids as they were formulated with deep fried *R. differens* exposed to harshest processing technique.

5.8 Fatty Acid Profiles of the Cookies

Methyl hexadecanoate, Methyl (*Z*)-9-Octadecenoate and Methyl (9*Z*,12*Z*)-Octadecadienoate were the principal FAs in SFAs, MUFAs and PUFAs respectively. Furthermore, the proportions of SFAs, MUFAs and PUFAs ranged 24.6-49.4%, 36.3-49.8% and 0.8-30.0% respectively, for the insect-based cookies and 52.8%, 45.8% and 1.4% respectively for the control cookies. The identified dominant fatty acids and the proportions of SFAs, MUFAs and PUFAs in the insect-based cookies reflected the pattern demonstrated in their respective processed insects.

Fascinatingly, blanched and boiled *R. differens*-based cookies displayed higher levels of PUFAs and lower levels of SFA while toasted and deep-fried *R. differens*-based cookies exhibited higher SFAs but lower PUFAs. These findings were consistent with fatty acids patterns observed in cookies formulated from different insect oils (Cheseto *et al*., 2020) as they were originally influenced by the different processing methods. It can therefore be alleged that baking had insignificant impact on the FAs patterns. The appearing and disappearing of certain FAs in cookies relative to the processed *R. differens* may be as a consequence of imperfect uniformity of dough formulation and the influence imposed by the baking conditions. In the control cookies, the proportions of FAs followed the increasing order: SFA>MUFA>PUFA. This implies that eggs, which were incorporated in the control cookies, contained appreciably higher levels of SFAs than MUFAs and PUFAs. The presence of PUFAs; methyl (12,15)-Octadecadienoate and methyl (9*Z*,12*Z*)-Octadecadienoate, methyl (9*Z*,12*Z*,15*Z*)-octadecatrienoate in the insect-based cookies and the abundance of MUFA, methyl (*Z*)-9-Octadecenoate, guarantees possibility of insect-neophobic consumers' accessing the health benefits of oils endowed in insects through developed products. Methyl (*Z*)-9-Octadecenoate is a cholesterol-lowering MUFA that also has anticancer and anti-inflammatory properties. It also supports cell viability, acts as a source of energy, and is a precursor for the biosynthesis of linoleic acid, which is required for the formation of arachidonic acid, which is important in cell regulation. Methyl (9*Z*,12*Z*)-Octadecadienoate is well-known for its function in the synthesis of arachidonic acid, which is a precursor for a variety of hormones such as prostaglandins, thromboxanes, and leukotrienes that regulate a variety of physiological processes (Cheseto *et al*., 2020).

5.9 Mineral Profiles of the Cookies

Ruspolia differens has been reported to furnish considerable levels of macro minerals K, P and Mg which other than Ca, have been reported to compare favourably with animal products such as meat, pork and eggs (Ssepuuya *et al*., 2019). This assertion is well confirmed in Table 4.9 where, the levels of the minerals depicted minimal variations in all the cookies except for deep-fried *R. differens*-based cookies. Ca levels were significantly higher in control cookies than in the insect-based cookies. This is because *R. differens* has lower levels of Ca attributable to the lack of mineralized skeleton (Kinyuru *et al*., 2010) as opposed to eggs laid by hens. *R. differens* also manifested appreciable amounts of trace elements Fe, Zn, Cu and Mn in amounts exceeding those reported in in pork and chicken (Ssepuuya *et al*., 2019). This study reinforces this claim by

showing significant differences in relation to the levels of these trace minerals between the control cookies and insect-based cookies (mainly Blanched, Boiled and Toasted *R. differens*-based cookies) (Table 4.9). These findings replicate reports by Ayensu *et al*. (2019) where the levels of trace minerals proportionally increased with increasing levels of palm weevil incorporation. This suggests that the average minerals in the final baked products mirrors the amounts and patterns of minerals in the respective ingredients. Therefore, cookies can be used to combat micronutrient related deficiency among populations unaccustomed to entomophagy.

5.10 Headspace Volatile Organic Compounds (VOCs) of the Cookies

Volatile organic compounds are one of the characteristics that dictate the perception and acceptability of foods, including edible insects, as they contribute to odour, flavour and taste. The aroma compounds that have been identified in insects include acids, alcohols, aldehydes, alkenes, amines, terpenes, ketones and esters (Cheseto *et al*., 2020; Ssepuuya *et al*., 2020; Tzompa-Sosa *et al*., 2019) which translate to savory, umami, buttery, meaty, bacony, sweet, herbal, or fruity flavours (Mishyna *et al*., 2019). The principal volatile organic compounds which contributed largely to differentiation of the cookies corroborates the profiles detected in boiled and roasted *R. differens* (Ssepuuya *et al*., 2020) and *R. differens* oil (Cheseto *et al*., 2020). The levels of hexanal and 2-pentylfuran were higher in boiled *R. differens*-based cookies formulated with boiled *R. differens*. This underpins the findings by Ssepuuya *et al*. (2020) that boiling *R. differens* enhances the two compounds. The predominant volatiles in raw *R. differens* have been reported to be limonene (0.17µg/g) and heptanol (0.0012µg/g) (Ssepuuya *et al*., 2020). In this study, the concentrations of limonene (Control cookies: 1.10µg/g, Insect based cookies: 0.62-0.97µg/g) and 1-heptanol (only present in boiled *R. differens*-based cookies:0.88µ/g) were comparably lower. The higher levels of hexanal and 2-pentylfuran in boiled *R. differens*-based cookies are consonant with findings that evinced the two aroma compounds as the predominating volatile organic compounds in boiled *R. differens* (Ssepuuya *et al*., 2020). Higher concentrations of methyl pyrazine were detected in toasted and deep-fried *R. differens*-based cookies formulated with toasted and deep-fried *R. differens*, respectively. During frying, Strecker aldehydes and α-amrinones are produced by the oxidation decarboxylation of reducing sugars and amino acids which then condense to form alkane pyrazines, serving as aroma compounds (Liu *et al*., 2022). Thermal decomposition of unsaturated fatty acid has been reported to produce 2*E*,4*E* -decadienal, and 2*E*-heptenal (Gasior & Wojtycza, 2016). In this study, the higher 2*E*,4*E*-decadienal detected

in toasted and deep-fried *R. differens*-based cookies may be linked to its formation during high temperature toasting and deep-frying of *R. differens*. The relatively lower concentrations of aldehydes nonanal, hexanal, 2*E*,4*E*-dodecadienal, pentanal and octanal, associated with fat, meaty flavour, nutty, sweet and almond-like aroma (Domínguez *et al*., 2014; Starowicz, 2021) and methyl pyrazine, furfurals, benzaldehyde and 2-pentyl furan which are associated with desirable flavours; nutty, cocoa, roasted meat, almond-like and sweet may have contributed to the low sensory scores regarding flavour of the blanched *R. differens*-based cookies compared with the other cookie types. This may be hypothesized to originate from partial processing (blanching) of *R. differens* used for its formulation characterized by inadequate chemical reactions to yield the flavour profiles. This can be confirmed from Figure 4.6A where there is no volatile organic compounds associated with blanched *R. differens*-based cookies from identified influential profiles in Figure 4.6C. Elsewhere, fermentation of *Allomyrina dichotoma* larvae using *Saccharomyces cerevisiae*, markedly subdued indole, a compound associated with fecal odour while simultaneously introducing new set of aroma compounds; 2-undecanone, 2-methyl-1-butanol, 2-nonanone, 3-methyl-1-butanol, isopentyl acetate, and ethyl acetate and enhancing others (Kim *et al*., 2021). This is a clear demonstration that processing procedures can be used to modify insect aroma from the native and objectionable ones to new pleasant profiles in order to enhance entomophagy.

5.11 *In vitro* Protein Digestibility of Cookies Enriched with Differently Processed *R. differens* Flours

The influence of protein digestibility of the enriched cookies mirrored the pattern realized in their respective processed *R. differens*. Hence, it can be argued that the limiting or enhancing factors to digestibility in the processed *R. differens* may have still played the same roles in the protein digestibility of the cookies. This is in agreement with the findings by Akullo *et al*. (2018) which attributed the variations in protein digestibility of crackers to the influence effected by processing conditions the termites were subjected to. Elsewhere, the protein digestibility of non-wheat cookies significantly increased with increasing substitution levels of Bambara ground nut flours of high protein digestibility (Kiin-kabari & Giami, 2015). Contrastingly, the protein digestibility of the enriched cookies was slightly lower than that of their respective counterpart processed *R. differens*. In the same respect, Abdel-Aal (2008) reported enhanced digestibility in a two-step enzyme digestibility of biscuits but, a decreased digestibility in a one-step enzyme

metabolism. The former phenomenon was linked to enhanced accessibility of proteins by enzymes upon baking whereas the latter was associated to pH change induced by buffering capacity of biscuit mix. This may also be accredited to the higher levels of digestible protein in the processed *R. differens* (7.8-44.7% Table 4.1) compared to the cookies (7.7-11.1 % Table 4.6). Higher digestibility of control cookies was as result of inclusion of eggs into the formulation with omission of the insect flours. Eggs has been reported to portray high protein digestibility (95%) (Kouřimská & Adámková, 2016). This is explicable by the fact of eggs lacking chitin which negatively correlates to protein digestibility (Manditsera *et al*., 2019) and the presence of highly soluble proteins.

