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COLLEGE OF BASIC AND APPLIED SCIENCES

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PROCESS OPTIMIZATION OF *ZOOM KOOM*

BY

EMMANUEL TEI-MENSAH

(10875457)

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INTEGRI PROCEDAMUS

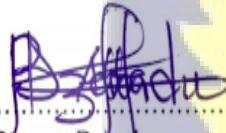
DECLARATION

I do hereby declare that this thesis is the result of my own research except for references to works of others that have been duly cited under the supervision of Dr. Bennett Dzandu, Dr. Idolo Ifie and Prof. John Owusu. This work either in whole or part has not been presented for another degree elsewhere.



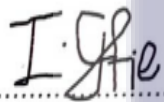
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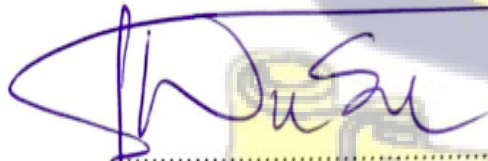
.....
Dr. Bennett Dzandu
(Supervisor)

27/04/2023
.....
Date



.....
Dr. Idolo Ifie
(Co-Supervisor)

27/04/2023
.....
Date



.....
Prof. John Owusu
(Co-Supervisor)

27/04/2023
.....
Date



DEDICATION

I dedicate this work to my mentor Mr. Ebenezer Nartey for making the completion of this MPhil. programme successful. I also thank Mr. Francis Cosmos Baiden for his support both in prayers and finances. My special thanks also go to my lovely wife Akua Nana Safo, also go to Emmanuel Tei-Mensah Junior and daughter Adwoa Akyaa Narkie Mensah for their care and understanding throughout this programme. A special dedication to my late mum, Faustina Yaa Amanfo who really sacrificed for me.



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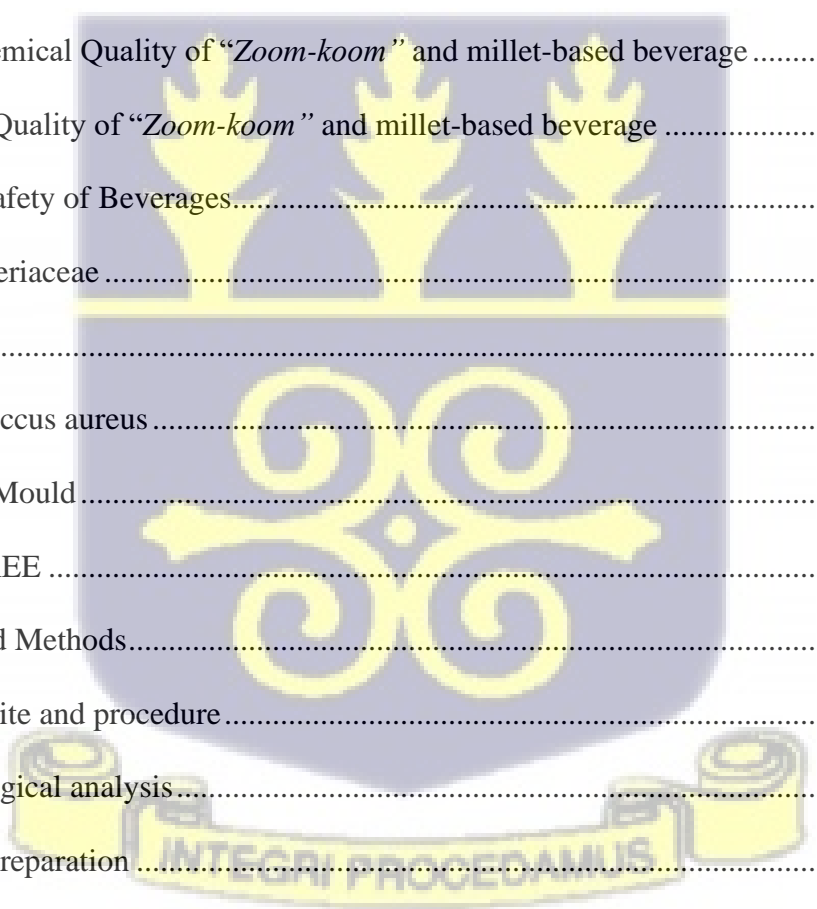
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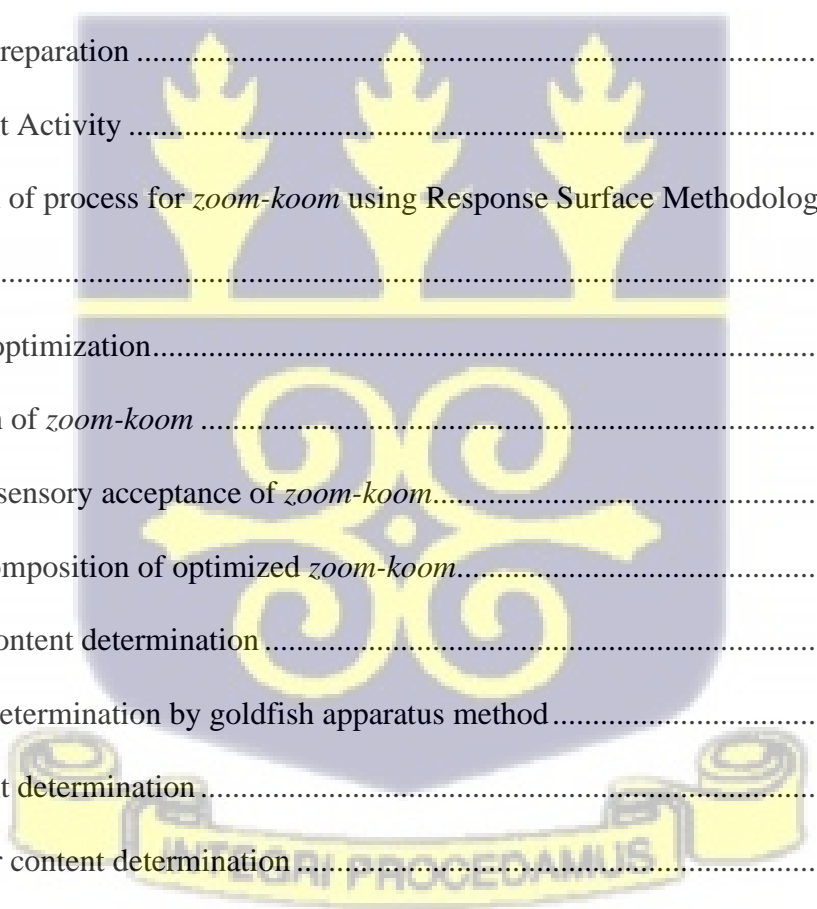
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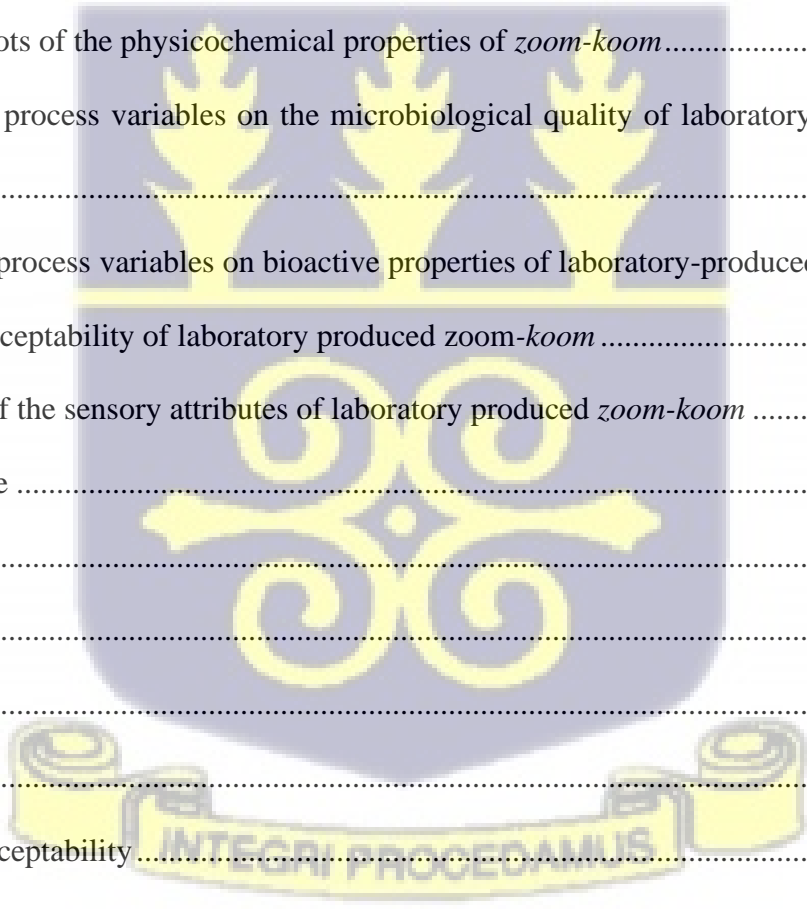
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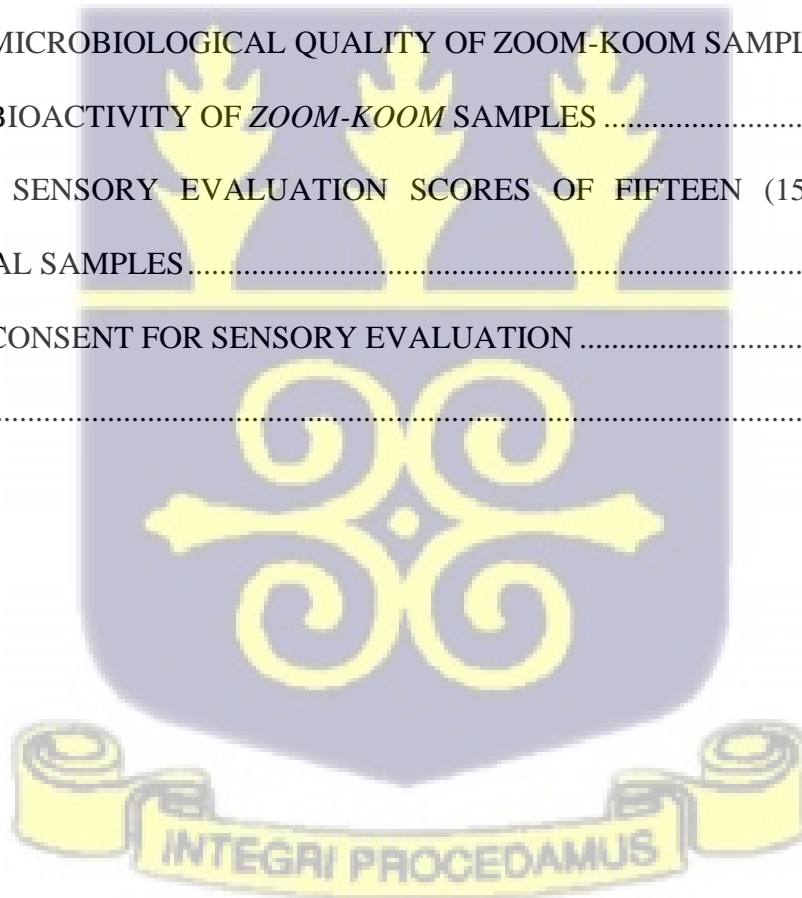
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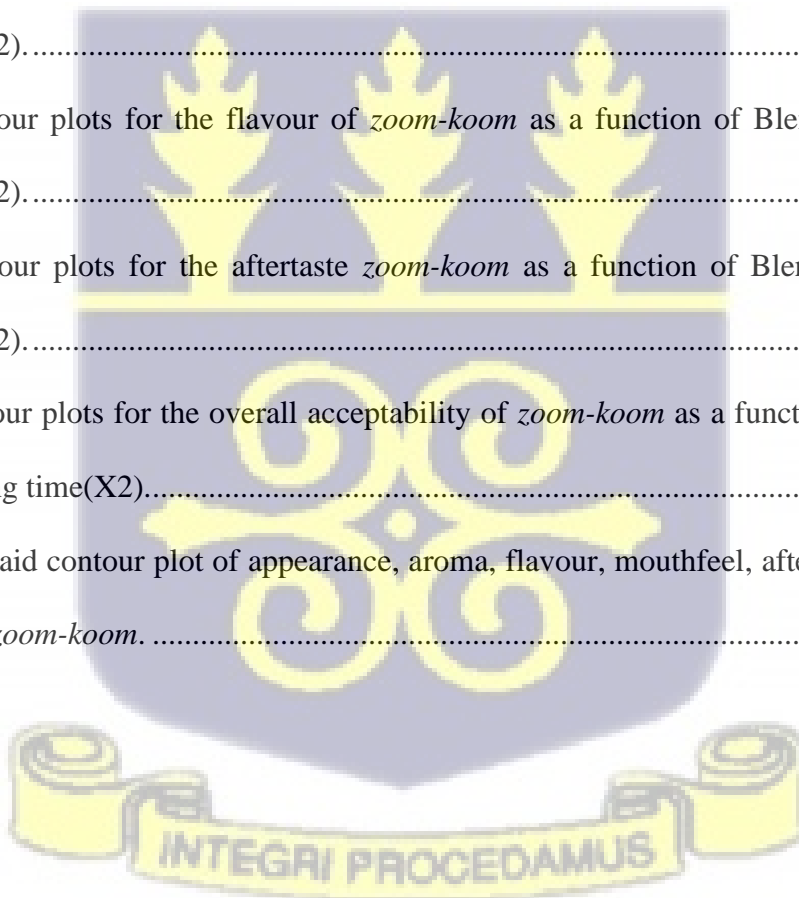
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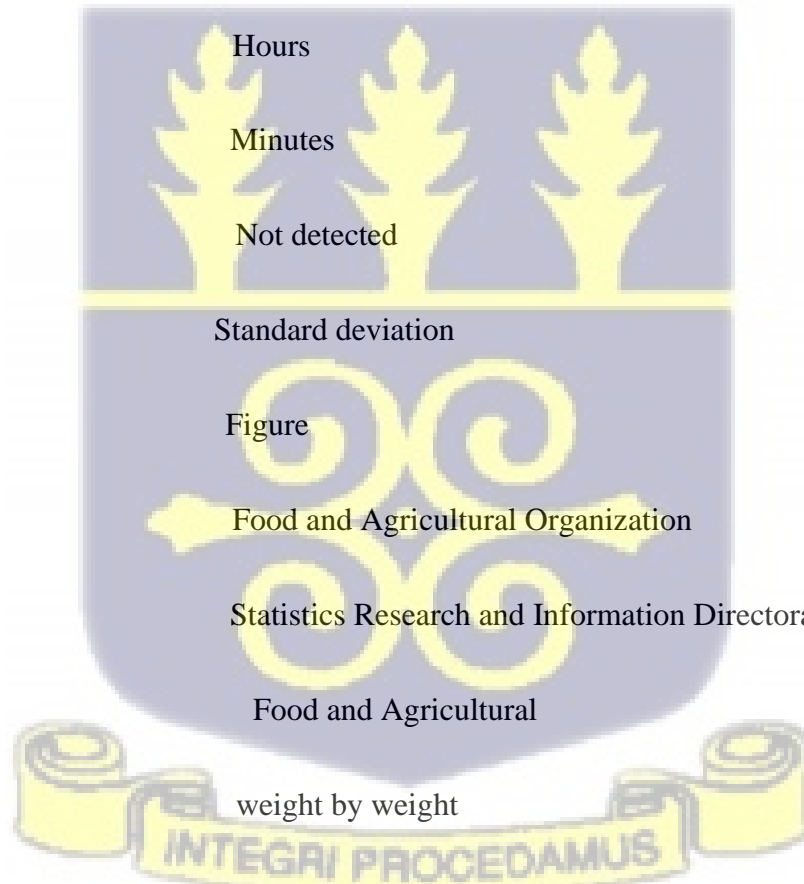
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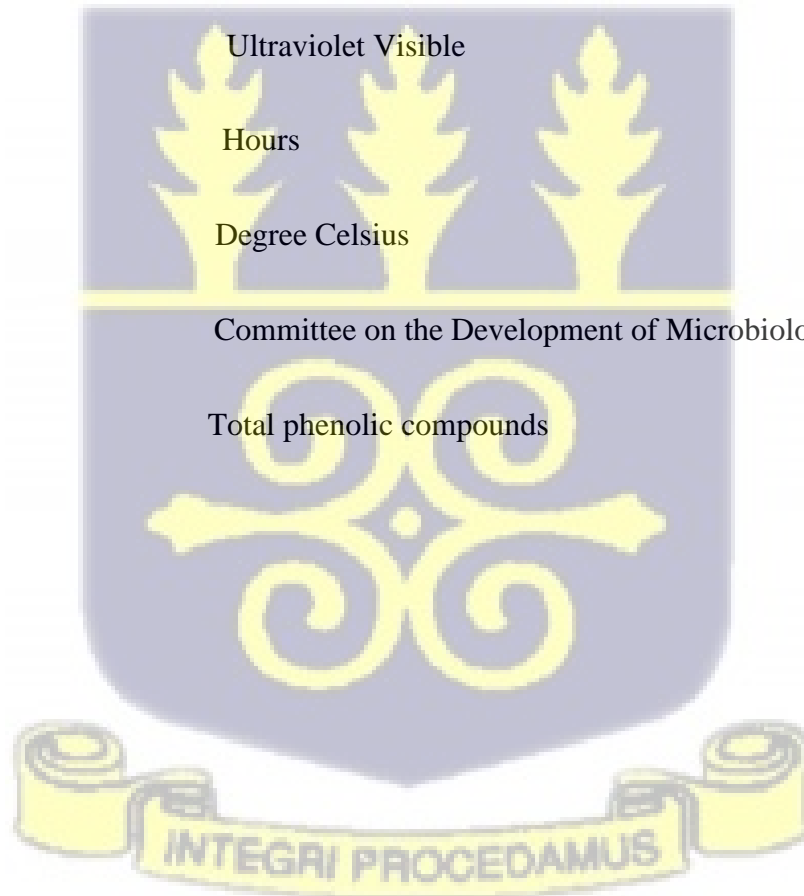