5.12 Microbial Levels of Cookies Enriched with Differently Processed *R. differens* Flours

The cookies expressed permissible microbial levels compliant with KEBS recommendations (KEBS, 2020) and can be regarded safe for consumption. Similar results were reported on biscuits fortified with palm weevil (Ayensu *et al*., 2019) and energy dense-biscuits enriched with silkworm pupae and locusts (Olamide *et al*., 2020). However, edible insects, applied as ingredients in these products are known to harbour *Salmonella spp*. and *Staphylococcus Spp*. and waterborne pathogens of public health concerns (Belluco *et al*., 2013; Montowska *et al*., 2019) and are still the key challenges in the use of insects as food (Caparros-Megido *et al*., 2017). The achievement of permissible microbial levels in cookies may be due to factors such as use of pre-treated insect ingredients and maintenance of high level of personal an environmental hygiene during ingredient formulations. Further, the baking temperatures (180℃/15 min) may have subdued majority of the microorganisms under examination. Lastly, post-baking handling of the cookies followed a strict adherence to hygienic standards coupled with prompt packaging into sterile zip lock bags to avoid contamination from the environment. The low water activities and pH in cookies are also restraining conditions to bacterial growth (Khan *et al*., 2017).

5.13 Sensory Acceptance of Cookies Enriched with Differently Processed *R. differens* Flours

In as much as appreciable level of insect familiarity has been achieved through inclusion into modern food products and intensified awareness, a good number of consumers still oppose the idea. There is a general tendency of negative correlation established between increasing levels of insects' incorporation into products and their acceptability has been established by authors

(Adeboye *et al*., 2016; Awobusuyi *et al*., 2020; González *et al*., 2019; Ogunlakin *et al*., 2018; Ojinnaka *et al*., 2015; Osimani *et al*., 2018). Of great influence to acceptability of edible insects is disgust, as it has been cited the major deterrent factor to entomophagy adoption (Gmuer *et al*., 2016; Hamerman, 2016; Wendin & Nyberg, 2021). Disgust is provoked by sensory characteristics such as aroma, texture and general appearance (Jensen & Lieberoth, 2019). Contrary to Bawa *et al*. (2020), Ojinnaka *et al*. (2015) and Adeboye *et al*. (2016) reporting significant difference in flavour perception of 10% insect enriched cookies relative to the control, the current study revealed no significant difference in boiled, toasted and deep-fried *R. differens*-based cookies but not blanched *R. differens*-based cookies (Table 4.12). This may be ascribed to process-induced transformation of original abhorrent flavours in the raw insects to give new sets of appealing flavours. Blanched *R. differens*-based cookies were enriched with blanched *R. differens* which was short-lived and may have not fully transformed the flavour compounds. Disgust has been cited the most common factor hindering receptiveness of edible insects to consumers. Sensory properties of insects such as flavours, appearance and texture have been identified as the prime elicitors (Evans *et al*., 2016; Sogari & Mora, 2019) and dictates acceptance or rejection of an insect-based product (Ojinnaka *et al*., 2015). However, in this study, flavour was weakly correlated to the overall acceptability (Figure 4.8 & 4.9), probably because of the eliminated unpleasant flavours, presenting insignificant influence on their acceptability. The colour rankings of control cookies were significantly different from blanched, boiled and toasted *R. differens*-based cookies. This may be caused by the higher available proteins and probably peptides and amino acids recorded in blanched, boiled and toasted *R. differens* for Maillard reactions (Mainley, 2011; Ojinnaka *et al*., 2015). Hence, may have led to the darker colours in blanched, boiled and toasted *R. differens*-based cookies (Figure 3.4). Notably, there was no significant difference in colour scores between control and deep-fried *R. differens*-based cookies which may be related to deep-frying destruction of proteins and amino acids of the *R. differens* used in deep-fried *R. differens*-based cookies formulation. Significant differences in texture scores between the control cookies and the insect-based cookies may be associated to exoskeleton of the ground *R. differens* incorporated in the insect-based cookies (Ojinnaka *et al*., 2015). Mouth-feel was highly correlated with the overall acceptability (R=0.99) meaning that the majority of the panellists relied on physical sensation of the cookies while in the mouth to gauge the acceptability. Generally, control cookies were the most

preferred. This is in line with reports from other similar studies (Akullo *et al*., 2018; Ogunlakin *et al*., 2018; Ojinnaka *et al*., 2015).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

i. Blanched *R. differens*-based cookies and boiled *R. differens*-based cookies have better nutritional quality in terms of higher levels of protein (11.09%), amino acids; alanine (24.13 mg/g), glutamic acid (21.54mg/g) and valine (22.41mg/g), minerals; Fe (7.11mg/100g), Zn (4.33mg/100g), Cu (301.30µg/100g) and Mn (808.71mg/100g), and significantly higher *in vitro* protein digestibility of 80.4%.

ii. Initial processing of *R. differens* followed by baking in cookie formulations drastically suppresses *Staphylococcus aureus*, *Salmonella spp*. and yeast and moulds counts to undetectable levels yielding insect-based cookies compliant with Kenya Bureau of Standards guidelines for insect enriched products.

iii. The sensory preferences of colour, flavour, mouthfeel, texture and overall acceptability of control cookies are higher than blanched *R. differens*-based cookies. Low flavour ratings of blanched *R. differens*-based cookies may be attributed to insufficient thermal transformation of original *R. differens* flavours during blanching. However, sensory characteristics of deep-fried *R. differens*-based cookies compare favourably with control egg-based cookies.

6.2 Recommendations

This study recommends that;

i. Blanching and boiling should be adopted for preparation of *R. differens* for products enrichment owing to its nutrient preservation. Further, the wet heat processing techniques like blanching and boiling of *R. differens* for products formulations should be adopted to ensure high digestibility of the added insect proteins.

ii. Pre-processing of edible insects prior to incorporation into products should be encouraged to ensure end-products of high sanitary levels.

iii. Artificial flavours should be added to mask the unpleasant flavours of blanched *R. differens*-based cookies since it displayed satisfactory nutritional composition. Other processing method like boiling may be adopted to transform the residual abhorrent volatile compounds to boost product likings.

REFERENCES

Abdel-Aal, E. S. (2008). Effects of baking on protein digestibility of organic spelt products determined by two in vitro digestion methods. *LWT-Food Science and Technology*, *41*(7), 1282–1288. https://doi.org/10.1016/j.lwt.2007.07.018

Adeboye, A., Bolaji, T., & Fatola, O. (2016). Nutritional composition and sensory evaluation of cookies made from wheat and palm weevil larvae flour blends. *Annals. Food Science and Technology*, *17*(2), 543–547.

Adeyeye, S. A. O., Adebayo-Oyetoro, A. O., & Tiamiyu, H. K. (2017). Poverty and malnutrition in Africa: a conceptual analysis. *Nutrition & Food Science*, *47*(6), 754-764.

Adeyeye, S. A. O., Ashaolu, T. J., Bolaji, O. T., Abegunde, T. A., & Omoyajowo, A. O. (2023). Africa and the Nexus of poverty, malnutrition and diseases. *Critical Reviews in Food Science and Nutrition*, *63*(5), 641-656.

Ahmad, R. S., Imran, A., & Hussain, M. B. (2018). Nutritional composition of meat. *Meat Science and Nutrition*, *10*(5772), 61–77. https://doi.org/10.5772/intechopen.77045

Akullo, J., Nakimbugwe, D., Obaa, B. B., Okwee-Acai, J., & Agea, J. G. (2018). Development and quality evaluation of crackers enriched with edible insects. *International Food Research Journal*, *25*(4), 1592–1599.

Ali, M. . A., Najmaldien, A. H. A., Latip, R. A., Othman, N. H., Majid, F. A. A., & Salleh, L. M. (2013). Effect of heating at frying temperature on the quality characteristics of regular and high-oleic acid sunflower oils. *Acta Scientiarum Polonorum Technologia Alimentaria*, *12*(2), 159–167.

Amadi, E. N., & Kiin-Kabari, D. B. (2016). Nutritional composition and microbiology of some edible insects commonly eaten in Africa, hurdles and future prospects: A critical review. *Journal of Food: Microbiology, Safety & Hygiene*, *1*(1), 1000107.

Aniebo, A. O., & Owen, O. J. (2010). Effects of age and method of drying on the proximate composition of housefly larvae (*Musca domestica* Linnaeus) Meal (HFLM). *Pakistan Journal of Nutrition*, *9*(5), 485–487. https://doi.org/10.3923/pjn.2010.485.487

AOAC. (2000). *Official Methods of AOAC International* (W. Horwitz, Ed.). AOAC International.

Attia, Y. A., Al-harthi, M. A., & Korish, M. A. (2020). Protein and amino acid content in four brands of commercial table eggs in retail markets in relation to human requirements. *Animals*, *10*(3), 406–416.

Awobusuyi, T. D., Pillay, K., & Siwela, M. (2020). Consumer acceptance of biscuits supplemented with a sorghum–insect meal. *Nutrients*, *12*(4), 895–907. https://doi.org/10.3390/nu12040895

Awobusuyi, T. D., Siwela, M., & Pillay, K. (2020). Sorghum–insect composites for healthier cookies: nutritional, functional, and technological evaluation. *Foods*, *9*(10), 1427–1441.

Ayensu, J., Lutterodt, H., Annan, R. A., Edusei, A., & Loh, S. P. (2019). Nutritional composition and acceptability of biscuits fortified with palm weevil larvae (*Rhynchophorus phoenicis* Fabricius) and orange-fleshed sweet potato among pregnant women. *Food Science & Nutrition*, *7*(5), 1807–1815. https://doi.org/10.1002/fsn3.1024

Ayieko, M., Oriaro, V., & Nyambuga, I. A. (2010). Processed products of termites and lake flies: improving entomophagy for food security within the lake victoria region. *African Journal of Food, Agriculture, Nutrition and Development*, *10*(2), 2085–2098. https://doi.org/10.4314/ajfand.v10i2.53352

Barreto Ferreira, T. H., Mizuno Florizo, G. K., & Sanjinez Argondoña, E. J. (2020). Shelf life of cookies made from baru (*Dipteryx alata* Vog.) pulp under different storage conditions. *Journal of Food Processing and Preservation*, *44*(9), 14702–14708. https://doi.org/10.1111/jfpp.14702

Bawa, M., Songsermpong, S., Kaewtapee, C., & Chanput, W. (2020). Nutritional, sensory, and texture quality of bread and cookie enriched with house cricket (*Acheta domesticus*) powder. *Journal of Food Processing and Preservation*, *44*(8), 14601–14609. https://doi.org/10.1111/jfpp.14601

Belluco, S., Losasso, C., Maggioletti, M., Alonzi, C., Paoletti, M., & Ricci, A. (2013). Edible insects in a food safety and nutritional perspective: A critical review. *Comprehensive Reviews in Food Science and Food Safety*, *12*(3), 296–313. https://doi.org/10.1111/1541-4337.12014

Bordin, K., Tomihe Kunitake, M., Kazue Aracava, K., & Silvia Favaro Trindade, C. (2013). Changes in food caused by deep fat frying - A review. *Archivos Latinoamericanos de Nutricion*, *63*(1), 5–13.

Caparros-Megido, R. C., Desmedt, S., Blecker, C., Béra, F., Haubruge, É., Alabi, T., & Francis, F. (2017). Microbiological load of edible insects found in Belgium. *Insects*, *8*(1), 12–19. https://doi.org/10.3390/insects8010012

Caparros Megido, R., Gierts, C., Blecker, C., Brostaux, Y., Haubruge, É., Alabi, T., & Francis, F. (2016). Consumer acceptance of insect-based alternative meat products in Western countries.

Food Quality and Preference, *52*, 237–243. https://doi.org/10.1016/j.foodqual.2016.05.004

Chavan, U. D., Mckenzie, D. B., & Shahidi, F. (2001). Functional properties of protein isolates from beach pea (*Lathyrus maritimus* L.). *Food Chemistry*, *8146*(2), 177–187. https://doi.org/10.1016/S0308-8146(01)00123-6

Cheseto, X., Baleba, S., Tanga, C. M., Kelemu, S., & Torto, B. (2020). Chemistry and sensory characterization of a bakery product prepared with oils from African edible insects. *Foods*, *9*(6), 800–826. https://doi.org/10.3390/foods9060800

Cheseto, X., Kuate, S. P., Tchouassi, D. P., Ndung'u, M., Teal, P. E., & Torto, B. (2015). Potential of the desert locust *Schistocerca gregaria* (Orthoptera: Acrididae) as an unconventional source of dietary and therapeutic sterols. *PLoS ONE*, *10*(5), 127171–127183. https://doi.org/10.1371/journal.pone.0127171

Das, M., & Mandal, S. (2013). Assessment of nutritional quality and anti-nutrient composition of two edible grasshoppers (Orthoptera: Acrididae)—a Search for New Food Alternative. *International Journal of Medicine and Pharmaceutical Science*, *3*(5), 31–48.

Deroy, O., Reade, B., & Spence, C. (2015). The insectivore's dilemma, and how to take the West out of it. *Food Quality and Preference*, *44*, 44-55.

De Smet, J., Wynants, E., Cos, P., & Van Campenhout, L. (2018). Microbial community dynamics during rearing of black soldier fly larvae (*Hermetia illucens*) and impact on exploitation potential. *Applied and Environmental Microbiology*, *84*(9), e02722-17. https://doi.org/10.1128/AEM.02722-17

Dewanto, V., Wu, X., Adom, K. K., & Liu, R. H. (2002). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *Journal of Agricultural and Food Chemistry*, *50*(10), 3010–3014. https://doi.org/10.1088/0022-3727/31/22/002

Dias-Martins, A. M., Cappato, L. P., Trombete, F. M., Silva, T. L., Gamallo, O. D., Keller, L. M., Augusta, I. M., & Saldanha, T. (2021). Effects of thermal processing on fatty acids, phytosterols and other chemical properties of black table olives. *Scientia Plena*, *16*(12), 121501–121507. https://doi.org/10.14808/sci.plena.2020.121501

Dinh, T. T., Thompson, L. D., Galyean, M. L., Brooks, J. C., Patterson, K. Y., & Boylan, L. M. (2011). Cholesterol content and methods for cholesterol determination in meat and poultry. *Comprehensive Reviews in Food Science and Food Safety*, *10*(5), 269–289. https://doi.org/10.1111/j.1541-4337.2011.00158.x

Domínguez, R., Gómez, M., Fonseca, S., & Lorenzo, J. (2014). Effect of different cooking methods on lipid oxidation and formation of volatile compounds in foal meat. *Meat Science*, *97*(2), 223–230. https://doi.org/10.1016/j.meatsci.2014.01.023

Egan, B. A., Addo-Bediako, A., Masoko, P., Mphosi, M., Olivier, P. A. S., Toms, R., & Minter, L. R. (2014). Nutritional significance of the edible insect, *Hemijana variegata* Rothschild (Lepidoptera: Eupterotidae), of the Blouberg region, Limpopo, South Africa. *African Entomology*, *22*(1), 15–23. https://doi.org/10.4001/003.022.0108

Encyclopaedia Britannica (Ed.) (2015, July 3). *Masaka*. *Encyclopedia Britannica*. https://www.britannica.com/place/Masaka

Evans, J., Alemu, M. H., Flore, R., Frøst, M. B., Halloran, A., Jensen, A. B., Maciel-Vergara, G., Meyer-Rochow, V. B., Münke-Svendsen, C., Olsen, S. B., Payne, C., Roos, N., Rozin, P., Tan, H. S. G., van Huis, A., Vantomme, P., & Eilenberg, J. (2015). 'Entomophagy': an evolving terminology in need of review. *Journal of Insects as Food and Feed*, *1*(4), 293–305. https://doi.org/10.3920/jiff2015.0074

Evans, J., Müller, A., Jensen, A. B., Dahle, B., Flore, R., Eilenberg, J., & Frøst, M. B. (2016). A descriptive sensory analysis of honeybee drone brood from Denmark and Norway. *2*(4), 277–283. https://doi.org/10.3920/JIFF2016.0014

FAO. (2010). Forest insects as food: humans bite back. In *Proceedings of a workshop on Asia-Pacific resources and their potential for development, Chiang Mai, Thailand, 19-21 February, 2008*. Food and Agriculture Organization of the United Nations (FAO). http://www.doc-developpement-durable.org/file/Elevages/Insectes/edible%20forest%20insects.pdf

FAO. (2003). Food energy–Methods of analysis and conversion factors. In *Food and agriculture organization of the united nations technical workshop report (Vol. 77, pp. 8-9).* Agricultural Research Service, US Department of Agriculture. https://www.ars.usda.gov/research/publications/publication/?seqNo115=172524

FAO, IFAD, & WFP. (2015). *The state of food insecurity in the world 2015: meeting the 2015 international hunger targets: taking stock of uneven progress*. https://academic.oup.com/advances/article-abstract/6/5/623/4558110

Finke, M. D. (2007). Estimate of chitin in raw whole insects. *Zoo Biology*, *26*(2), 105–115. https://doi.org/10.1002/zoo.20123

Finke, M. D. (2015). Complete nutrient content of four species of commercially available feeder insects fed enhanced diets during growth. *Zoo Biology*, *34*(6), 554–564. https://doi.org/10.1002/zoo.21246

Florence, S. P., Urooj, A., Asha, M. R., & Rajiv, J. (2014). Sensory, physical and nutritional qualities of cookies prepared from pearl millet (*Pennisetum typhoideum*). *Journal of Food Processing & Technology*, *5*(10), 1-6.

Fombong, F. T., van Der Borght, M., & Vanden Broeck, J. (2017). Influence of freeze-drying and oven-drying post blanching on the nutrient composition of the edible insect *Ruspolia differens*. *Insects*, *8*(3), 102-115. https://doi.org/10.3390/insects8030102

Gahukar, R. T. (2011). Entomophagy and human food security. *International Journal of Tropical Insect Science*, *31*(3), 129–144. https://doi.org/10.1017/S1742758411000257

Gasior, R., & Wojtycza, K. (2016). Sense Of Smell And Volatile Aroma compounds and their role in the evaluation of the quality of products of animal origin - A review. *Annals of Animal Science*, *16*(1), 3–31. https://doi.org/10.1515/aoas-2015-0047

Gatheru, J. W., Khamis, F. M., Ombura, F. L. O., Nonoh, J., Tanga, C. M., Maina, J., Mohamed, S. A., Subramanian, S., Ekesi, S., & Fiaboe, K. K. M. (2019). Impact of processing methods on microbial load of reared and wild-caught edible crickets (*Scapsipedus icipe* and *Gryllus bimaculatus*) in Kenya. *Journal of Insects as Food and Feed*, *5*(3), 171–183. https://doi.org/10.3920/jiff2018.0042

Gere, A. (2017). Insect Based Foods a Nutritional Point of View. *Nutrition & Food Science International Journal*, *4*(2), 1–5. https://doi.org/10.19080/nfsij.2017.04.555638

Gmuer, A., Nuessli Guth, J., Hartmann, C., & Siegrist, M. (2016). Effects of the degree of processing of insect ingredients in snacks on expected emotional experiences and willingness to eat. *Food Quality and Preference*, *54*, 117–127. https://doi.org/10.1016/j.foodqual.2016.07.003

González, C. M., Garzón, R., & Rosell, C. M. (2019). Insects as ingredients for bakery goods. A comparison study of *H. illucens, A. domestica* and *T. molitor* flours. *Innovative Food Science & Emerging Technologies, 51*, 205-210.