LIST OF ACRONYMS

CACS	College of Agriculture and Consumer Sciences
GSA	Ghana Standards Authority
TTA	Titrateable Acidity
cfu	Coliform forming unit
Y&M	Yeasts and moulds
E.	Escherichia
Hrs	Hours
Min	Minutes
N.D	Not detected
SD	Standard deviation
Fig.	Figure
FAO	Food and Agricultural Organization
SRID-MOFA	Statistics Research and Information Directorate-Ministry of Food and Agricultural
w/w	weight by weight
CGIAR	Consultative Group on International Agricultural Research
USDA	United State Department of Agriculture



GDP	Gross Domestic Product
USCDC	United States Centre for Disease Control
µg/ml	Microgram per millilitres
mg/ml	Milligram per millilitres
P.E. T	Polyethylene terephthalate
ANOVA	Analysis of Variances
DPPH	2,2-Diphenylpicrylhydrazyl
UV-Vis	Ultraviolet Visible
H	Hours
°C	Degree Celsius
CECMA	Committee on the Development of Microbiological Criteria
TPC	Total phenolic compounds



ABSTRACT

The production process traditional beverages such as *zoom-koom* are usually characterized by lack of standardization, inconsistency, inefficiency and unsanitary conditions. A detailed analysis of *zoom-koom* and its production can help to optimize the process that will ensure the production of the product with reliable quality to meet consumer demand. This study aimed at determining *zoom-koom* product and its consumer acceptability to guide the standardization and optimization of the traditional production process. A Box - Behnken design was used to optimize the production process. Blend ratio of spices and steeped millet (700:50, 700: 100 and 700:150) and steeping time (2, 7 and 12 hrs) and steeping temperature (25, 35 and 45^oC) was considered for this study. An optimum region of blend ratio of spices and steeped millet (700:50 to 700:100), steeping time between (2 to 6 hrs) and steeping temperature of 35^oC was determine as optimize parameters obtain. Low counts of aerobic bacteria, yeast and mould and the absence of coliform, *E. coli* and *Staphylococcus aureus* in the experimental samples can be attributed to the sufficient hygienic measures implore during the processing. The commercially processed *zoom-koom* were acidic with pH ranging from 3.08 to 3.59. The acidity, TSS, colour (*L**, *b** and *a**) were from 0.04 to 0.09, 6.80 to 9.63, 21.27 to 26.49, 0.53-4.29 and 3.78 to 11.4, respectively. The total colour change (ΔE) ranged from 20.22 to 21.46 for the commercially produced *zoom-koom*. *E. coli*, total coliform *S. aureus* were not detected in the fifteen experimental *zoom-koom* samples. There was significant difference in the *Enterobacteriaceae* and *E. coli* counts of the commercial and experimental samples. Different experimental combinations should be explored to further optimize and standardize the traditional beverage.

CHAPTER ONE

1.0 INTRODUCTION

1.1 General Overview

Consumer awareness of the impact of diet on their health has developed a rising concern across the world. This is becoming more and more popular, especially in Africa where the majority of people want foods that reflect their traditional identities, cultural traditions and religious beliefs. There are several such foods in Ghana, depending on the location, traditional group, and tribal environment. In addition to the diversification of meals brought about by urbanization, it is challenging to designate any one particular dish as the national delicacy due to numerous preparation techniques and widespread consumption of several indigenous foods. Food, a requirement for all living things, not only gives us energy and nourishment, but also represents our attitudes and the way we think about ourselves (Roberts, 2001). It is necessary for basic level for our survival. In fact, it is a basic requirement for human survival (Baker et al., 2011). Ghana's native food come in a variety of forms, including liquid, solid, and semi-solid. The majority of Ghana's basic foods are indigenous, and the country's culture and history are strongly reflected in how they are prepared (Sefa-Dedeh, 1993). The same meal preparations in this modern day employ the same concepts and techniques as current food technology. However, the scale and application differ because they are essentially artisanal in nature. Despite their basic nature, these skills are utilized to produce a varied variety of processed and semi-processed traditional staples to fulfil the needs of consumers from diverse socio-economic classes.

Cereals as the main source of energy for humans have become increasingly popular over the world (FAO, 2020), with millet ranking among the most significant cereal grains. *Eleusine coracana* (L.), often known as millet, is a major food crop in northern Ghana and a minor cereal

crop in Ethiopia, Nigeria, Burkina Faso, and Niger is currently underutilized. Typically, the crop is grown in India, Asia, and other African nations like Ghana, Nigeria, etc. According to SRID-MoFA (2011), the majority of Ghana's millet is grown in the Northern Region. For small-scale farmers it is a significant staple food crop (Rooney & Serna-Saldivar, 2000) grown in areas that are not prone to floods or on soils with little capacity to retain water. Millet, a typical dryland crop may also tolerate harsh weather conditions (FAO, 2001). Millet can therefore be cultivated in a variety of environments, including semi-arid to sub-humid agro-ecosystems that are prone to drought (Chandra et al., 2016).



In African communities, traditional meals, especially drinks, play a significant role as they serve as a source of income for processors in both rural and urban locations (Oduro-Yeboah, 2015). *Zoom-koom* is one of Ghana's non-fermented beverages that is usually consumed in the Northern part of the country and also some parts of the capital city (Accra) especially Nima and in Koforidua in the Eastern Region. The beverage which is usually consumed in a liquid form is also native to some West African countries such as Nigeria and Burkina Faso with their different ways of producing it based on the country of origin. The beverage is commonly made from pearl millet. It is gluten-free, non-acid-forming, and most of all easy to digest with low glycemic index (Muthamilarasan et al., 2016; Manjula & Visvanathan, 2014). It also helps to control blood sugar levels since it is believed to have a low glycemic index, making it an appropriate choice for people with diabetes and celiac disease (a condition brought on by eating gluten-containing cereal proteins) (Jideani & Jideani, 2011). Sorting, washing and steeping the millet grains in water that is twice as much as their mass (2:1, w/w) are the steps in the preparation of *zoom-koom*. According to observations made at micro-workshops, the typical steeping period is 12 hours. Before wet milling, flavouring and aromatizing spices are combined with the soaked millet grains (at a rate of 3 g/100 g for mint and 6 g/100 g for ginger). A muslin fabric (0.5 mm) is then used to filter the suspension after adding three times the mass of the wet dough in water to give a very fine wort. Finally, a sugar solution is poured to the filtrate to produce a fresh *zoom-koom* (Soma et al., 2019). Traditional beverages continue to be an indispensable aspect of Ghanaian culture, but disposition towards branded beverages is progressively rising (Veitch, 2021). There is therefore the need to upgrade the quality of traditional beverages through improved processing techniques. It has been suggested that there is a correlation between a product's characteristics and its performance in terms of meeting consumers' needs and expectations (Peri, 2006). Currently, there is limited

documentation on the production methods and product characteristics of *zoom-koom* and many of such traditional products. Also, various issues including processing operations and safety are of concern and need to be addressed (Amoa-Awua *et al.*, 2007). A thorough and systematic analysis of *zoom-koom* and its production process can help produce an optimized process that will assure the production of a product with consistent quality. With the present trends in urbanization, and the increasing acceptance of traditional beverages such as *zoom-koom* among consumers, it has become necessary to address concerns with and industrially scale up *zoom-koom* production in order to achieve consistent and predictable quality. Based on these considerations, this study explored the optimization of some key processing parameters of *zoom-Koom* so as to produce *zoom-koom* that would meet both local and international consumer demand and acceptability.



1.2 Rational for the Study

Zoom-koom production, like many other traditional food processes, is considered as small-scale operations with little or no documentation on the product. Production of the beverage depends on the knowledge of the producer from handling the product over a period (Addo *et al.*, 2016). As a result, there is always a difference in the quality of *zoom-koom* produced between different processors and even between batches from the same processor. *Zoom-Koom* have become a highly consumed beverages in some parts of West Africa including Ghana where it is becoming popular (especially in the Northern Regions and in the Accra, the capital city of Ghana) due its associated health benefits (Kannan *et al.*, 2013). However, processing of *zoom-koom* is spontaneous, uncontrolled and usually made with varied steeping time, temperature and spices (Soma *et al.*, 2017). A recent study of *zoom-Koom* production using the traditional method resulted in an increase in enterobacteria counts making the final product unsafe for consumption (Soma *et al.*, 2017). This menace further reduces the possible large-scale commercialization and quality standardization of the beverage, which has been a main source of income for its producers and subsequently boost the country's economy. Therefore, optimization of the process of the production will provide a way of standardizing the product and this can also potentially improve the nutrient profile, organoleptic and sanitary quality of the *zoom-Koom*. Furthermore, bioactivity of the *zoom-Koom* beverage may be extended because of the polyphenols and terpenoids in the spices since these are known to possess antimicrobial and bioactive properties (Ojewole *et al.*, 2004). This study seeks to clearly explain how the *zoom-koom* beverage is produced in Ghana and how the production process influences the quality of the final product. Essentially, this work tackles the optimization of some key processing parameters of *zoom-koom* production such that the final product will satisfy local and international consumer demand and acceptability.

1.3 Main objective

To produce and optimize the production process for *zoom-koom* using Response Surface Methodology.

1.4 Specific Objectives

1. Evaluate the physicochemical, bioactive and microbial quality of *zoom-koom* obtained from traditional processors.
2. Optimize the *zoom-koom* production process using response surface methodology.
3. Determine the composition of the optimized *zoom-koom*.



CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Introduction

Traditional foods are prepared from fresh materials native to a specific setting, area or locality. They are prepared utilizing outmoded methods and techniques that have been handed down through the generations. According to Hamm and Bellows (2002), the right to provide one's own labour, agriculture, fisheries, food supply as well as land policies that are environmentally, socially and that is practicably suitable to one's own conditions constitutes the individual right to exercise food authority hence the widespread eating of indigenous foods leads to food sovereignty. According to Marshall and Mejia-Lorio (2012), food sovereignty refers to people's innate capabilities for obtaining and generating food, which implies that everyone has a right to foods that are both safe and healthy as well as socially and culturally acceptable. According to Shobana et al. (2013), nutrition and wellbeing are justifiable forces that maximize and improve health development of human potential energy. Comparatively, traditional foods cost less than exotic ones and this may be partly due to the fact that they are made using local food products and raw processing techniques. Sorghum, maize, and millet are indigenous crops well-known to be extremely significant and valuable essential foods in some African nations like Nigeria, Ghana, and Sudan (Taofeek et al., 2014). Depending on the region and culture, they are used to make a range of meals, including "tuo-zafi" (a dish from Ghana), porridge, various baked products and drinks (both alcoholic and non-alcoholic).

2.2 'Zoom-Koom' (millet beverage)

Zoom-koom is a non-alcoholic drink, made from millet and rarely from sorghum, and much valued by consumers in Burkina Faso, Ghana and Nigeria. In Ghana, *zoom-koom* is thought to have originated from the Northern Region. Due to the traditional nature of its processing, the beverage is not produced on large scale, it is not pre-packaged and only few processors are into its commercial production. Traditional processors commonly produce the beverage on a small scale and sell it in plastic containers mixed with ice cubes to keep the beverage chilled. The beverage is usually served upon purchase into packaging materials such as clear polyethene bags and Polyethylene terephthalate bottles. Flavourings such as vanilla flavour, are commonly added to the beverage upon request. The beverage is consumed at any time of the day but usually consumed in the afternoon. It also usually served to farmers in the Northern region before lunch time. The *zoom-koom* beverage is sold at affordable prices to consumers considering the value of the end-product. Traditional beverages like *zoom-koom* have socio-cultural relevance in Ghana. In recent times there has been a rapid increase in the patronage of common traditional beverages such as *zoom-koom*, *aliha*, *sobolo*, palm wine at ceremonies and occasions such as weddings, engagement, naming ceremonies, funerals and parties. Classically, at occasions, the *zoom-koom* beverage is served in dispensers. For the traditional processing of *zoom-koom*, millet (pearl) is first sorted, cleaned and then steeped in water. The steeped grains are finely ground upon addition of spices such as ginger, cloves and black pepper, then water is added, it is then mashed and filtered to obtain the wort. An approximately amount of sugar is added to it to obtain fresh *zoom-koom*.

In Ghana, there are other common traditional millet beverages similar to *zoom-koom* such as *Frofro* and *Fura*. *Frofro* and *Fura* are thought to have originated from the Northern Region of

Ghana. A distinguished difference between *Frofro* and *Fura* is the nature of their end product after processing. *Fura* needs to be mixed with water after processing whereas the latter is always in a liquid form even after processing. The unit operations involved in the processing of this traditional millet beverage include steeping, wet milling, wort extraction after which sugar is added to the wort (Soma et al.,2014). The existing documented knowledge on this traditional millet beverages in Nigeria and Burkina have only focused on their physico-chemical and microbiological quality. Soma et al., (2020) conducted research focused on evaluating the microbial quality of *zoom-koom* sold in some selected schools, health centres and markets in Ouagadougou in Burkina Faso. Results showed that most of the commercial *zoom-koom* samples collected from schools and health centres in twelve (12) different districts were found not have met the microbiological quality criteria for enterobacteria and yeasts and moulds.

Soma et al. (2020) studied the microbes associated with spoilage in *zoom-koom* during storage. The study showed that, *Lactobacillus plantarum subsp. plantarum* *Pediococcus pentosaceus*, and *Lactobacillus fermentum* were linked to *zoom-koom*. Until now limited research has focused on characterizing and optimizing the process of producing *zoom-koom* as well as determining its nutrient profile.

2.2.1 Processing of Zoom-koom

2.2.1.1 Steeping/Soaking

Zoom-koom is produced from the Pearl type of millet or the Finger type of millet. Steeping or soaking of the millet grains is the first stage in the process of making *zoom-koom*. Depending on the processor, the step's duration and conditions might vary greatly, which has an impact on the value of the final product. According to the processor, the grains are traditionally soaked in an

amount of water for a few hours until an acceptable moisture level is obtained. This, in the opinion of Woonton et al. (2005), helps in the elimination of specific colours, bacteria, and chemicals that can result in bitterness in the chosen grains. To get rid of any dirt in the soaked millet grains, it is subsequently sieved. Traditional *zoom-koom* preparation involves physically sorting and lightly cleaning the grains before steeping them in large bowls, buckets, or pots that may or may not be covered. As a result, there is a good chance that foreign objects including stones, sand, hair, mice, insects, and other debris will be found in the grains during steeping.

2.2.1.2 Wort extraction

Following a number of operation units, wet milling is done followed by the preparation of the wort and the extraction. A combination of the steeped millet grain, spices (such as ginger, cloves, and black pepper), and water is added in a ratio according to the processor. The finished combination is given some time to stand. The mixture's insoluble components settle to the bottom and is subsequently filtered. Most people refer to this as "mashing" (Glover, 2007). Different varieties of *zoom-koom* exist in Ghana due to variations in the millet used, the mashing process, and the additives being used.

2.2.1.3 Addition of sweeteners and storage

Addition of sweetener is the final step in *zoom-koom* processing. After the wort has been extracted, a quantity of sugar is added to the wort based on a ratio of 10:1. *Zoom-koom* processing is a spontaneous, and unfermented process as reported by Soma et al. (2019). Certain biochemical changes occur during its storage causing the beverage to undergo some type of fermentation.

According to Singh and Raghuvanshi (2012), these modifications can include an increase in amino nitrogen, the breakdown of proteins, and the elimination of any inhibitors that could be present. The bacteria of the genus *Lactobacillus* and *Weisella* species, for instance, have been revealed as considerable contributors to the acidity of the *zoom-koom* beverage during its first souring process. (Soma et al., 2019). The beverage is put into jugs and bottles for storage and sale once the sugar has been added. It is standard practice for the majority of *zoom-koom* producers to offer the beverage chilled. This enables the substance to remain stable without going through fermentation.

2.3 Other Traditional millet-based beverages

2.3.1 *Sur*

It is a fermented beverage made primarily from finger millet (*Eleusine coracana*) that is made in the Kullu district's Lug valley, the Kangra district's Bhangal and Luharti valleys, the Mandi district's Balh and Barot valleys, and the Sirmour region of India (Joshi et al., 2015; Kumar, 2013). Fermentation is carried out using a mixture (inocula) of roasted barley and regional herbs known as "dhaeli." The millet flour is combined with water to produce a dough, which is then allowed to naturally ferment for 7-8 days in a container. The fermented flour is cut into rotis, baked for half the time, then chilled. Then, the roti pieces, powdered dhaeli, and enough water and jaggery are then added to earthen pots that have been treated with smoke and allowed to ferment for 10 days under cover. After completion of fermentation, the product is filtered and stored in special earthen pots that are airtight. 5–10% alcohol has reportedly been found in the product (Kumar, 2013).

2.3.2 *Madua*

In Arunachal Pradesh, India, one of the most well-liked finger-millet-based drinks is called *madua*. The millet is first roasted for 30 minutes, then cooled and cooked till tender. The starting culture is added to the softened grains, which are then left to ferment for 4–7 days in a perforated basket covered in *ekam* leaves. Hot water is poured from the top and collected in a container after the fermentation process is finished. The liquid is referred to as *madua*. A high-quality *madua* has a golden hue, a sweet flavour, and outstanding alcohol compatibility. Other finger millet-based alcoholic beverages manufactured and consumed in Arunachal Pradesh, India, including *temsing*, *rakshi*, *mingri*, and *lohpani* (Shrivastava et al., 2012).

2.3.3 *Kunnu-zaki*

Kunnu-zaki, is another traditional non-alcoholic fermented beverage commonly consumed in Nigeria (Obadina et al., 2008; Adeleke & Abiodun, 2010; Agarry et al., 2010; Nwachukwu et al., 2010; Sekwati-Monang, 2011). Due to its stimulating property, it is accepted in other parts of the country (Amusa & Ashaye, 2009). It is produced from either millet (*Pennisetum tyroidum*), sorghum (*Sorghum bicolor*), or maize (*Zea mays*) (Akoma et al., 2006). Like other traditional fermented non-alcoholic beverages, *kunnu-zaki* is consumed anytime of the day by children and adults, served to entertain visitors and at community gatherings (Amusa & Ashaye, 2009; Ndulaka et al., 2014). Sugar or honey with some number of sweet potatoes and spices (such as ginger, black pepper or cloves) added for taste and flavour (Elmahmood & Doughari, 2007). The method of preparing *kunnu-zaki* differs among different cultures and still produced on a small scale (Ndulaka et al., 2014). Typically, *kunnu zaki* is processed by soaking sorghum, millet or maize, wet milling, sieving and partial gelatinization of the slurry (Ndulaka et al., 2014). It takes about five (5) days

for the whole process to be completed and can be stored for up to three (3) days under refrigeration condition (Ndulaka et al., 2014).

2.3.4 Oshikundu

Oshikundu is a traditional sour-sweet beverage from Namibia that is made from grain. Both an alcoholic and non-alcoholic version of it is produced. It is brewed with water, local sorghum (*Sorghum bicolor*), bran, and pearl millet (*Pennisetum glaucum*) meal. *Oshikundu* is brewed at home by rural women for daily consumption as well as for sale in several cities in northern Namibia's open markets. Boiling water is added to the *mahangu* meal during production, and the mixture is then allowed to cool to room temperature while being stirred occasionally. The mixture is then supplemented with bran and malted sorghum meal. Depending on the availability and preference of utilizing bran in brewing, the bran adding phase is optional. Some already fermented oshikundu is added after the mixture has been prepared. The resulting mixture is then diluted with water according to the amount of starting material used and the desired volume of the finished product. *Oshikundu* is then prepared by allowing the mixture to ferment for an average of one and a half hours at room temperature. Malt sorghum is fermented by yeast, which results in the production of alcohol. It is a perishable beverage that must be consumed the same day because its shelf life is less than 6 hours (Werner et al., 2012).

2.3.5 Koozh

In Tamil Nadu, India, ethnic people primarily eat *koozh*, another fermented beverage prepared with millet flour and rice (Ilango and Antony, 2014). Although pearl millet has been mentioned in other places, finger millet (*Eleusine corcana*) is the primary ingredient used in its preparation. Two

fermentation phases are included in the *koozh* preparation steps. The millet is first ground into flour, combined with water, and then left to ferment for an entire night. The overnight fermented millet slurry is combined with broken rice (20% by weight of millet) and cooked the next day to create *noyee*, a thick porridge. This porridge ferments for 24 hours, producing *kali*, a semi-solid porridge to which the necessary amount of drinkable water is added (1:6 w/v), and salt is manually mixed in to produce *koozh* (Ndulaka et al., 2014).

2.4 Millet and its nutritional value

In Sub-Saharan Africa, millet is a dominant crop for ensuring food security. According to Girish et al. (2014), millet has an exceptional capacity to grow and survive harsh environmental factors such as low soil fertility, insufficient rainfall, and land topography. As a result, growing millet in the Northern region of Ghana is not as difficult. India, Nigeria, Niger, China, Burkina Faso, Mali and Sudan are some of the countries with millet as a major import crop (Singh & Raghuvanshi, 2012). In Ghana, it is a chief cereal crop and essential food cultivated in the Northern part of the country. It is the second cereal crop after sorghum in terms of production area, with about 1.2 million hectares (FAO STAT, 2016). Since ancient times, Millet has been grown in Africa and the Indian subcontinent. It is thought that millet originated in Africa and was later introduced in India. According to the earliest archaeological evidence, millet was likely domesticated in Africa before moving to India around 2000 BC (Malik et al., 2002).

Singh & Raghuvanshi (2012) emphasizes on the the four most common millet varieties namely; Pearl millet (*Pennisetum glaucum*), Foxtail millet (*Setaria italica*), Proso millet or white millet (*Panicum miliaceum*), and finger millet (*Eleusine coracana*). Pearl millet accounts for 40% of the world's production. The millet kernel's high dietary fiber content is mostly due to the seed

coat, embryo (germ), and endosperm (FAO, 1995), which also has a hypoglycaemic impact when ingested. Complex carbohydrate in high fiber diets in the product is also gradually processed and absorbed, leading to a decrease postprandial glucose level (Singh & Raghuvanshi, 2012) when consume. Although there are variants available such as yellow, white, tan, red, brown, or violet in colour, but only the red types are typically grown around the world (Shobana et al., 2013). According to Ajiboye et al. (2014), addition of ancient based cereals such as sorghum and millet in our daily meals can decrease the risk of long-lasting disease, making them vital crops in Ghana. Aside from *pito*, millet is used in Ghana to make a wide range of dishes, including hausa *koko*, weaning foods, *tuo zafi*, and numerous other baked goods. According to Singh and Raghuvanshi (2012), millet contains a total carbohydrate content of roughly 72% to 79.5% and a range of 5.6% to 12.7% in protein. According to Mbithi et al. (2000), dark seeded varieties have higher protein levels than white seeded varieties. They also claimed that the necessary amino acids in a protein determine its quality. Due to the high levels of lysine, threonine, and valine contents in finger millet, in contrast, the essential amino acid balance is significantly better (Ravindran, 1992). Ravindran (1992) found an inverse relationship between the amounts of the amino acid's lysine and methionine in the finger millet grain and its protein content. With a ratio of 2 between leucine and isoleucine content, finger millet almost has the same amount of isoleucine as rice and wheat (Ravindran, 1992). Grain cereals supply essential dietary elements to people all over the world, making plant nutrients extremely important in the food sector (Shobana et al., 2013). Proteins can be altered to modify their structure, and presumably its physicochemical and functional properties by employing physical, chemical, and biological approaches like fermentation or enzymatic treatment (Amadou et al., 2013). Magnesium and phosphorus are present in millet in reasonable proportions (Girish et al., 2014). Magnesium can lessen the impact of heart-related problems and

migraines. On the other hand, phosphorus is an essential part of a precursor to adenosine triphosphate (ATP), which is necessary for the body to produce energy and function normally. A spontaneously fermented millet-based food (koko) was organically utilized as a probiotic therapy for diarrhoea in young children in an innovative intervention (Lei et al. 2006). According to Shobana et al. (2013), the millet grain contains full of phytochemicals, including phytic acid, which is known to lower cholesterol levels. Also, phytate is believed to be effective in cancer risk reduction. The variety of potential chemo-preventive molecules known as phytochemicals, which include antioxidants present in very high amounts in foods like millet, are responsible for these health advantages (Izadi et al., 2012).

2.4.1 Structure and Chemical Composition of Millet

The kernel structure of millet is comparable to that of sorghum. The pearl millet is caryopsis, where the pericarp is completely linked to the endosperm, and it is made up of the pericarp, germ, and endosperm. However, the endosperm and the pericarp-like sack are only weakly attached at one point in the finger millet. These millet kernels are referred to as utricles because their pericarp can easily separate from the testa, which serves as a shield for the endosperm (McDonough et al., 2000).

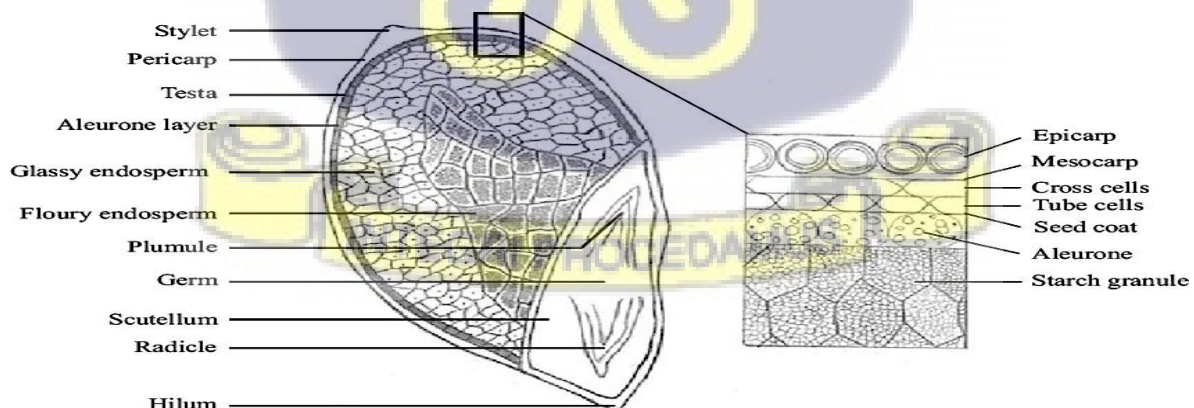


Figure 1: Typical Structure of Millet Seed (Singh & Raghuvanshi, 2012)

The relative distribution of the three components of the kernel are 8.4% of the pericarp, 75% of the endosperm, and 16.5% of the germ. As a result, endosperm to germ is about 4.5:1 in pearl millet, but 8.4:1 in sorghum in proportion. Due to the finger millet's small germ, the endosperm to germ ratio is smaller than that of sorghum and pearl millet, ranging from 11:1 to 12:1. The 1000 kernel weight for finger millet is relatively modest, and there are differences between the visual colours of pearl and finger millets and hence the texture of the millets should be taken into consideration when preparing them (Abdelrahman, 1984). Dry milling corneous kernel types yields more grain than soft floury kernel types do. When producing thick porridge, cultivars with higher levels of corneous endosperm are desirable, also, in the baking process either fermented or unfermented bread, the flour obtained from soft endosperm are much preferred (Rooney, 1986).

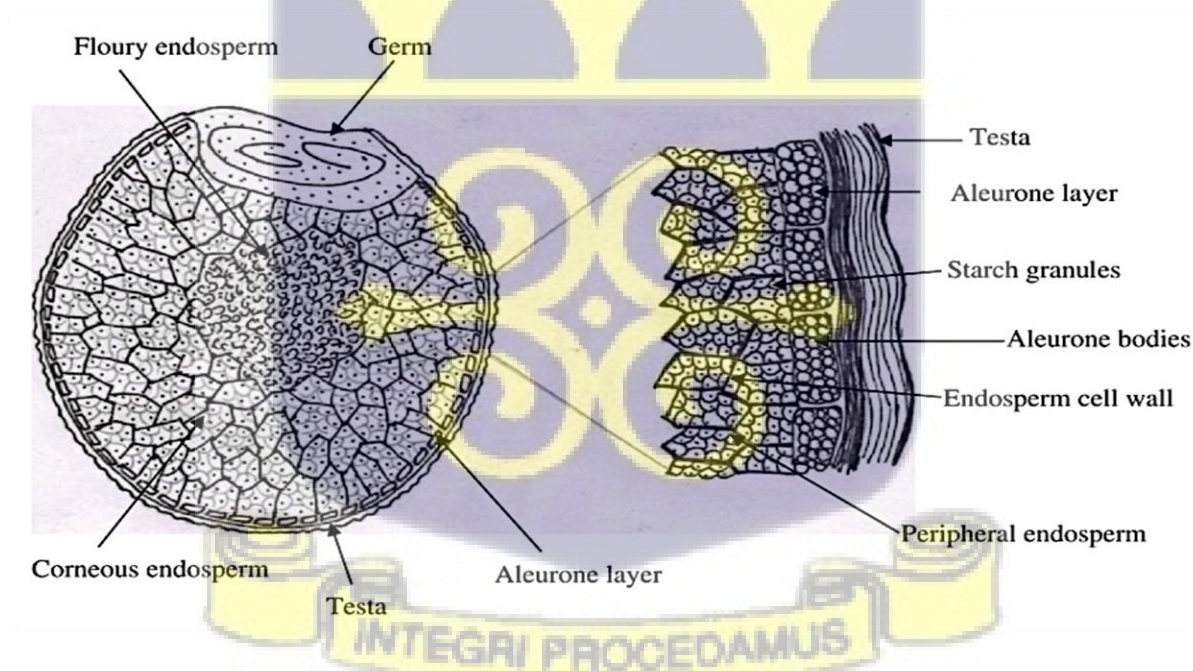


Figure 2: Cross section diagram of millet grain (Singh & Raghuvanshi, 2012).

2.4.2 Millet Production and consumption in Ghana

In Northern Ghana, millet farming has existed around 1459 BC (D'Andrea et al., 2001). According to SRID-MoFA (2011), millet is mostly grown in Ghana's Northern, Upper East, and Upper West regions, accounting for 29% of the country's total land area. In Northern Ghana, millet grows better than other crops because it grains yields in hot, dry weather and on soils with little water retention capacity (CGIAR, 1996). The value of millet as a commercial crop is secondary to its importance as a food crop. It is a customary crop that is cultivated by the majority of homes for food and is only ever sold to make money as a last option. Millet is also known to be the first crop to be harvested following a prolonged dry season, which is why it is considered a hunger-buster grain (Kudadjie et al., 2004). According to USDA (2022), Ghanaians consume 223 pounds of millet domestically, with a growth rate of -3.43%. Millet is turned into a number of products in Ghana, including *koko*, *fura*, and *maasa* (Lei & Jakobsen, 2004). According to the (MoFA 2018 Annual Report), millet has also generated revenue source for individuals and Ghana as a whole by contributing its fair share to the country's Gross Domestic Product. The annual production of millet in Ghana from 2006 to 2020 is shown in Table 1. Some of the high yielding varieties of millet cultivated in Upper East and Upper West Regions of Ghana are *Kaanati*, *Akad-kom*, *Naad-kohblug*, *Afribeh-naara* and *Waapp-naara* (MoFA 2018).

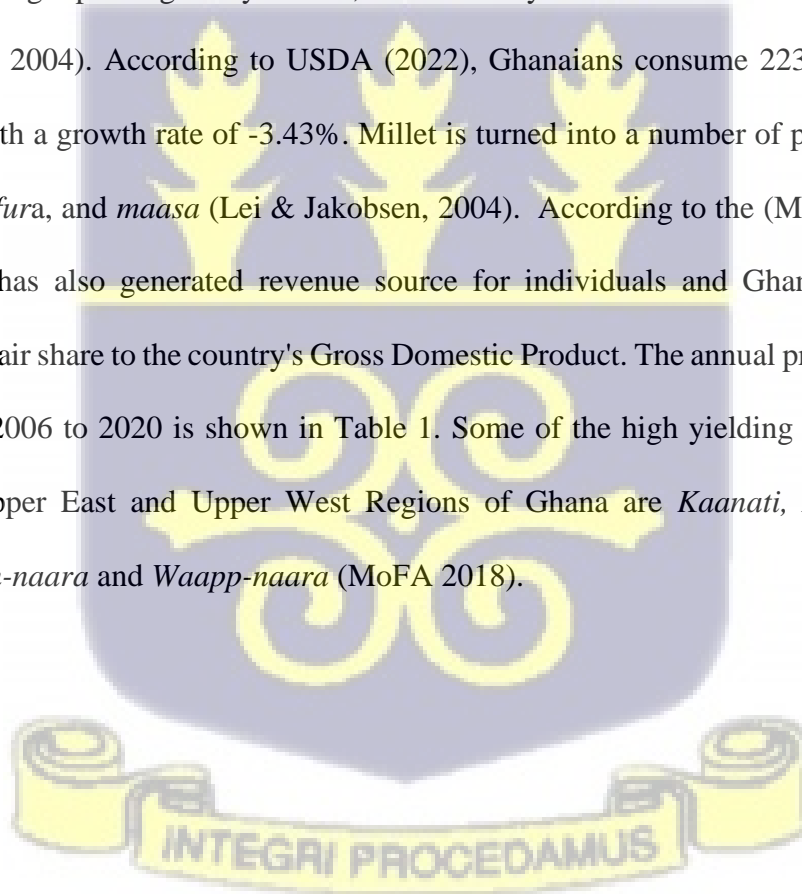
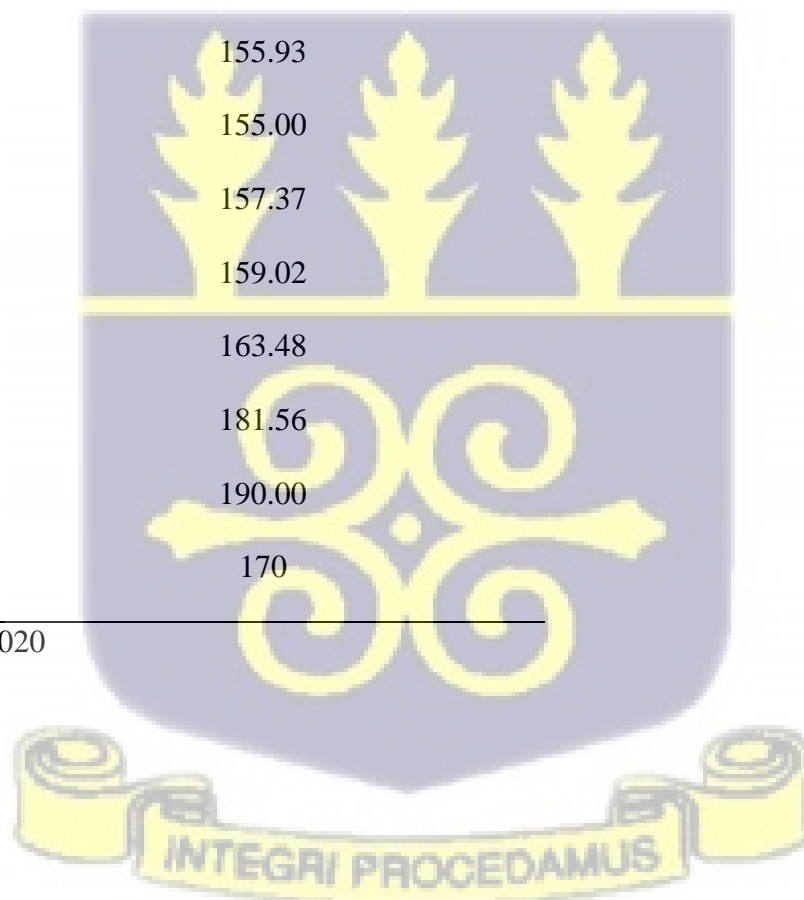


Table 1: Production of Millet in Ghana from 2006 to 2020

Year	Volume (1000 metric tonnes)
2006	165
2007	113.04
2008	193.84
2009	245.55
2010	218.95
2011	183.92
2012	179.68
2013	155.93
2014	155.00
2015	157.37
2016	159.02
2017	163.48
2018	181.56
2019	190.00
2020	170

Source: FAO, 2020



2.5 Nutrient Profile of millet – based beverages

2.5.1 Phenolic compounds and Antioxidant activity

The extremely diverse class of chemicals known as phenolic compounds include the phenol functional group as a primary constituent. These are conveniently divided into phenolic acids, flavonoids, and tannins. The subcategories of phenolic acids include hydroxybenzoic, hydroxycinnamic, hydroxyphenylacetic, and hydroxyphenylpropanoic acids. By using HPLC-DAD-ESI-MSⁿ (Dykes &Rooney, 2006; Chandrasekara and Shahidi 2011) identified and categorize the free, hydrolyzed (esterified and etherified), and bound phenolic chemicals in millets. The soluble fraction of finger millet has the highest concentrations of flavonoids (1896 g/g) and hydroxybenzoic acid derivatives (62.2 g/g). Little millet (173 g/g) and foxtail millet (171 g/g) had the highest quantities of hydroxycinnamic acid and its derivative in soluble form. The insoluble bound phenolics that are affixed to the cell wall make up the largest portion of the overall phenol content. Free form flavonoids are more common.