Government of the republic of Kenya (2020). *Implementation status of the big four agenda 2018/2019*. https://monitoring.planning.go.ke/wp-content/uploads/2020/10/Big-Four-Agenda-Report-2018_19.pdf

Grabowski, N. T., & Klein, G. (2017a). Microbiology of cooked and dried edible Mediterranean field crickets (*Gryllus bimaculatus*) and superworms (*Zophobas atratus*) submitted to four different heating treatments. *Food Science and Technology International*, *23*(1), 17–23. https://doi.org/10.1177/1082013216652994

Grabowski, N. T., & Klein, G. (2017b). Microbiology of processed edible insect products – Results of a preliminary survey. *International Journal of Food Microbiology*, *243*, 103–107. https://doi.org/10.1016/j.ijfoodmicro.2016.11.005

Guclu, G., Sevindik, O., Kelebek, H., & Selli, S. (2016). Determination of volatiles by odor activity value and phenolics of cv. ayvalik early-harvest olive oil. *Foods*, *5*(3), 46–58. https://doi.org/10.3390/foods5030046

Hamerman, E. J. (2016). Cooking and disgust sensitivity influence preference for attending insect-based food events. *Appetite*, *96*, 319–326. https://doi.org/10.1016/j.appet.2015.09.029

Hassanien, M. F. R. (2013). Plant sterols and tocols profile of vegetable oils consumed in Egypt. *International Journal of Food Properties*, *16*(3), 574–585. https://doi.org/10.1080/10942912.2011.557758

Hlongwane, Z. T., Slotow, R., & Munyai, T. C. (2020). Nutritional composition of edible insects consumed in Africa: A systematic review. *Nutrients*, *12*(9), 1–28. https://doi.org/10.3390/nu12092786

Ho, L. H., & Abdul-Latif, N. W. B. (2016). Nutritional composition, physical properties, and sensory evaluation of cookies prepared from wheat flour and pitaya (*Hylocereus undatus*) peel flour blends. *Cogent Food & Agriculture*, *2*(1), 1–10. https://doi.org/10.1080/23311932.2015.1136369

Hrušková, M., & Švec, I. (2015). Cookie making potential of composite flour containing wheat, barley and hemp. *Czech Journal of Food Sciences*, *33*(6), 545–555. https://doi.org/10.17221/9/2015-CJFS

Hussein, L., Abbassy, M., Arafa, A., & Morcos, S. R. (1979). The amino acid composition and protein quality of biscuits. *Zeitschrift Für Ernährungswissenschaft*, *249*, 245–249.

Hwang, S., & Choi, S. (2015). Quality characteristics of muffins containing mealworm (*Tenebrio molitor*). *Culinary Science and Hospitality Research*, *21*(3), 104–115.

Igiehon, N. O., Babalola, O. O., Cheseto, X., & Torto, B. (2021). Effects of *Rhizobia* and *Arbuscular mycorrhizal* fungi on yield, size distribution and fatty acid of soybean seeds

grown under drought stress. *Microbiological Research*, *242*, 126640–126655. https://doi.org/10.1016/j.micres.2020.126640

Irina, I., & Mohame, G. (2012). Biological activities and effects of food processing on flavonoids as phenolic antioxidants. In M. Petre (Ed.), *Advances in Applied Biotechnology* (pp. 101–124). InTech. https://doi.org/10.5772/30690

ISO 5492 Sensory Analysis. (2008). *General Guidance for the Selection, Training and Monitoring of Assessors 2008*. ISO.

Jensen, N. H., & Lieberoth, A. (2019). We will eat disgusting foods together – evidence of the normative basis of Western entomophagy-disgust from an insect tasting. *Food Quality and Preference*, *72*, 109–115. https://doi.org/10.1016/j.foodqual.2018.08.012

Jongema, Y. (2017). World list of edible insects. *Wageningen University*, 1–75. https://doi.org/10.1049/iet-spr.2016.0169

Karimian-Khosroshahi, N., Hosseini, H., Rezaei, M., Khaksar, R., & Mahmoudzadeh, M. (2016). Effect of different cooking methods on minerals, vitamins, and nutritional quality indices of rainbow trout (*Oncorhynchus mykiss*). *International Journal of Food Properties*, *19*(11), 2471–2480. https://doi.org/10.1080/10942912.2015.1039028

KEBS. (2020). *Edible insects-specification part 1: edible insects' products.* Kenya Standard, KS 2922-1:2020. https://www.kebs.org/images/standards/public_review_standards/2020/june/dks_2922part_1_2020_edible_insects_products_pr.pdf

Kelemu, S., Niassy, S., Torto, B., Fiaboe, K., Affognon, H., Tonnang, H., Maniania, N. K., & Ekesi, S. (2015). African edible insects for food and feed: inventory, diversity, commonalities and contribution to food security. *Journal of Insects as Food and Feed*, *1*(2), 103–119. https://doi.org/10.3920/jiff2014.0016

Khan, S., Hashmi, S., & Saleem, Q. (2017). Microbial spoilage of bakery products and its control by preservatives. *International Journal of Pharmaceutical & Biological Archives*, *3*(1), 38–48.

Kiin-kabari, D. B., & Giami, S. Y. (2015). Physico chemical properties and in-vitro protein digestibility of non-wheat cookies prepared from plantain flour and bambara groundnut protein concentrate. *Journal of Food Research*, *4*(2), 78–86. https://doi.org/10.5539/jfr.v4n2p78

Kim, J., Eun, H., Kim, Y., Yang, J., Lee, S., & Hoon, Y. (2021). Development of a post-processing method to reduce the unique off-flavor of *Allomyrina dichotoma* : Yeast fermentation. *LWT*, *150*, 111940–111946. https://doi.org/10.1016/j.lwt.2021.111940

Kim, T. K., Yong, H. I., Kim, Y. B., Kim, H. W., & Choi, Y. S. (2019). Edible insects as a protein source: A review of public perception, processing technology, and research trends. *Food Science of Animal Resources*, *39*(4), 521–540. https://doi.org/10.5851/kosfa.2019.e53

Kinyuru, J. N. U. (2014). *Nutrient composition and utilization of edible termites (Macrotermes subhylanus) and grasshoppers (Ruspolia differenes) from Lake Victoria Region of Kenya* [Doctoral dissertation, Jomo Kenyatta University of Agriculture and Technology]. Jomo Kenyatta University of Science and Technology Digital Repository. http://hdl.handle.net/123456789/1407

Kinyuru, J. N. (2018). The Role of Edible Insects in Diets and Nutrition in East Africa. In A. Halloran, R. Flore, P. Vantomme, & N. Roos (Eds.), *Edible Insects in Sustainable Food Systems* (pp. 93–108). Springer. https://doi.org/10.1007/978-3-319-74011-9

Kinyuru, J. N. (2021). Oil characteristics and influence of heat processing on fatty acid profile of wild harvested termite (*Macrotermes subhylanus*) and long-horned grasshopper (*Ruspolia differens*). *International Journal of Tropical Insect Science, 41*(2), 1427-1433.

Kinyuru, J. N., Kenji, G. M., Muhoho, S. N., & Ayieko, M. (2010). Nutritional potential of longhorn grasshopper (*Ruspolia differens*) consumed in Siaya District, Kenya. *Journal of Agriculture, Science and Technology*, *12*, 32–46.

Kinyuru, J. N., Kenji, G. M., Njoroge, S. M., & Ayieko, M. (2010). Effect of processing methods on the in vitro protein digestibility and vitamin content of edible winged termite (*Macrotermes subhylanus*) and grasshopper (*Ruspolia differens*). *Food and Bioprocess Technology*, *3*, 778–782. https://doi.org/10.1007/s11947-009-0264-1

Kinyuru, J. N., Mogendi, J. B., Riwa, C. A., & Ndung'u, N. W. (2015). Edible insects-A novel source of essential nutrients for human diet: Learning from traditional knowledge. *Animal Frontiers*, *5*(2), 14–19. https://doi.org/10.2527/af.2015-0014

Klunder, H. C., Wolkers-Rooijackers, J., Korpela, J. M., & Nout, M. J. R. (2012). Microbiological aspects of processing and storage of edible insects. *Food Control*, *26*(2), 628–631. https://doi.org/10.1016/j.foodcont.2012.02.013

Kmiecik, D., Fedko, M., Rudzińska, M., Siger, A., Gramza-Michałowska, A., & Kobus-Cisowska,

J. (2021). Thermo-oxidation of phytosterol molecules in rapeseed oil during heating: The impact of unsaturation level of the oil. *Foods*, *10*(1), 50-63. https://doi.org/10.3390/foods10010050

Kouřimská, L., & Adámková, A. (2016). Nutritional and sensory quality of edible insects. *NFS Journal*, *4*, 22–26. https://doi.org/10.1016/j.nfs.2016.07.001

Kröger, T., Dupont, J., Büsing, L., & Fiebelkorn, F. (2022). Acceptance of insect-based food products in western societies: a systematic review. *Frontiers in Nutrition*, *8*, 759885.

Kröncke, N., Grebenteuch, S., Keil, C., Demtröder, S., Kroh, L., Thünemann, A. F., Benning, R., & Haase, H. (2019). Effect of different drying methods on nutrient quality of the yellow mealworm (*Tenebrio molitor* L.). *Insects*, *10*(4), 1–13. https://doi.org/10.3390/insects10040084

Kwiri, R., Winini, C., Muredzi, P., Tongonya, J., Gwala, W., Mujuru, F., & Gwala, S. T. (2014). Mopane Worm (*Gonimbrasia belina*) Utilisation, a potential source of protein in fortified blended foods in Zimbabwe: A review. *Global Journal of Science Frontier Research: D Agriculture and Veterinary*, *14*(10), 55–66.

Labu, S., Subramanian, S., Khamis, F. M., Akite, P., Kasangaki, P., Chemurot, M., Tanga, C. M., Ombura, F. L. O., & Egonyu, J. P. (2021). Microbial contaminants in wild harvested and traded edible long-horned grasshopper, *Ruspolia differens* (Orthoptera: Tettigoniidae) in Uganda. *Journal of Insects as Food and Feed*, *7*(7), 1131-1141. https://doi.org/10.3920/jiff2020.0069

Heymann, H., & Lawless, H. T. (2013). *Sensory evaluation of food: principles and practices*. Springer Science & Business Media.

Lee, S., Choi, Y., Jo, K., Kim, T., Yong, H. I., & Jung, S. (2020). Quality characteristics and protein digestibility of *Protaetia brevitarsis* larvae. *Journal of Animal Science and Technology*, *62*(5), 741–752.