According to Dykes and Rooney (2006), millet's phenols exhibit antioxidant, anti-mutagenic, anti-oestrogenic, anti-inflammatory, antiviral, and platelet aggregation inhibitory properties. Total antioxidant capacity of finger millet, little, foxtail and proso millets is high due to their high total carotenoid and tocopherol content which varied from 78 to 366 and 1.3 to 4.0 mg/100 g respectively, in different millet varieties (Dykes &Rooney, 2006). The chemical characteristics of millet are suppressed by phenolics during the enzymatic breakdown of complex carbohydrates, delaying the absorption of glucose and ultimately controlling the postprandial blood glucose level. These chemical qualities include amylase and -glucosidase activities.

Table 2: Phenolic compound content ($\mu\text{g/g}$ defatted meal) in different types of millets

Phenolic compound	Pearl	Finger	Proso	Foxtail	Kodo
Methyl vanillate	19.8	–	–	–	–
Protocatechuic acid	11.8 ^a	23.1 ^a , 48.2	69.7	10.2	39.7
p-Hydroxybenzoic acid	22 ^a	8.9 ^a , 1.7	55.4	14.6 ^a , 5.63	10.5
Vanillic	16.3 ^a , 7.08	15.2 ^a ,	85.8	87.1 ^a , 22.1	40.1
Syringic	17.3 ^a	7.7 ^a	–	93.6 ^a	–
Gentisic acid	96.3 ^a	61.5 ^a	–	21.5 ^a	–
Cafeic acid	21.3 ^a	16.6 ^a , 11	–	10.6 ^a , 34	276
p-Coumaric acid	268.9 ^a , 53.5	36	1188	2133.7 ^a , 848	767
Trans-ferulic acid	637	331	332	631	1844
Cis-ferulic acid	81.5	65.3	18.6	101	100
8,8'-Aryl ferulic acid	–	–	–	19.6	94.8
5,5'-Di ferulic acid	57	11.8	5.44	62.2	173
Flavonoids ^b	7.1	1896	1.9	169	179

Kumar et al., 2018. (Adapted from Chandrasekara and Shahidi (2011) (content of phenolic compounds in bound form).

values are taken from Dykes and Rooney (2006) (expressed as μg phenolic acid/mg samples)

b Content of phenolic compounds in soluble fraction of millet grains

c Data not available



The antioxidant activity of millets is attributed to the presence of high polyphenol and tannin content (Rao et al., 2017, Rathore et al., 2019). Plants with antioxidant qualities scavenge free radicals which are the root cause of some deadly illnesses and disorders like cancer, diabetes, liver disease, renal failure, and degenerative diseases. Free radicals are organic substances that interfere with the regular functioning of body metabolism, and millet varieties are known sources of antioxidants that prevent their build up in the body (Sies, 1993; Oboth and Rocha, 2007).

2.5 Physicochemical and Microbial Quality of “Zoom-koom”

2.5.1 Physicochemical Quality of “Zoom-koom” and millet-based beverage

Physicochemical properties are the intrinsic physical and chemical characteristics of a food substance, this includes pH, Acidity, Total Soluble solids and colour. According to Tapsoba et al. (2017), who investigated the physicochemical properties of *zoom-koom* and the impact of fermentation on the microbial community during processing in Burkina Faso, except for the millet *zoom-koom* ranged from 4.2 to 4.1 for the fermented *zoom-koom* without and with sugar, respectively, the pH of the unfermented *zoom-koom* did not significantly decrease. The study also showed a non-significant variation in the pH between the unfermented *zoom-koom* and the sugar- and tamarind-sweetened *zoom-koom*, ranging from 5.2 to 3.5 respectively.

With the exception of the millet *zoom-koom*, the titratable acidity of the unfermented *zoom-koom* likewise showed a non-significant decline, ranging from 0.25 to 0.39% for the fermented *zoom-koom* without and with sugar, respectively. The study also reveals non-significant differences of the acidity with respect to the unfermented *zoom-koom* ranging from 0.19 to 0.24% for the *zoom-koom* without sugar, with sugar and Tamarind respectively.

Additionally, Soma et al. (2019) investigated into how adding a starting culture of *lactobacillus* to liquid *zoom-koom* and instant flour *zoom-koom* can enhance their nutritional, hygienic, and sensory qualities. The liquid *zoom-koom* samples had titratable acidities of between 1.21 and 1.89 g of lactic acid per 100 g of product, with a pH range of 5.02 to 5.13. Both forms of *zoom-koom* are acidic drinks (pH 6), according to the findings. Food acidification was a method used to stabilize the products. The "Kounou" samples were kept at room temperature and in the refrigerator for 4 days before the TSS values were read, according to Bayoi et al. (2022), who studied the Physicochemical Changes of Commercial "Kounou"- a millet beverage During Short Term Storage at Room and Refrigerated Temperatures. The TSS fell considerably ($p \leq 0.05$) in the ambiently stored samples, but not statistically significantly ($p \geq 0.05$) in the samples kept in the refrigerator. Between the first and fourth days, the TSS changed from 7.26 °Brix to 4.98 °Brix and 8.78 °Brix to 8.13 °Brix. A natural lacto-alcoholic fermentation process, which is the primary characteristic of the indigenous African beverages consumed while they are still in an active state of fermentation, was indicated by the reduction of TSS after storage (Tusekwa et al., 2000). This process gradually degrades soluble solids like sugars. Additionally, research showed that samples kept at ambient temperature had lower total soluble solids values than samples kept in a cold environment. A similar fermented beverage made from cereal that was produced in Nigeria and kept at the same temperature was found to exhibit the same pattern (Noah et al., 2013). Abiodun et al. (2017) studied the physicochemical, microbial and sensory properties of *kunuzaki* beverage sweetened with black velvet tamarind (*Dialium guineense*) and observed that the lightness, L^* value ranged from 38.23 for the control to 33.32 for the treatment with the highest amount of velvet tamarind, indicating a reduced lightness with increment in the incorporation with the velvet

tamarind. They also noted that the redness, a^* and yellowness, b^* values increased with the addition of the tamarind.

2.5.2 Microbial Quality of “Zoom-koom” and millet-based beverage

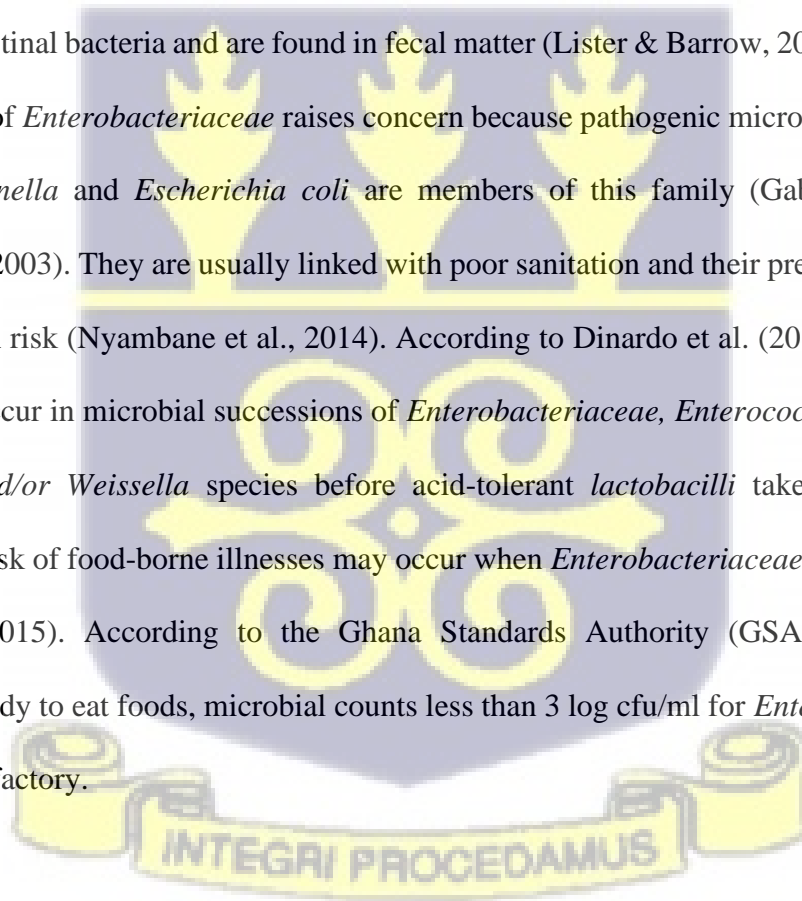
Soma et al. (2019), investigated into how adding a starter culture of *Lactobacillus* to liquid *zoom-koom* and instant flour *zoom-koom* can enhance their nutritional, sanitary, and organoleptic qualities. According to the microbiological quality findings, the amount of plate count (aerobic) in the liquid *zoom-koom* samples ranged from 1.0×10^6 cfu/ml to 1.0×10^8 cfu/ml. The concentration of lactic acid bacteria varied from 9.5×10^5 to 1.5×10^8 cfu/ml. Total coliforms ranged from 1.5×10^2 to 4.9104×10^4 cfu/ml. The concentration of the thermotolerant coliforms ranged from 9.0×10^1 to 4.9×10^4 cfu/ml. Yeast and mould concentrations ranged from 4.0×10^1 to 7.6×10^4 cfu/ml. The *zoom-koom* made and sold in Ouagadougou city contained high levels of coliforms, according to earlier studies (Barro et al., 2003; Bsadjo-Tchamba et al., 2014) and those on beverages with a similar composition, such as the traditional millet-based drink *kunun-zaki* from Nigeria (Gaffa et al., 2002; Elmahmood and Doughari 2007). Additionally, there is no pasteurization stage in the *zoom-koom* processing in Burkina Faso to guarantee the products' safety (Soma et al., 2017; Tapsoba et al., 2017). According to Tapsoba et al. (2017), starters could greatly enhance the hygiene of the *zoom-koom*. According to the recommendations made by CECMA (2009), the quantities of aerobic mesophilic bacteria and total coliforms in unfermented liquid *zoom-koom* and instant flour *zoom-koom* on the one hand, and in fermented liquid *zoom-koom* and fermented instant flour *zoom-koom* on the other hand, were determined to be non-conforming. However, it was discovered that the levels of yeast and moulds in all of the *zoom-koom* samples complied with CECMA's (2009) requirements.

2.6. Microbial Safety of Beverages

Along the food value chain, risk factors for poor quality and safety include inadequate food safety knowledge, the use of contaminated raw materials, polluted water, unhygienic practices and environments, unstandardized production processes, mixed-culture processing, poor packaging, and inadequate preservation techniques (Adekoya et al., 2019).

2.6.1 *Enterobacteriaceae*

Enterobacteriaceae are a group of gram-negative aerobic or facultatively anaerobic, asporogenous, rod-shaped bacteria (Lister & Barrow, 2008). Some genera of *Enterobacteriaceae* are resident intestinal bacteria and are found in fecal matter (Lister & Barrow, 2008). The presence and dominance of *Enterobacteriaceae* raises concern because pathogenic microorganisms such as *Shigella*, *Salmonella* and *Escherichia coli* are members of this family (Gabaza et al., 2019; Blandino et al., 2003). They are usually linked with poor sanitation and their presence could mean a possible health risk (Nyambane et al., 2014). According to Dinardo et al. (2019), natural cereal fermentations occur in microbial successions of *Enterobacteriaceae*, *Enterococcus*, *Leuconostoc*, *Pediococcus* and/or *Weissella* species before acid-tolerant *Lactobacilli* takes over. In cereal beverages, the risk of food-borne illnesses may occur when *Enterobacteriaceae* persists (Todorov & Holzappel, 2015). According to the Ghana Standards Authority (GSA) microbiological standards for ready to eat foods, microbial counts less than 3 log cfu/ml for *Enterobacteriaceae* is considered satisfactory.



2.6.2 *E. coli*

E. coli is a fecal coliform that is naturally found in the intestines of humans and vertebrates. According to Pachepsky et al. (2016), *E. coli* like other fecal contamination indicators such as the thermotolerant coliforms are used by monitoring bodies to check for the presence of fecal and pathogenic infection. It has been shown that *E. coli* pathovars cause enteric diseases such as bloody and non-bloody diarrhoea and chronic sequelae such as hemolytic uremic syndrome (Buchholz et al., 2011; Gault et al., 2011). In Doza et al. (2017)'s study, it was found that pathogenic *E. coli* genes were found in 14% of *E. coli*-positive food and 2% of *E. coli* positive flies after a multiplex PCR test of a subset of *E. coli* positive food and fly samples.

2.6.3 *Staphylococcus aureus*

Staphylococcus aureus is a naturally occurring pathogen found in the environment and on human skin and mucous membranes of most healthy humans (Rasigade & Vandenesch, 2014). Humans are significant hoarders of this microorganism (Boucher & Corey, 2008; Chambers, 2005). It does not usually cause infections on healthy skin but if it enters the blood stream it could cause a variety of infections (Rasigade & Vandenesch, 2014). They are commonly associated with community acquired infections and hospital-acquired infections in which treatment is still a challenge due to the emergence of Methicillin-Resistant-*S. aureus* (USCDC, 2003; Boucher & Corey, 2008). Hundred thousand (100,000) cfu/ml of *S. aureus* in food is needed to produce 1 µg of toxin and the temperature range for *Staphylococcus aureus* to form toxin is between 10 to 45°C and optimal at around 35 to 40°C. Standard refrigeration temperature can therefore limit the development of *Staphylococcal enterotoxin* (Centre for Food Safety, Food and Environmental Hygiene Department, Hong Kong, 2014). According to Kalita et al. (2017), *S. aureus* can cause health

complications like septic shock, multiple organ failure, microvascular abnormalities development, disseminated intravascular coagulation and necropsy.