Lehtovaara, V. J., Roininen, H., & Valtonen, A. (2018). Optimal temperature for rearing the edible *Ruspolia differens* (Orthoptera: Tettigoniidae). *Journal of Economic Entomology*, *111*(6), 2652-2659.

Leonard, A., Egonyu, J.P., Tanga, C.M., Kyamanywa, S., Tonnang, H.Z., Azrag, A.G., Khamis, F.M., Ekesi, S., & Subramanian, S. (2021). Predicting the current and future distribution of the edible long-horned grasshopper *Ruspolia differens* (Serville) using temperature-

dependent phenology models. *Journal of Thermal Biology*, *95*, 102786. https://doi.org/10.1016/j.jtherbio.2020.102786

Lewis, J. (2019). *Codex nutrient reference values.* FAO and WHO.

Liu, C., & Zhao, J. (2018). Insects as a Novel Food. In *Encyclopedia of Food Chemistry* (pp. 428-436). Elsevier. https://doi.org/10.1016/b978-0-08-100596-5.21782-4

Liu, M., Zhao, X., Zhao, M., Liu, X., Pang, Y., & Zhang, M. (2022). Characterization of the key aroma constituents in fried tilapia through the sensorics concept. *Foods*, *11*(4), 494–507.

Lu, X., Brennan, M. A., Serventi, L., & Brennan, C. S. (2018). Incorporation of mushroom powder into bread dough—effects on dough rheology and bread properties. *Cereal Chemistry*, *95*(3), 418–427. https://doi.org/10.1002/cche.10043

Mainley, D. (Ed.). (2011). *Technology of biscuits, crackers and cookies* (4th ed.). Woodhead Publication.

Mandal, F. B., & Dutta, M. (2022). The potential of entomophagy against malnutrition and ensuring food sustainability. *African Journal of Biological Sciences, 4*(4), 15-22. https://doi.org/10.33472/AFJBS.4.4.2022.15-22

Mancini, S., Moruzzo, R., Riccioli, F., & Paci, G. (2019). European consumers' readiness to adopt insects as food. A review. *Food Research International*, *122*, 661-678.

Manditsera, F. A., Luning, P. A., Fogliano, V., & Lakemond, C. M. M. (2019). Effect of domestic cooking methods on protein digestibility and mineral bioaccessibility of wild harvested adult edible insects. *Food Research International*, *121*, 404–411. https://doi.org/10.1016/j.foodres.2019.03.052

MarkManuel, D. P., & Godwin, J. (2020). Effects of Culinary Methods on The Proximate Composition of an Edible Insect (*Rhynchophorus phoenicis*) Larvae Obtained From Bayelsa State, Nigeria. *European Journal of Agriculture and Food Sciences, 2*(4), 1–7. https://doi.org/10.24018/ejfood.2020.2.4.67

Matojo, N. D., & Yarro, J. G. (2010). Variability in polymorphism and sex ratio of the conehead *Ruspolia differens* Serville (Orthoptera: Conocephalidae) in north-west Tanzania. *International Journal of Integrative Biology*, *9*(3), 131-136.

Mekonnen, B., Cheseto, X., Pirk, C., Yusuf, A., Ekesi, S., Deletre, E., & Torto, B. (2021). Re-analysis of abdominal gland volatilome secretions of the African weaver ant, *Oecophylla longinoda* (Hymenoptera Formicidae). *Molecules*, *26*(4), 871–882.

Melgar-Lalanne, G., Hernández-Álvarez, A. J., & Salinas-Castro, A. (2019). Edible insects processing: traditional and innovative technologies. *Comprehensive Reviews in Food Science and Food Safety*, *18*(4), 1166–1191. https://doi.org/10.1111/1541-4337.12463

Menéndez-Carreño, M., Ansorena, D., & Astiasarán, I. (2008). Stability of sterols in phytosterol-enriched milk under different heating conditions. *Journal of Agricultural and Food Chemistry*, *56*(21), 9997–10002. https://doi.org/10.1021/jf802000m

Menozzi, D., Sogari, G., Veneziani, M., Simoni, E., & Mora, C. (2017). Eating novel foods: An application of the Theory of Planned Behaviour to predict the consumption of an insect-based product. *Food Quality and Preference*, *59*, 27–34. https://doi.org/10.1016/j.foodqual.2017.02.001

Milanović, V., Osimani, A., Pasquini, M., Aquilanti, L., Garofalo, C., Taccari, M., Cardinali, F., Riolo, P., & Clementi, F. (2016). Getting insight into the prevalence of antibiotic resistance genes in specimens of marketed edible insects. *International Journal of Food Microbiology*, *227*, 22–28. https://doi.org/10.1016/j.ijfoodmicro.2016.03.018

Mishyna, M., Chen, J., & Benjamin, O. (2019). Sensory attributes of edible insects and insect-based foods – Future outlooks for enhancing consumer appeal. *Trends in Food Science & Technology*, *95*, 141–148. https://doi.org/10.1016/j.tifs.2019.11.016

Mmari, M. W. (2017). *Nutrient composition and consumer acceptability of soybean- sweet potato based complementary food fortified with longhorn grasshopper (Ruspolia differens)* [Doctoral dissertation, Jomo Kenyatta University of Agriculture and Technology]. Jomo Kenyatta University of Science and Technology Digital Repository. http://hdl.handle.net/123456789/3912

Mmari, M. W., Kinyuru, J. N., Laswai, H. S., & Okoth, J. K. (2017). Traditions, beliefs and indigenous technologies in connection with the edible longhorn grasshopper *Ruspolia differens* (Serville 1838) in Tanzania. *Journal of Ethnobiology and Ethnomedicine*, *13*, 1–11. https://doi.org/10.1186/s13002-017-0191-6

Mohamed, E. H. (2015). Fatty acids contents of the edible migratory locust *Locusta migratoria*, Linnaeus, 1758 (Orthoptera: Acrididae). *International Journal of Advances in Pharmacy, Biology and Chemistry*, *4*, 746–750.

Montowska, M., Rybicka, I., & Fornal, E. (2019). Nutritional value , protein and peptide composition of edible cricket powders. *Food Chemistry*, *289*, 130–138.

https://doi.org/10.1016/j.foodchem.2019.03.062

Montoya, C., Cochard, B., Flori, A., Cros, D., Lopes, R., Cuellar, T., Espeout, S., Syaputra, I., Villeneuve, P., Pina, M., Ritter, E., Leroy, T., & Billotte, N. (2014). Genetic architecture of palm oil fatty acid composition in cultivated oil palm (*Elaeis guineensis* Jacq.) compared to its wild relative *E. oleifera* (H.B.K) Cortés. *PLoS ONE*, *9*(5), 95412–95424. https://doi.org/10.1371/journal.pone.0095412

Musundire, R., Osuga, I. M., Cheseto, X., Irungu, J., & Torto, B. (2016). Aflatoxin contamination detected in nutrient and anti-oxidant rich edible stink bug stored in recycled grain containers. *PLoS ONE*, *11*(1), 1–16. https://doi.org/10.1371/journal.pone.0145914

Mutungi, C., Irungu, F. G., Nduko, J., Mutua, F., Affognon, H., Nakimbugwe, D., Ekesi, S., & Fiaboe, K. K. M. (2019). Postharvest processes of edible insects in Africa: A review of processing methods, and the implications for nutrition, safety and new products development. *Critical Reviews in Food Science and Nutrition*, *59*(2), 276–298. https://doi.org/10.1080/10408398.2017.1365330

Mwangi, K. W., Nduko, J. M., Kingori, A., Toroitich, F., & Faraj, A. (2019). Development and microbiological load of composite flours from locusts, grasshoppers and malted finger millet. *African Journal of Food Science and Technology*, *10*(1), 13–20. https://doi.org/10.14303/ajfst.2019.004

Ng'ang'a, J., Imathiu, S., Fombong, F., Ayieko, M., Vanden Broeck, J., & Kinyuru, J. (2019). Microbial quality of edible grasshoppers *Ruspolia differens* (Orthoptera: Tettigoniidae): From wild harvesting to fork in the Kagera Region, Tanzania. *Journal of Food Safety*, *39*(1), 12549–12554. https://doi.org/10.1111/jfs.12549

Niu, Y., Liu, Y., & Xiao, Z. (2020). Evaluation of perceptual interactions between ester aroma components in langjiu by GC-MS, GC-O, sensory analysis, and vector model. *Foods*, *9*(2), 183–200.

Noor-Aziah, A. A., Mohamad-Noor, A. Y., & Ho, L. H. (2012). Physicochemical and organoleptic properties of cookies incorporated with legume flour. *International Food Research Journal*, *19*(4), 1539–1543. https://doi.org/10.1080/23311932.2016.1172389

Nyangena, D. N., Mutungi, C., Imathiu, S., Kinyuru, J., Affognon, H., Ekesi, S., Nakimbugwe, D., & Fiaboe, K. K. (2020). Effects of traditional processing techniques on the nutritional and microbiological quality of four edible insect species used for food and feed in East Africa.

Foods, *9*(5), 574–589. https://doi.org/10.3390/foods9050574

Odongo, W., Okia, C. A., Nalika, N., Nzabamwita, P. H., Ndimubandi, J., & Nyeko, P. (2018). Marketing of edible insects in Lake Victoria basin: The case of Uganda and Burundi. *Journal of Insects as Food and Feed*, *4*(4), 285–293. https://doi.org/10.3920/JIFF2017.0071

Ogbe, R. J., Ochalefu, D. O., Mafulul, S. G., & Olaniru, O. B. (2015). A review on dietary phytosterols: their occurrence, metabolism and health benefits. *Pelagia Research Library Asian Journal of Plant Science and Research*, *5*(4), 10–21.