2.6.4 Yeast and Mould

The uses of fungi (yeast and mould) are quite enormous; it can be a pathogen and act as causative agent of diseases and infections (Prescott, 2002). In most cases, food spoilage happens or occurs through moulds (Mariott and Gravani, 2006). Fungi can serve as a source of antibiotic; it can serve as a fruiting agent as in the case of mushrooms. There are fungi diseases, which adversely affects both humans and animals. There will be an adverse consequence on food supplies, health, the economy etc. if fungi degenerate.



CHAPTER THREE

3.0 Materials and Methods

3.1.1 Sampling site and procedure

Samples of *Zoom-koom* (20) were obtained from 5 processors conveniently selected from the Ashaiman-Tulaku, Amansaman, Nima-main market (Greater Accra Region) and Koforidua Zongo (Eastern Region). Each commercial processor was visited to study the production process for *zoom-koom* and samples of about 100ml was collected from each processor for analysis. Samples were transported under chilled conditions to the laboratory in the Department of Nutrition and Food Science at the University of Ghana for microbiological, bioactivity and physicochemical analysis. The control sample was also produced in the laboratory based on how the local processors produced their *zoom-koom*. It was produced in the laboratory because of safety and hygiene reasons.

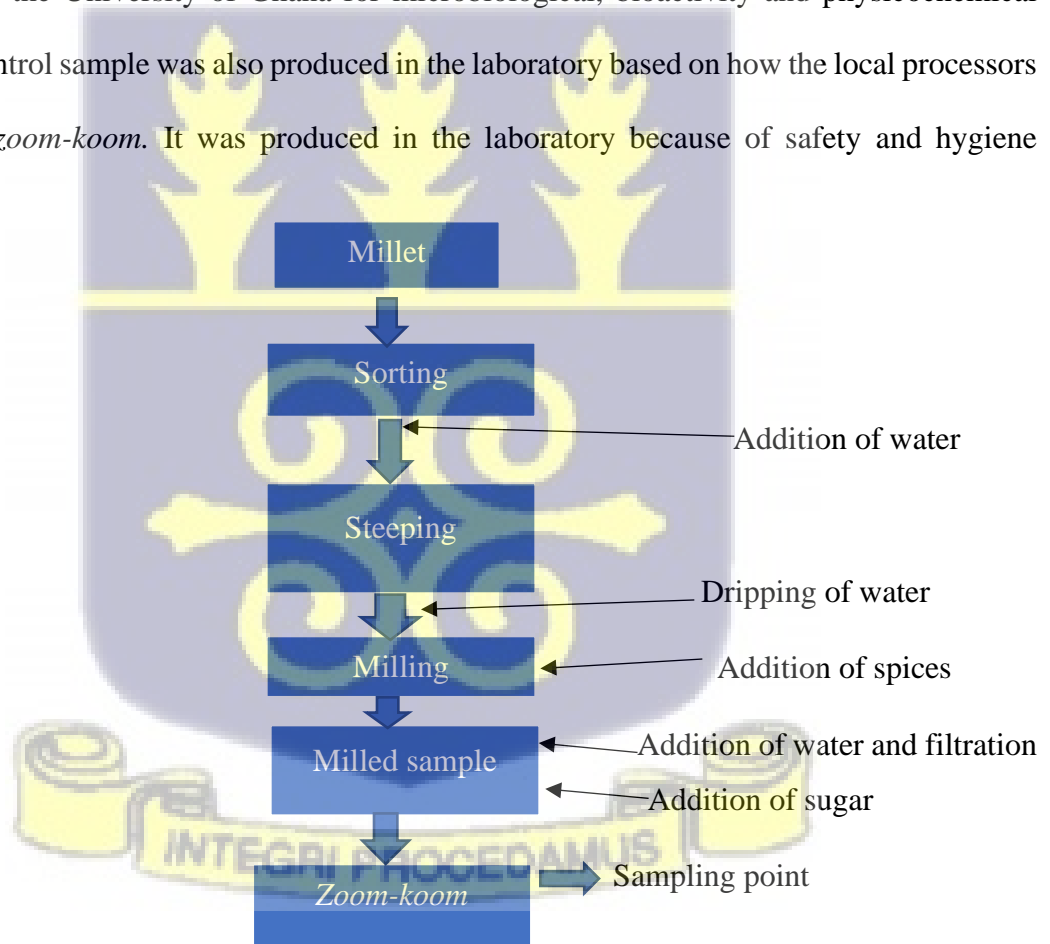


Figure 3: Process for commercial production of *zoom-koom*. (Soma et al., 2015)

Table 3: Analysis of zoom-koom from commercial processors

Commercial processors	Physicochemical and chemical analysis	Microbiological analysis
Nima (5) Koforidua (5) Ashaiman (5) Amansaman (5)	Colour, pH, Titratable Acidity, brix, Total phenolic compound, Antioxidant activity	Total plate counts, yeasts and moulds, Total coliform, <i>E. coli</i> , <i>Staphylococcus aureus</i> .

3.1.2 Microbiological analysis

3.1.2.1 Sample preparation

Ten grams of the samples was measured and homogenized in a sterile stomacher bag for one minute using a stomacher blender (Steward Stomacher blender 400 circulator) in 90ml of 0.1 % peptone water to suspend the microorganisms. The stock preparation was serially diluted from 10^1 to 10^{-9} and the appropriate dilution used.

3.1.2.2 Media preparation

All microbiological media used: Potato Dextrose agar for yeasts and moulds, MacConkey agar for *E. coli*, Mannitol Salt Agar for *Staphylococcus aureus* and Violet Red Bile Glucose Agar for *Enterobacteriaceae* (*E. coli*) were prepared following the instructions specified by the manufacturer. The media were sterilized in an autoclave for 15 min at 121°C and tempered at 50°C. The plates were checked for sterility by incubating uninoculated agar plates to check for growth. Peptone water (0.1%) used as a diluent was also prepared following manufacturer's instructions and incubated at 121°C for 15 min (Benulah et al.,2022).

3.1.2.3 Enumeration of Total bacteria, yeasts and moulds, total coliform, *Escherichia coli* and *Staphylococcus aureus*

Aliquot (0.1ml) of appropriate dilutions were pipetted unto duplicate sterile agar plates using the spread plate technique. The MacConkey agar, Mannitol Salt Agar and Violet Red Bile Glucose Agar plates were incubated in an inverted position at 37°C for 24h while Potato Dextrose agar plates were incubated at 25°C for 3-5 days. The agar plates of De Man Rogosa Sharpe agar were incubated anaerobically in an anaerobic jar at 37°C for 24h. After incubation, the bacterial colonies were observed and counted using a colony counter. The number of microorganisms counted were multiplied by the reciprocal of the dilution factor to give the count per gram. The method used was a modification from Morello et al. (2003).

3.2 Physicochemical Analysis

3.2.1 pH

The pH of the samples was evaluated using an Orion 2-star pH meter in accordance with AACC (2000) procedure. The pH of 60ml of sample was measured and recorded using the probe of the pH meter already calibrated according to manufacturer instructions.

3.2.2 Titratable Acidity

The titratable acidity, given as percent lactic acid, was calculated using the AACC (2000) method, which involved titrating 10 ml of sample against 0.1 N NaOH with 1% phenolphthalein (2 - 3 drops) as an indicator until a light pink color appeared (endpoint). Triplicate determination per sample were made. The following formula was used to compute the titratable acidity:

$$\text{Titrateable Acidity(\% Lactic acid)} = (V \times N \times 9.08)/(W \times 10)$$

Where V = titre value

N = normality of the titrant

W = sample weight

9.08 = Equivalent weight of predominant acid (lactic acid)

3.2.3 Brix

Brix was determined using a digital refractometer (Hanna digital refractometer model HI 96801) at room temperature.

3.2.4 Colour

The colour of *zoom-koom* samples was determined using a colourimeter (Digital handheld colorimeter-FRU[®] 10QC160226). Samples of *zoom-koom* were poured into a plastic petri dish. The measuring head of the colourimeter was cautiously positioned on top of the petri dish and the colour measurement taken. Three different colour readings were done on each sample and the mean calculated by the CIE L^* a^* & b^* colour value index. Colour change (ΔE) was calculated

using the formular: $\Delta E = \sqrt{(L_o^* - L^*)^2 + (a_o^* - a^*)^2 + (b_o^* - b^*)^2}$

The control *zoom-koom* sample was the reference point, with colour parameters L^* a^* and b^* denoted by L_o , a_o & b_o , where L is lightness, a is the positive value of red and negative value of green; b is the positive value of yellow and the negative value of blue (Sumnu et al., 2005).

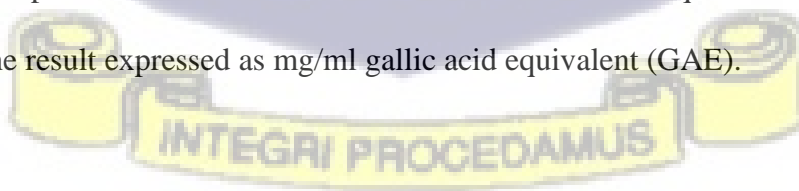


3.3 Chemical Analysis

3.3.1 Determination of total phenol compounds

3.3.1.1 Sample preparation

Using the Folin-Ciocalteu reagent and a slightly modified Adusei et al. (2019) method, the total phenol content of *zoom-koom* drink was measured, the calibration curve was plotted using gallic acid as a reference standard. 1 ml aliquot of 10, 20, 40, 80, and 100 mg/ml gallic acid solutions was mixed with 2 ml Folin-Ciocalteu reagent (diluted 1:10 with de-ionized water) and neutralized with 4 ml sodium carbonate solution (7.5 percent w/v). For colour development, the reaction mixture was incubated at room temperature for 30 minutes with intermittent shaking. A single beam UV-VIS spectrophotometer (Ultraspec Pro) was used to quantify the absorbance of the ensuing blue colour at a wavelength of 765 nm. For sample determination, 1 ml of the test sample was mixed with 80% methanol (v/v). The sample mixture was then vortexed using a (Standard mini Vortexer 009060) for 2 minutes, after which the sample was centrifuged using an (Eppendorf centrifuge 5417C) for 5 mins at a speed of 10000rpm. The supernatant was then discarded into a test and 2ml of the Folin-Ciocalteu and sodium carbonate reagents were then added to the test sample. The mixture was then incubated for 30mins with intermittent shaking and absorbance values read at wavelength of 765nm. All measurements were performed in triplicate for each analysis. The total phenols content was determined from the linear equation of a standard gallic acid curve and the result expressed as mg/ml gallic acid equivalent (GAE).



3.3.2 Antioxidant Activity

Radical Scavenging Activity of DPPH.) The DPPH assay was carried out according to Pandey et al., (2018). The sample (1 mL) was combined with 3 mL of DPPH solution (4 mL of stock DPPH solution in 96 mL of 80 percent methanol) and maintained at room temperature for 30 minutes. A UV-Vis spectrophotometer was used to measure the mixture's absorbance at 520 nm (Ultraspec Pro). As a blank, a mixture of 1 mL methanol and 3 mL DPPH solution was utilized. The percent inhibition of the extract's antioxidant activity was calculated using the following equation:

$$\text{Inhibition} = (A \text{ control} - A \text{ sample}) / (A \text{ control}) \times 100$$

where $A \text{ control}$ = is the blank's absorbance value and $A \text{ sample}$ = the absorbance of extract and DPPH solution

3.4 Optimization of process for *zoom-koom* using Response Surface Methodology

3.4.1 Materials

The local variety of millet (pearl), ginger, black pepper and gloves were obtained from the local market in Koforidua, Ghana.

3.4.2 Design of optimization

The Box - Behnken design was used to identify the combinations of process variables that provided the best quality characteristics for sensory acceptability, physicochemical properties, chemical properties, and microbial quality of *zoom-koom* (Box and Behnken, 1960; Montgomery, 2001). These factors include the blend ratio of steeped millet and spices, steeping time, and steeping temperature. The variables utilized in the experiment are listed in Table 4 along with their values.

Table 4: Variables and their levels used in the Box-Behnken design

Variables	Symbols	Levels		
		Coded	-1	0
Blend ratio (g) of steeped millet and spices	X1	700:50	700:100	700:150
Steeping time (hrs)	X2	2	6	12
Steeping temperature (°C)	X3	25	35	45

The number of experimental runs (N) in a Box-Behnken design was calculated using the formula $N = K^2 + K + C$, where (k) is the number of components and C_0 is the number of replications at the center point (Aslan and Cebeci, 2006). For Box-Behnken designs, $N = 2k(k-1) + C_0$, was used to determine N, however for central composite designs, $N = 2k + 2k + C_0$ was used (Ferreira et al., 2007), where k is the number of components, and C_0 is the number of centre points. So, for the three component Box-Behnken design in this work, a total of 15 experimental runs was used (Table 5). In order to construct the design matrix (factor combinations per experimental unit) for the Box-Behnken design, the level of one of the components was set at the centre point while mixtures of all levels of the other factors were used (Myers and Montgomery 2002). According to Table 4, all levels of factors X1 and X2 were merged after determining the level of component X3 (the steeping temperature), and the same methods were then applied for the factors X2 and X1, respectively. The final rows of the design matrix contained the three replicate center positions (Table 5). The data from the trials were fixed into the second order polynomial model (Montgomery 2001):

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + \dots\dots\dots$$

The independent variables in this equation are X_1 , X_2 , and X_3 . The linear coefficients are b_1 , b_2 , and b_3 . The interaction coefficients are b_{12} , b_{13} , and b_{23} . The quadratic coefficients are b_{11} , b_{22} , and b_{33} (Montgomery, 2001). Y is the response and b_0 is the model constant.