Ogunlakin, G. O., Oni, V. T., & Olaniyan, S. A. (2018). Quality evaluation of biscuit fortified with edible termite (*Macrotermes nigeriensis*). *Asian Journal of Biotechnology and Bioresource Technology*, *4*(2), 1–7. https://doi.org/10.9734/ajb2t/2018/43659

Ogunwole, O. A., Yinka, A., & Ojelade, P. (2015). Proximate composition and physical characteristics of eggs from laying chickens fed different proprietary vitamin-mineral premixes under two rearing systems during storage. *International Journal of Food Science and Nutrition Engineering*, *5*(1), 59–67. https://doi.org/10.5923/j.food.20150501.08

Ojinnaka, M. C., Ofoelo, M. U., & Ezenwa, L. I. (2015). Nutritional evaluation of wheat cakes enriched with edible African termites (*Macrotermes nigeriensis*). *Agro-Science*, *12*(3), 35–42. https://doi.org/10.4314/as.v12i3.5

Okoye, J. O., Ojimelukwe, P., & Ukom, A. N. (2016). Amino acid composition and protein quality of wheat flour biscuits fortified with soybean and bambara groundnut flours. *Global Journal of Science Frontier Research: D Agriculture and Veterinary*, *3*(4), 8–19.

Olamide, A., Samuel, O., Adeniyi, V., & Taiwo, S. (2020). Silkworm pupae (*Bombyx mori*) and locusts as alternative protein sources for high-energy biscuits. *Journal of Asia-Pacific Entomology*, *23*(1), 234–241. https://doi.org/10.1016/j.aspen.2020.01.003

Omoba, O. S., & Omogbemile, A. (2013). Physicochemical properties of sorghum biscuits enriched with defatted soy flour. *British Journal of Applied Science & Technology*, *3*(4), 1246–1256.

Oonincx, D. G. A. B., & Finke, M. D. (2020). Nutritional value of insects and ways to manipulate their composition. *Journal of Insects as Food and Feed*, *7*(5), 639–659. https://doi.org/10.3920/jiff2020.0050

Osimani, A., Milanović, V., Cardinali, F., Roncolini, A., Garofalo, C., Clementi, F., Pasquini, M., Mozzon, M., Foligni, R., Raffaelli, N., Zamporlini, F., & Aquilanti, L. (2018). Bread enriched

with cricket powder (*Acheta domesticus*): A technological, microbiological and nutritional evaluation. *Innovative Food Science and Emerging Technologies*, *48*, 150–163. https://doi.org/10.1016/j.ifset.2018.06.007\

Parniakov, O., Mikhrovska, M., Wiktor, A., Alles, M., Ristic, D., Bogusz, R., Nowacka, M., Devahastin, S., Mujumdar, A., Heinz, V. and Smetana, S. (2022). Insect processing for food and feed: A review of drying methods. *Drying Technology*, *40*(8), 1500-1513.

R Core Team. (2020). *A language and environment for statistical computing* (Version 1.3.1093–1) [Computer software]. R Foundation for Statistical Computing.

Raba, D. N., Chambre, D. R., Copolovici, D. M., Moldovan, C., & Copolovici, L. O. (2018). The influence of high-temperature heating on composition and thermo-oxidative stability of the oil extracted from Arabica coffee beans. *PLoS ONE*, *13*(7), 200314–200326. https://doi.org/10.1371/journal.pone.0200314

Ramashia, S. E., Tangulani, T., Mashau, M. E., & Nethathe, B. (2020). Microbiological quality of different dried insects sold at Thohoyandou open market, South Africa. *Food Research*, *4*, 2247-2255.

Réhault-Godbert, S., Guyot, N., & Nys, Y. (2019). The golden egg: Nutritional value, bioactivities, and emerging benefits for human health. *Nutrients*, *11*(3), 684–709. https://doi.org/10.3390/nu11030684

Roe, M., Pinchen, H., Church, S., & Finglas, P. (2013). *Nutrient analysis of eggs: Analytical Report*.https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/167973/Nutrient_analysis_of_eggs_Analytical_Report.pdf

Rumpold, B. A., & Schlüter, O. K. (2013). Nutritional composition and safety aspects of edible insects. *Molecular Nutrition and Food Research*, *57*(5), 802–823. https://doi.org/10.1002/mnfr.201200735

Rumpold, B., & Schlüter, O. (2013). Potential and challenges of insects as an innovative source for food and feed production. *Innovative Food Science and Emerging Technologies*, *17*, 1–11. https://doi.org/10.1016/j.ifset.2012.11.005

Sabolová, M., Adámková, A., Kouřimská, L., Chrpová, D., & Pánek, J. (2016). Minor lipophilic compounds in edible insects. *Potravinarstvo*, *10*(1), 400–406. https://doi.org/10.5219/605

Sainan, H., & Kapute, F. (2017). Effect of cooking method on proximate and mineral composition of Lake Malawi tilapia (*Oreochromis karongae*). *African Journal of Food, Agriculture,*

Nutrition and Development, *17*(4), 12589–12599. https://doi.org/10.18697/ajfand.80.16715

Schlüter, O., Rumpold, B., Holzhauser, T., Roth, A., Vogel, R. F., Quasigroch, W., Vogel, S., Heinz, V., Jäger, H., Bandick, N., Kulling, S., Knorr, D., Steinberg, P., & Engel, K. H. (2017). Safety aspects of the production of foods and food ingredients from insects. *Molecular nutrition & food research*, *61*(6), 1600520. https://doi.org/10.1002/mnfr.201600520

Sengendo, F., Subramanian, S., Chemurot, M., Tanga, C. M., & Egonyu, J. P. (2021). Efficient harvesting of safe edible grasshoppers: evaluation of modified drums and light-emitting diode bulbs for harvesting *Ruspolia differens* (Orthoptera: Tettigoniidae) in Uganda. *Journal of Economic Entomology*, *114*(2), 676-683.

Shadung, K. G., Mphosi, M. S., & Mashela, P. W. (2012). Influence of drying method and location on proximate chemical composition of African metallic wood boring beetle, *Sternocera orissa* (Coleoptera: Buprestidae) in Republic of South Africa. *African Journal of Food Science*, *7*(46), 6130–6135. https://doi.org/10.5897/ajfs11.146

Skotnicka, M., Karwowska, K., Kłobukowski, F., Borkowska, A., & Pieszko, M. (2021). Possibilities of the development of edible insect-based foods in Europe. *Foods*, *10*(4), 766.

Sogari, G., & Mora, C. (Eds.). (2019). *Edible Insects in the Food Sector*. Springer Nature.

Soni, N., Kulkarni, A. S., & Patel, L. (2018). Studies on development of high protein cookies. *Int. J. Chem. Stud*, *6*, 439-444.

Ssepuuya, G., Mukisa, I. M., & Nakimbugwe, D. (2017). Nutritional composition, quality, and shelf stability of processed *Ruspolia nitidula* (edible grasshoppers). *Food Science and Nutrition*, *5*(1), 103–112. https://doi.org/10.1002/fsn3.369

Ssepuuya, G., Nakimbugwe, D., De Winne, A., Smets, R., Claes, J., & Van Der Borght, M. (2020). Effect of heat processing on the nutrient composition, colour, and volatile odour compounds of the long-horned grasshopper *Ruspolia differens* Serville. *Food Research International*, *129*, 108831–108841. https://doi.org/10.1016/j.foodres.2019.108831

Ssepuuya, G., Smets, R., Nakimbugwe, D., Van Der Borght, M., & Claes, J. (2019). Nutrient composition of the long-horned grasshopper *Ruspolia differens* Serville: Effect of swarming season and sourcing geographical area. *Food Chemistry*, *301*, 125305–125313. https://doi.org/10.1016/j.foodchem.2019.125305

Ssepuuya, G., Wynants, E., Verreth, C., Crauwels, S., Lievens, B., Claes, J., Nakimbugwe, D., & Van Campenhout, L. (2019). Microbial characterisation of the edible grasshopper *Ruspolia*

differens in raw condition after wild-harvesting in Uganda. *Food Microbiology*, *77*, 106–117. https://doi.org/10.1016/j.fm.2018.09.005

Starowicz, M. (2021). Analysis of volatiles in food products. *Separations*, *8*(9), 157–170.

Stoops, J., Crauwels, S., Waud, M., Claes, J., Lievens, B., & Van Campenhout, L. (2016). Microbial community assessment of mealworm larvae (*Tenebrio molitor*) and grasshoppers (*Locusta migratoria* Migratorioides) sold for human consumption. *Food Microbiology*, *53*, 122–127. https://doi.org/10.1016/j.fm.2015.09.010

Stull, V. J. (2021). Impacts of insect consumption on human health. *Journal of Insects as Food and Feed*, *7*(5), 695–713. https://doi.org/10.3920/JIFF2020.0115

Thanh, T. T., Vergnes, M. F., Kaloustian, J., El-Moselhy, T. F., Amiot-Carlin, M. J., & Portugal, H. (2006). Effect of storage and heating on phytosterol concentrations in vegetable oils determined by GC/MS. *Journal of the Science of Food and Agriculture*, *86*(2), 220–225. https://doi.org/10.1002/jsfa.2322

Tiencheu, B., Womeni, H. M., Linder, M., Mbiapo, F. T., Villeneuve, P., Fanni, J., & Parmentier, M. (2013). Changes of lipids in insect (*Rhynchophorus phoenicis*) during cooking and storage. *European Journal of Lipid Science and Technology*, *115*(2), 186–195. https://doi.org/10.1002/ejlt.201200284

Traksele, L., Speiciene, V., Smicius, R., Alencikiene, G., Salaseviciene, A., Garmiene, G., Zigmantaite, V., & Grigaleviciute, R. (2021). Investigation of in vitro and in vivo digestibility of black soldier fly (*Hermetia illucens* L.) larvae protein. *Journal of Functional Foods*, *79*, 104402–104408. https://doi.org/10.1016/j.jff.2021.104402

Turfani, V., Narducci, V., Durazzo, A., Galli, V., & Carcea, M. (2017). Technological, nutritional and functional properties of wheat bread enriched with lentil or carob flours. *LWT - Food Science and Technology*, *78*, 361–366. https://doi.org/10.1016/j.lwt.2016.12.030

Tzompa-Sosa, D. A., Yi, L., Van Valenberg, H. J. F., & Lakemond, C. M. M. (2019). Four insect oils as food ingredient : physical and chemical characterisation of insect oils obtained by an aqueous oil extraction. *Journal of Insects as Food and Feed*, *5*(4), 279–292. https://doi.org/10.3920/JIFF2018.0020

United Nations. (2017). *Sustainable development goals report 2017*. United Nations Publications. https://unstats.un.org/sdgs/files/report/2017/thesustainabledevelopmentgoalsreport2017.pdf

van der Spiegel, M., Noordam, M. Y., & van der Fels-Klerx, H. J. (2013). Safety of novel protein

sources (insects, microalgae, seaweed, duckweed, and rapeseed) and legislative aspects for their application in food and feed production. *Comprehensive Reviews in Food Science and Food Safety*, *12*(6), 662–678. https://doi.org/10.1111/1541-4337.12032

van Huis, A., Klunder, H., Van Itterbeeck, J., Merten, E., Halloran, A., Muir, G., & Vantomme, P. (2013). *Edible insects: Future prospects for food and feed security*. Food and Agriculture Organization of the United Nations. https://doi.org/10.1017/CBO9781107415324.004

van Huis, Arnold, & Oonincx, D. G. A. B. (2017). The environmental sustainability of insects as food and feed. A review. *Agronomy for Sustainable Development*, *37*(5), 43–56. https://doi.org/10.1007/s13593-017-0452-8

Vandeweyer, D., Lenaerts, S., Callens, A., & Van Campenhout, L. (2017). Effect of blanching followed by refrigerated storage or industrial microwave drying on the microbial load of yellow mealworm larvae (*Tenebrio molitor*). *Food Control*, *71*, 311–314. https://doi.org/10.1016/j.foodcont.2016.07.011

Vandeweyer, D., Lievens, B., & Van Campenhout, L. (2015, September). *Microbial quality of edible insects reared on industrial scale in Belgium and the Netherlands* [Paper presentation]. Innovations in Food Packaging, Shelf Life and Food Safety. Munich, Germany.

Vispute, P., & Dabhade, S. (2018). Refining of palm oil: A review on palm oil refining process, 3- MCPD esters in refined palm oil, and possible reduction tactics for 3-MCPD esters. *International Journal of Agricultural Engineering*, *11*, 81–85. https://doi.org/10.15740/has/ijae/11.sp.issue/81-85

Wang, X., Gao, W., Zhang, J., Zhang, H., Li, J., He, X., & Ma, H. (2010). Subunit , amino acid composition and in vitro digestibility of protein isolates from Chinese kabuli and desi chickpea (*Cicer arietinum* L.) cultivars. *Food Research International*, *43*(2), 567–572. https://doi.org/10.1016/j.foodres.2009.07.018

Wendin, K. M. E., & Nyberg, M. E. (2021). Factors influencing consumer perception and acceptability of insect-based foods. *Current Opinion in Food Science*, *40*, 67–71. https://doi.org/10.1016/j.cofs.2021.01.007

Wu, K., Li, W., Song, J., & Li, T. (2015). Production, purification, and identification of cholest-4-en-3-one produced by cholesterol oxidase from *Rhodococcus sp*. in Aqueous/Organic Biphasic System. *Biochemistry Insights*, *8*, 1–8. https://doi.org/10.4137/bci.s21580

Yadav, N., Kaur, D., Malaviya, R., Singh, M., Fatima, M., & Singh, L. (2018). Effect of thermal

and non-thermal processing on antioxidant potential of cowpea seeds. *International Journal of Food Properties*, *21*(1), 437–451. https://doi.org/10.1080/10942912.2018.1431659

Yang, D. S., Shewfelt, R. L., Lee, K. S., & Kays, S. J. (2008). Comparison of odor-active compounds from six distinctly different rice flavor types. *Journal of Agricultural and Food Chemistry*, *56*(8), 2780–2787.

Zielińska, E., Baraniak, B., Karaś, M., Rybczyńska, K., & Jakubczyk, A. (2015). Selected species of edible insects as a source of nutrient composition. *Food Research International*, *77*, 460–466. https://doi.org/10.1016/j.foodres.2015.09.008

APPENDICES

Appendix A: Sensory Evaluation Questionnaire

Panelist Code: _____

Date: __ __ 2021

A) CONSENT FORM

You are invited to participate in a research study on the perception of an insect-based bakery product. Keenly read through this form and ask any questions for clarity before agreeing to be enrolled in this study. This exercise is purely voluntary thus, promptly notify us of any allergenicity or intolerance to insect-based food products so that you are excluded from this study. The results of your assessment as a panelist will be kept strictly confidential. Kindly fill in your details in the section below.

I, (Name)……., have read the information pertaining to my involvement in this study and comfortably confirm that my concerns have been addressed to satisfaction. I hereby give my voluntary consent for participation in this study.

Gender

Male	**[ ]**	Female	**[ ]**

Age Bracket

Less or equal to 20	**[ ]**	21-25	**[ ]**
26-30	**[ ]**	31-35	**[ ]**
36-40	**[ ]**	41 and above	**[ ]**

Signature : _____

B) SENSORY EVALUATION

INSTRUCTIONS

You have been provided with **five (5) coded** samples of insect-based cookies. Please take a sip of water to cleanse your palate before and after tasting each sample. Taste the samples and hold in the mouth while chewing for 5 sec. Please look and taste each of the (6) coded cookies samples. Rate the attributes of each coded sample against the scale of 1-5 provided below. Each number in the scale denotes the degree of likeness. Put the appropriate number in the table against each attribute with reference to the scale below:

- 5- Like extremely
- 4 - Like
- 3- Neither like nor dislike
- 2- Dislike
- 1-Dislike extremely

Attributes	**Sample Codes**				
	ITS	**ILC**	**IBL**	**IBC**	**IDF**
Colour					
Flavour					
Mouthfeel					
Texture					
Overall Acceptability					

Additional comments:

……
……
……

Thank you for participating in the study.

Appendix B: Abstract of Publications

1st Publication

Food Chemistry 383 (2022) 132397

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Dynamics in nutrients, sterols and total flavonoid content during processing of the edible Long-Horned grasshopper (*Ruspolia differens* Serville) for food

Brian O. Ochieng [a,b,*], Joseph O. Anyango [b], John M. Nduko [b], Xavier Cheseto [a], Cynthia M. Mudalungu [a], Fathiya M. Khamis [a], Changeh J. Ghemoh [d], Peter J. Egonyu [a], Sevgan Subramanian [a], Dorothy Nakimbugwe [c], Geoffrey Ssepuuya [c], Chrysantus M. Tanga [a,*]

[a] International Centre of Insect Physiology and Ecology (icipe), P.O. BOX 30772 – 00100, Nairobi, Kenya
[b] Department of Dairy and Food Science and Technology, Egerton University, P.O. Box 536 – 20115, Njoro, Kenya
[c] Department of Food Technology and Nutrition, School of Food Technology, Nutrition and Bioengineering, Makerere University, P.O. Box 7062, Kampala, Uganda
[d] Centre for African Bio-Entrepreneurship (CABE), P.O. Box 25535-00603, Lavington, Nairobi, Kenya

ARTICLE INFO

Keywords:
Ruspolia differens
Tettigoniidae
Post-harvest processing
Total flavonoid
Sterols
Nutrients
Food and nutritional security

ABSTRACT

Long-horned grasshopper (*Ruspolia differens* Serville) is a tasty delicacy in over 20 African countries. This study evaluated the impact of diverse post-harvest thermal treatment (blanching, boiling, toasting, and deep-frying) on the nutrients, total flavonoid content and sterols preservation of *R. differens* products. Crude protein, ash, and fibre of *R. differens* was drastically reduced by deep-frying technique. There was increase in Omega-3 (α-linolenic acid), Omega-6 fatty acid (linoleic and arachidonic acids) and sterols [(22*Z*)-27-Norergosta-5,22-dien-3β-ol, cholesterol, campesterol, cholest-4-ene-3-one and β-sitosterol] and flavonoids (2–3 folds) during blanching compared to other techniques. The iron and zinc content increased significantly in blanched and boiled products of *R. differens*. Thus, losses of nutrients, total flavonoid content and sterols during processing of *R. differens* for food can be mitigated by employing blanching technique, which is cheaper and least time-consuming. The implications of these dietary and therapeutic compounds on human nutrition and health are discussed.

2nd Publication

LWT - Food Science and Technology 184 (2023) 115012

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Nutritional characteristics, microbial loads and consumer acceptability of cookies enriched with insect (*Ruspolia differens*) meal

Brian O. Ochieng [a, b, **], Joseph O. Anyango [b], Fathiya M. Khamis [a], Sunday Ekesi [a], James Peter Egonyu [a], Sevgan Subramanian [a], John M. Nduko [b], Dorothy Nakimbugwe [c], Xavier Cheseto [a], Chrysantus M. Tanga [a, *]

[a] International Centre of Insect Physiology and Ecology (icipe), P.O. BOX 30772, 00100, Nairobi, Kenya
[b] Department of Dairy and Food Science and Technology, Egerton University, P.O. Box 536, 20115, Njoro, Kenya
[c] Department of Food Technology and Nutrition, School of Food Technology, Nutrition and Bioengineering, Makerere University, P.O. Box 7062, Kampala, Uganda

ARTICLE INFO

Keywords:
Edible grasshoppers
Ruspolia differens processing
Microbial quality
Nutritional composition
Safe cookie products

ABSTRACT

Utilization of *Ruspolia differens* Serville as functional food ingredient is rapidly gaining popularity. This study evaluated the nutrient quality, microbial safety and consumers' acceptability of cereal-based cookies fortified with various processed products of *R. differens* meals. Cookies fortified with blanched, boiled, and toasted *R. differens* meals had higher protein, fat and energy levels, respectively, than the control cookies. Enrichment of cookies with differentially processed *R. differens* meals had elevated levels of isoleucine and leucine. Omega-3 fatty acid, methyl (9Z,12Z,15Z)-octadecatrienoate, was detected only in cookies prepared from wheat-insect meals blends. Blanched and boiled *R. differens* meal significantly ($p < 0.05$) boosted iron (1.70-folds) and zinc (1.12–1.16-folds) contents of the cookies. The cookie products had reduced *Enterobacteriaceae*, *S. aureus*, yeast and mould with permissible exposure limits for human consumption. The overall acceptability of insect-enriched cookie product by male and female respondents ranged between 57 and 80%. The survey revealed that the flavour, colour, mouthfeels and texture of the cookie products were important motivation for consumers to accept grasshoppers as a food source. Further research on the flavour of cookie products enriched with grasshopper meal would be required to increase acceptability to market-driven consumer appealing food products.