Table 5: Box-Behnken Design matrix of variables (k=3) for optimization of the zoom-koom

Variants	X1	X2	X3	Blend ratio (g)	Steeping time (hrs)	Steeping temperature (°C)
1	-1	-1	0	700:50	2	35
2	1	-1	0	700:150	2	35
3	-1	1	0	700:50	12	35
4	1	1	0	700:150	12	35
5	-1	0	-1	700:50	7	25
6	1	0	-1	700:150	7	25
7	-1	0	1	700:50	7	45
8	1	0	1	700:150	7	45
9	0	-1	-1	700:100	2	25
10	0	1	-1	700:100	12	25
11	0	-1	1	700:100	2	45
12	0	1	1	700:100	12	45
13	0	0	0	700:125	7	35
14	0	0	0	700:125	7	35
15	0	0	0	700:125	7	35

3.4.3 Preparation of *zoom-koom*

One kilogram (1 kg) each of millet grains and the spices (ginger, gloves & black pepper) were washed and steeped in water with respect to time and temperature according to the experimental design (Table 4 and 5). Five (5%) sodium benzoate was added to the steep grain was added to the beverage to control the microorganism that may be present in the steeped sample (Terna and Ayo, 2002). The steeped grains were washed again and milled according to the blend ratio of the steeped millet and spices based on the experimental design using a multifunction blender robot (SHB-3088). The slurry was prepared by adding sterilized water in a ratio of 10:1 to the fresh extract and filtered using muslin cloth to obtain the wort. About 0.05kg/L of sugar was added to produce the final *zoom-koom*.

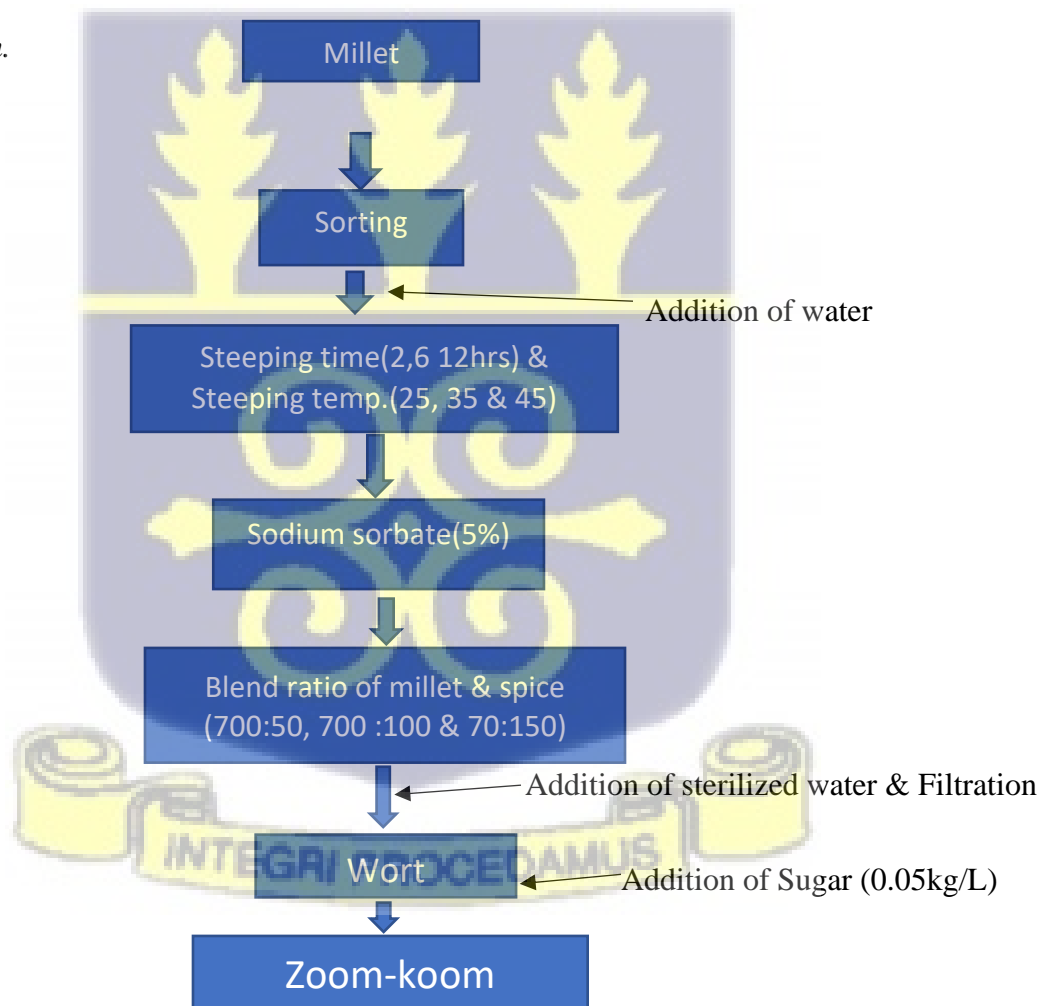


Figure 4: Process flow showing Laboratory Processing of *zoom-koom*

Table 6: Analysis of *zoom-koom* samples

Physicochemical	Microbiological Analysis	Bioactive properties
Colour, pH, TTA, °Brix	Total plate counts, yeasts and moulds, <i>E. coli</i> , <i>Staphylococcus aureus</i> , <i>Enterobacteriaceae</i>	Antioxidant Activity, Total phenolics.

3.4.4 Consumer sensory acceptance of *zoom-koom*

The sensory acceptability of the 15 *zoom-koom* samples excluding the control obtained as detailed in the preceding section was examined. The study used the balanced incomplete block design described by Cochran and Cox (1957) and Montgomery (2001) with 15 treatments (samples), $k = 5$ (samples per judge), $r = 7$ (replicates), $b = 21$ (number of blocks/judge), $t = 2$, and $N=105$. Each panellist was given the opportunity to examine five samples at a time because it would be increasingly challenging for a single consumer to evaluate all 15 *zoom-koom* samples in one session without using up valuable time. Panellists' ($n=21$, $k= 5$ samples per judge) from the University of Ghana were chosen at random amounting to a total number of 105 consumers. Measures for recruiting included ensuring that panellists were frequent *zoom-koom* consumers. The panellists were given the *zoom-koom* in disposable cups that were coded with randomly generated three-digit codes. panellists were asked to evaluate the samples based on the taste, aftertaste, colour, aroma, and overall acceptability using a 9-point hedonic scale (9 = like greatly, 5 = neither dislike nor like, and 1 = dislike significantly) (Prinyawiwatkul et al. 1997; Peryam and Pilgrim 1957).

3.5 Proximate composition of optimized *zoom-koom*

3.5.1 Moisture content determination

An amount of 3 g *zoom-koom* was weighed using an analytical balance (SARTORIUS B120S, GERMANY). The weight of the petri dish and each sample was determined and recorded.

The petri dish and its content were placed in a drying oven (FISHER Isotemp[®] Oven, SENIOR MODEL) at a temperature of 105°C for 12 h. It was then removed and placed in a desiccator and then weighed. The procedure was repeated for each sample in triplicates (AOAC, 2000). The

moisture content was determined as follows:
$$\text{Moisture (\%)} = \frac{(W1 - W2) \times 100}{W2}$$

Where: $W1$ = weight (g) of sample and crucible before drying and $W2$ = weight (g) of sample and crucible after drying.

3.5.2 Crude fat determination by goldfish apparatus method

An amount of 3 g *zoom-koom* with a known a moisture content was used. The beakers were weighed using an analytical balance (SARTORIUS B120S, GERMANY). The samples were placed into a thimble and placed in the holding chamber of the Goldfish apparatus. An amount of petroleum ether (25ml) was poured into each of the beaker. The beaker containing the solvents was also connected to the gaskets. The tap was then opened to allow free flow of water through the apparatus to facilitate the condensing of the solvent extracted within 4h. The beakers were and its content dried in an oven (FISHER Isotemp[®] Oven, SENIOR MODEL) for 30 min cooled in a desiccator for 30 min and weighed using an analytical balance (SARTORIUS B120S, GERMANY) to determine the difference in weight of the flask. The procedure was repeated for

each sample in triplicates (AOAC, 2000). The fat content was calculated using the formula: Crude

$$\text{Fat (\%)} = \frac{W_1 \times 100}{W_2}$$

Where: W_1 =Fat weight and W_2 = Sample weight

3.5.3 Ash content determination

An amount of 3 g *zoom-koom* was weighed using an analytical balance (SARTORIUS B120S, GERMANY). The weight of the crucible and each sample was determined and recorded. The crucible and its content were placed in a muffle furnace (THERMO SCIENTIFIC) at a temperature of 600°C for 2 h. The crucibles were removed and allowed to cool in a desiccator after which was weighed. The procedure was repeated for each sample in triplicates (AOAC, 2000). The formula

$$\text{used to calculate ash content: Ash (\%)} = \frac{\text{weight of ash sample} - \text{weight of empty crucible}}{\text{sample weight}} \times 100$$

3.5.4 Crude fiber content determination

The sample utilized for the fat analysis was also used for the study of the raw fiber. The defatted sample was put into a 500 ml Erlenmeyer flask, to which 0.5 g of asbestos and 200 ml of 1.25% boiling H_2SO_4 were added. The condenser was then attached, and the flask was placed on a heated plate. It was then be filtered and washed with boiling water till filtrate was no longer basic and 15ml alcohol was used to do a final washing and residues transferred into silica crucibles and dried in an electric oven (FISHER Isotemp[®] Oven, SENIOR MODEL) for one hour at 100°C. The weight loss was calculated after the crucibles and their contents have been heated for 30 minutes in a muffle furnace, cooled in a desiccator, and weighed. Each sample was done in triplicate (AOAC, 1990). The crude fibre content was calculated using the formula:

Crude fibre(%) = $\frac{C1-C2}{C3} \times 100$, where: $C1$ = Weight of dried sample, $C2$ = Weight of ashed sample, and $C3$ = Weight of defatted sample

3.5.5 Crude protein content determination

Using an analytical balance (SARTORIUS B120S, Germany), 3 g of *zoom-koom* were weighed and put into a digestion flask. Kjeldahal catalysts and concentrated H₂SO₄ amount to 25 milliliters. The digested sample was next filtered into a 100 ml volumetric flask, filled to the appropriate level with 60 ml of distilled water, and thoroughly mixed. The Kjeldhal apparatus was heated while containing 10 ml of sample and 17 ml of NaOH for the ammonia distillation process. Twenty-five millilitres of 4% boric acid were measured into the conical flask to receive the liberated ammonia gas. The nitrogen content was estimated by titrating the ammonium borate formed with standard 0.096N HCl using mixed indicator and titre values recorded. The procedure was repeated for each sample in triplicates (AOAC, 2000). Protein content was calculated using

the formula: Crude protein(%) = $\frac{(A-B) \times 14.007 \times 6.25}{W}$

Where: A= volume (ml) of 0.2 N HCL used sample titration, B= volume (ml) of 0.2 N HCL used sample titration, N= Normality of HCL, W= weight (g) of sample, 14.007= atomic weight of nitrogen, and 6.25= the protein – nitrogen conversion factor.

3.5.6 Carbohydrate /nitrogen free extract

The estimated amount of total carbohydrates was calculated by deducting the total of moisture, ash, protein, fat, and crude fiber from 100 and expressing the result as a percentage. (AOAC, 2000).

% Carbohydrate

$$= 100 - (\% \text{ moisture} + \% \text{ Ash} + \% \text{Crude fibre} + \% \text{Crude protein} + \% \text{Crude fat})$$

3.5.7 Energy

The energy content of *zoom-koom* was determined by multiplying a factor of four (4) to the protein, a factor of nine (9) to the fat and a factor of four (4) to the carbohydrate and the results summed up (AOAC, 1990). The energy content of *bread* was calculated using the formula:

$$\text{Energy (kcal)} = (4 \times \text{protein content}) + (9 \times \text{fat content}) + (4 \times \text{carbohydrate content})$$

3.6 Data and Statistical analysis

Mean \pm SD was used to summarize physicochemical, bioactive properties and microbiological values. ANOVA was used to check the significance of differences at an alpha level of 0.05 between the microbial counts and physicochemical values of the commercially processed samples. Differences were assessed using a post hoc Tukey test using Minitab software version 20.0. For the optimization, response surface regression techniques were used to analyse the experimental data, and the F-test was used to determine the significance of the regression coefficients, the collected data were fitted to polynomial models, and the models' fitness were assessed in terms of R² values, the absence of fit errors, and the prediction error sum of squares (PRESS).