3rd Publication

www.nature.com/scientificreports

scientific reports

Check for updates

OPEN

Aroma characterization and consumer acceptance of four cookie products enriched with insect (*Ruspolia differens*) meal

Brian O. Ochieng[1,2✉], Joseph O. Anyango[2], John M. Nduko[2], Cynthia M. Mudalungu[1], Xavier Cheseto[1] & Chrysantus M. Tanga[1✉]

This research aims to advance knowledge on the impact of four processing methods on volatile compounds from insect-based baked products (cookies) to provide insights on consumer acceptance. Samples were exposed to double step enzyme digestive test, volatiles characterized through headspace analysis, while semi-trained panelists were recruited for the sensory test. Blanched and boiled samples of *R. differens* had considerably higher digestibility (83.42% and 81.61%, respectively) ($p < 0.05$) than toasted and deep-fried samples. Insect-based cookie products integrated with blanched and boiled *R. differens* meal expressed higher digestibility (80.41% and 78.73%, respectively) that was comparable to that of commercial cookie products (control cookies-CTRC with 88.22%). Key volatile compounds common between the various cookie products included, nonanal, octanal, methyl-pyrazine, hexanal, tetradecane, 2-pentylfuran, 2-heptanone, 2*E*-octenal, 2*E*-heptenal and dodecane. Among the volatile compounds, pleasant aromas observed were 2*E*,4*E*-dodecadienal, pentanal, octanal, methyl pyrazine, furfurals, benzaldehyde, and 2-pentyl furan, which were more pronounced in cookies fortified with boiled, toasted and deep-fried *R. differens* meal. There was a greater resemblance of sensory characteristics between control cookies and those fortified with deep-fried *R. differens*. These findings underscore the significant influence of aroma compounds on consumer acceptability and preference for insect-based baked food products, which allows for future process-modification of innate aromas of insect-based meals to produce high-valued pleasant consumer driven market products.

Appendix C: Research Permit

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: 505234

Date of Issue: 25/January/2021

RESEARCH LICENSE

This is to Certify that Mr.. Brian Onyango Ochieng of Egerton University, has been licensed to conduct research in Nairobi on the topic: Nutritional Value, Microbial Quality, Shelf-life and Consumer Acceptability of Cookies Enriched with Rutpolita different Flours from Different Processing Methods for the period ending : 25/January/2022.

License No: NACOSTI/P/21/8303

505234

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

Appendix D: Samples of Statistical Data Analysis Output

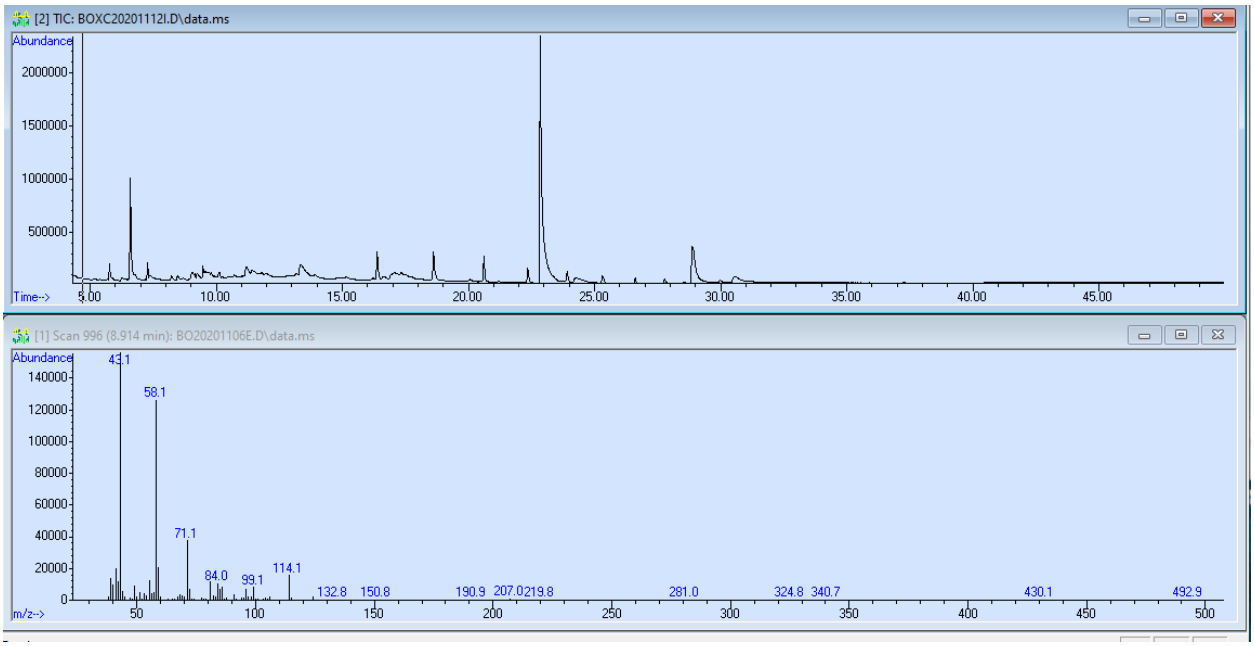
R Studio Output for amino acid Tyrosine

```
> model3<-lm(Tyr~Sample, data=aa.cookies) #Tyrosine
> Anova(model3)
Anova Table (Type II tests)

Response: Tyr
           Sum Sq Df F value    Pr(>F)
Sample    1.62476  4  28.077 2.043e-05 ***
Residuals 0.14467 10
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> marginal = lsmeans(model3, ~ Sample)
> pairs(marginal, adjust="tukey")
 contrast    estimate     SE df t.ratio p.value
 BCRC - BLRC  0.00682 0.0982 10  0.069  1.0000
 BCRC - CTRL  0.77553 0.0982 10  7.897  0.0001
 BCRC - DFRC  0.61389 0.0982 10  6.251  0.0007
 BCRC - TSRC  0.09754 0.0982 10  0.993  0.8526
 BLRC - CTRL  0.76870 0.0982 10  7.827  0.0001
 BLRC - DFRC  0.60707 0.0982 10  6.181  0.0008
 BLRC - TSRC  0.09072 0.0982 10  0.924  0.8814
 CTRL - DFRC -0.16164 0.0982 10 -1.646  0.5039
 CTRL - TSRC -0.67799 0.0982 10 -6.904  0.0003
 DFRC - TSRC -0.51635 0.0982 10 -5.258  0.0026

P value adjustment: tukey method for comparing a family of 5 estimates
> multcomp::cld(marginal, alpha   = 0.05, Letters = letters,  adjust  = "tukey")
Note: adjust = "tukey" was changed to "sidak"
because "tukey" is only appropriate for one set of pairwise comparisons
 Sample lsmean     SE df lower.CL upper.CL .group
 CTRL     3.41 0.0694 10     3.19     3.63  a
 DFRC     3.57 0.0694 10     3.35     3.79  a
 TSRC     4.08 0.0694 10     3.86     4.30   b
 BLRC     4.17 0.0694 10     3.96     4.39   b
 BCRC     4.18 0.0694 10     3.96     4.40   b
```

Volatile Organic Compound Peaks of Toasted *R. differens*-based cookies from Chemstation Output



R analysis of Sensory Attributes: Mean separation of Colour scores of the different cookies

```
#COMPOUNDS AND PROCESSING
attach(AnovaSensory)

#MANOVA
#packages
library(car)
library(multcompView)
library(lsmeans)


#ANOVA
model1<-lm(Colour ~Product_code, data=AnovaSensory) #
Anova(model1)
marginal = lsmeans(model1, ~ Product_code)
pairs(marginal, adjust="tukey")
multcomp::cld(marginal, alpha   = 0.05, Letters = letters,  adjust  = "tukey")

model1<-lm(Flavour ~Product_code, data=AnovaSensory) #
Anova(model1)
marginal = lsmeans(model1, ~ Product_code)
pairs(marginal, adjust="tukey")
multcomp::cld(marginal, alpha   = 0.05, Letters = letters,  adjust  = "tukey")
```

41:1 (Top Level)

Console | Terminal | Jobs

C:/Users/HP/Dropbox/DATA/COOKIES/Analyzed/Sensory Evaluation/

```
> multcomp::cld(marginal, alpha   = 0.05, Letters = letters,  adjust  = "tukey")
Note: adjust = "tukey" was changed to "sidak"
because "tukey" is only appropriate for one set of pairwise comparisons
 Product_code lsmean     SE  df lower.CL upper.CL .group
 IBL            3.68 0.0819 852     3.46     3.89  a
 IBC            3.83 0.0819 852     3.62     4.05  ab
 1RA            3.86 0.0819 852     3.64     4.08  ab
 ITS            3.92 0.0819 852     3.71     4.14  ab
 IDF            4.15 0.0819 852     3.93     4.36   bc
 ILC            4.47 0.0819 852     4.25     4.68    c

Confidence level used: 0.95
```