CHAPTER FOUR

4.0 Results and Discussion

4.1 Physicochemical properties of commercially processed *zoom-koom*

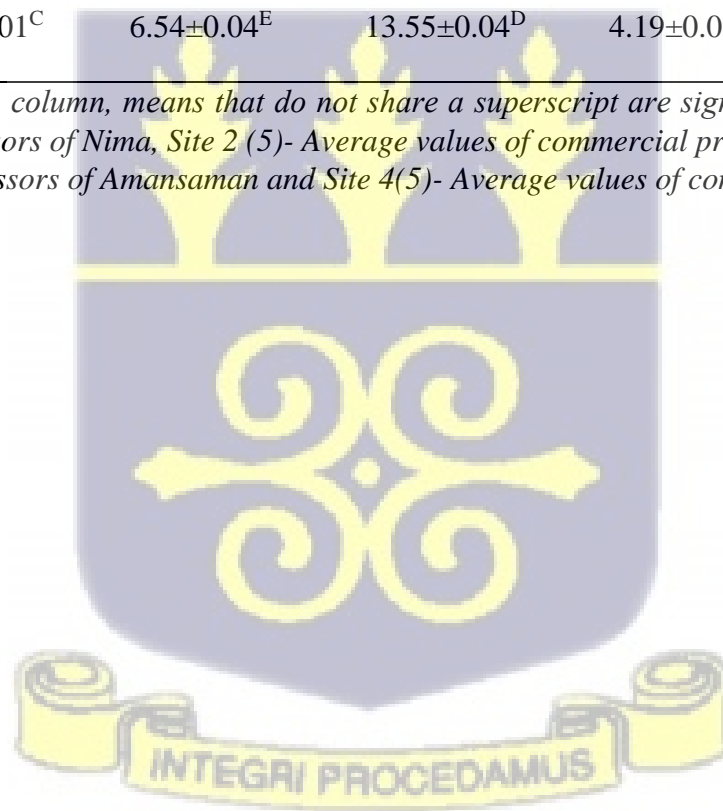
The physicochemical properties of *zoom-koom* samples along the commercial processing chain sourced from four commercial processors in the Greater Accra Region, one commercial processor in Koforidua in the Eastern Region of Ghana and the control sample treatment is presented in Table 7. Generally, there was a reduction in pH and a rise in TTA. The pH of the commercial *zoom-koom* range between 3.08 to 4.95 with the control sample having the highest value and product from Nima had the least pH value (Table 7). The differences in pH is a result of the variation in millet cultivar, variation in the type and amount of spices and the variation in *zoom-koom* processing.

The pH of the *zoom-koom* obtained from the commercial sources in this study were lower compared with 4.1-4.2 reported for fermented and unfermented *zoom-koom* produced in Burkina Faso (Tapsoba et al., 2017). The *zoom-koom* produced with *Lactobacillus fermentum* starter culture was reported to have pH in the range 5.02-5.13 (Soma et al., 2019), and this is very high compared with the values obtained in the present study. The titratable acidity (TTA) of the commercial *zoom-koom* generally showed an inverse relationship with pH. Other authors have also made this observation (Tapsoba et al., 2017; Soma et al., 2019). The TTA of the commercial *zoom-koom* were in the range 0.01-0.09 (% lactic acid). Higher values of 0.25 to 0.39% respectively for the fermented *zoom-koom* without sugar and the one with sugar (Tapsoba et al., 2017). A *zoom-koom* product made by using *Lactobacillus fermentum* starter culture recorded acidity of 1.21 and 1.89 g of lactic acid / 100 g of product (Soma et al., 2019). The lower TTA in the present study compared with the values reported by Soma et al. (2019) may be due to the differences in the starter culture used.

Table 7: Physicochemical properties of commercially processed *zoom-koom*

Sample	pH	Acidity (% Lactic acid)	TSS (°Brix)	<i>L</i> *	<i>a</i> *	<i>b</i> *	ΔE
Site 1 (5)	3.08±0.01 ^C	0.09±0.01 ^A	8.65±0.02 ^B	26.49±0.09 ^A	0.53±0.00 ^A	11.45±0.11 ^A	11±0.01
Site 2 (5)	3.14±0.01 ^C	0.06± 0.01 ^{AB}	6.80±0.010 ^D	21.29±0.03 ^C	4.27±0.01 ^B	10.19±0.08 ^B	6±0.02
Site 3 (5)	3.59±0.07 ^B	0.04±0.01 ^{BC}	9.63±0.04 ^A	21.27±0.02 ^C	4.29±0.03 ^B	10.39±0.13 ^B	7±0.11
Site 4 (5)	3.56±0.02 ^B	0.05±0.04 ^{ABC}	6.99±0.01 ^C	22.05±0.02 ^B	3.95±0.05 ^C	3.78±0.28 ^C	5±0.00
Control	4.95±0.05 ^A	0.01±0.01 ^C	6.54±0.04 ^E	13.55±0.04 ^D	4.19±0.03 ^{BC}	10.68±0.23 ^B	-

Key: Values are Means± SD. In Each column, means that do not share a superscript are significantly different at $\alpha < 0.05$. Site 1(5)- Average values of commercial processors of Nima, Site 2 (5)- Average values of commercial processors of Tulaku-Ashaiman, Site 3(5)- Average values of commercial processors of Amansaman and Site 4(5)- Average values of commercial processors of Koforidua.



The TSS value of the control was significantly lower ($P \leq 0.05$) than those of the *commercial zoom-koom* whose values ranged from 6.80-9.63°Brix. The *zoom-koom* from each location was significantly different, and may be due to the variation in processing. The TSS values were higher than 4.98-7.26°Brix reported for commercial “*kounou*” millet beverage (Tusekwa et al., 2000). All colour values were expressed by Hunter L^* , a^* b^* and ΔE principles representing lightness, redness, yellowness and colour difference respectively. The commercial *zoom-koom* had L^* values in the range 21.27-26.49. The control however, gave significantly lowest L^* value of 13.55. The results show that the control was darkest than all the commercial products. Among the commercial products there was generally significant difference in their L^* values. With the exception of the commercial *zoom-koom* from Nima, all the others gave a^* values which were not significantly different from the control. The b^* values for the *zoom-koom*, including the control ranged from 3.28-11.45. The b^* value of the control was not different ($P > 0.05$) from those obtained from Tulaku-Ashaiman and Amansaman. The colour difference, ΔE between the control and the commercial *zoom-koom* products ranged from 5-11. Since the values are more than 1, all the commercial products could be distinguished from the control in terms of colour. This result is comparable to (Dipika et al., 2020) who developed a millet based ready-to-drink beverage for geriatric population. The differences in colour may be due to the different varieties of millet (pearl and finger), proportion of spices, and dilution factor used in the *zoom-koom* formulation from the different locations.



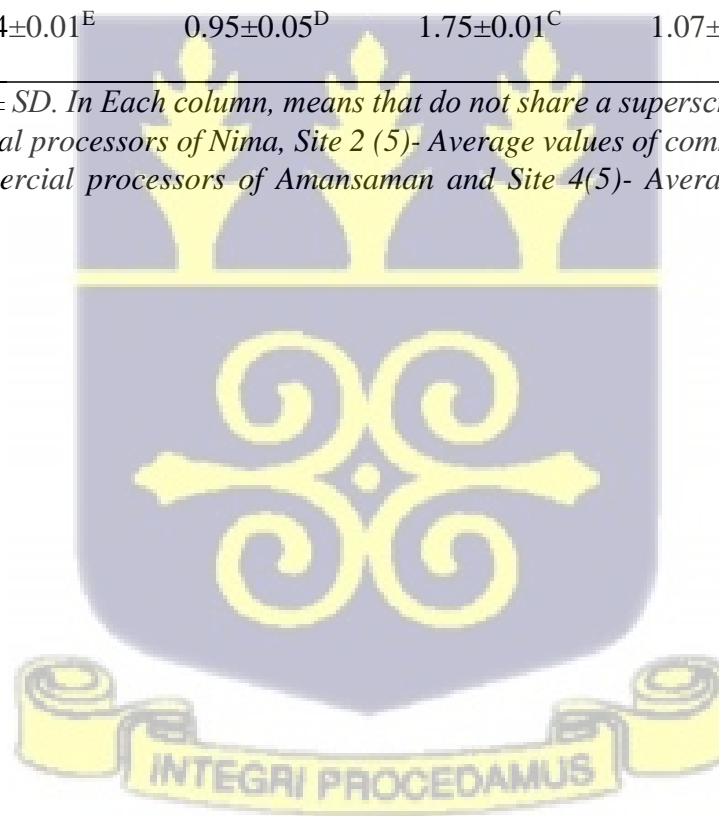
4.2 Microbial quality of commercially processed *zoom-koom*

The microbial quality of *zoom-koom* samples along the commercial processing chain sourced from commercial processors and the control sample is presented in Table 8. There was significant difference in the yeasts and moulds, total coliforms, *E. coli*, and *Staphylococcus aureus* counts of the commercial *zoom-koom* samples as well as the control. The aerobic plate counts of commercial *zoom-koom* ranged between 2.73 to 3.05 log cfu/ml whilst yeasts and moulds count ranged between 2.14 to 3.16 log cfu/ml. The results showed that the dominating microorganism in this study were the yeasts and moulds. This is in agreement with other studies on African traditional beverages where yeasts were found to be dominant (Adesulu & Awojobi, 2014; De Vuyst et al., 2016). In this study, *E. coli* counts in the commercial *zoom-koom* samples ranged between 0.77 to 2.12 log cfu/ml. With the exception of *zoom-koom* obtained from *Amansaman*, all other commercial *zoom-koom* and the one prepared in the laboratory (control) had lower *E. coli* counts less than 2log cfu/ml as required by the Ghana Standard Authority microbiological standards for ready to eat foods (GSA,2020). Other studies have reported the presence of pathogenic bacteria in popular traditional beverages even under acidic pH condition. For example, Adinsi et al. (2017) detected *Enterobacteriaceae* and *E. coli* in some ‘*gowe*’ samples (a Beninese traditional fermented non-alcoholic drink made from corn) collected from a market. Aboh and Oladosu (2014) collected samples of ‘*kunun-zaki*’ (a Nigerian traditional non-alcoholic beverage made from either maize, sorghum or millet) and assessed their microbiological quality in Abuja Nigeria). In a study by Oshoma et al. (2009) on the growth and survival of *E. coli* in ‘*kunun-zaki*’ during storage at different temperatures, it was shown that *E. coli* could at low pH although survival rate decreased with time. *Enterobacteriaceae* such as *E. coli* are usually linked with poor hygiene and their presence could mean a potential health risk (Nyambane et al., 2014).

Table 8: Microbial Quality of commercially processed *zoom-koom*

Sample	Aerobic plate Count	Yeast & Moulds	Total coliform	<i>E. coli</i>	<i>S. aureus</i>
Site 1 (5)	2.73±0.01 ^D	3.16±0.01 ^A	1.32±0.03 ^D	1.06±0.01 ^C	2.04±0.02 ^D
Site 2 (5)	3.05±0.05 ^A	3.13±0.03 ^{AB}	1.18±0.01 ^E	0.77±0.03 ^D	2.37±0.05 ^C
Site 3 (5)	2.97±0.02 ^B	3.07±0.01 ^B	2.76±0.01 ^A	2.12±0.07 ^A	2.81±0.01 ^B
Site 4 (5)	2.83±0.02 ^C	2.14±0.01 ^C	2.14±0.00 ^B	1.93±0.00 ^B	3.27±0.01 ^A
Control	1.44±0.01 ^E	0.95±0.05 ^D	1.75±0.01 ^C	1.07±0.02 ^C	2.04±0.05 ^D

Key: Values are mean Log CFU /ML ± SD. In Each column, means that do not share a superscript are significantly different at $\alpha < 0.05$. Site 1(5)- Average values of commercial processors of Nima, Site 2 (5)- Average values of commercial processors of Tulaku-Ashaiman, Site 3(5)- Average values of commercial processors of Amansaman and Site 4(5)- Average values of commercial processors of Koforidua.



In cereal beverages, the risk of food-borne illnesses may occur when *Enterobacteriaceae* persists (Todorov & Holzapfel, 2015). The result was comparable to a study conducted by Aboagye et al. (2020) on the microbial evaluation of 'asaana' where *E. coli* counts ranged between 0 and 1.14×10^8 cfu/ml (0 and 8.06 log cfu/ml). *E. coli* is a fecal coliform that is naturally found in the intestines of humans and vertebrates. *E. coli* like other fecal contamination indicators such as the thermotolerant coliforms are used to ascertain for the presence of fecal contamination and other pathogens (Pachepsky et al., 2016). Addo et al. (2016) attributed the occurrence of *E. coli* in 'olewonyo' samples to poor hygienic conditions under which the samples were prepared since the ones they produced in the laboratory were not contaminated with *E. coli*.

Staphylococcus aureus count in this study between 2.04 to 3.27 log cfu/ml. Mean log cfu/ml values below 3 log cfu/ml is considered satisfactory according to the Ghana Standards Authority (GSA) microbiological standards for ready to eat foods. Microbial load from the laboratory processed *zoom-koom* be as result of flavours and sweeteners, or dispensing of the extract into sieve meshes, nylons and bottles. Utensils and ladles used during the post-processing stages can also serve as a source of contamination. The source of contamination may also have come from the spices used as additives (Bakobie et al., 2017; Odamtten et al., 2018). Apart from the *zoom-koom* samples obtained from Koforidua, all others commercially processed *zoom-koom* gave *S. aureus* count less than 3log cfu/ml limit set by Ghana Standard Authority microbiological standards for ready to eat foods (GSA, 2020).

The presence of pathogenic bacteria such as *S. aureus* is associated with several factors affecting quality along the processing chain such as insufficient food safety knowledge, using soiled raw materials and polluted water, inadequate hygienic practices, production in unhygienic environments and unhygienic hawking activities (Adekoya et al., 2019).

4.3 Bioactive Properties of commercially processed *zoom-koom*

Table 9 shows the bioactive properties of *zoom-koom* samples along the commercial processing chain sourced from four commercial processors in the Greater Accra Region, one commercial processor in Koforidua in the Eastern region.

Table 9: Bioactivity of commercially processed *zoom-koom*

Sample	Total Phenolic Compound(mg/ml)	Antioxidant Activity (%Inhibition)
Site 1 (5)	29.92±0.03 ^A	84.06±0.15 ^D
Site 2 (5)	24.06±0.02 ^B	89.17±0.06 ^C
Site 3 (5)	30.10±0.90 ^A	91.88±0.10 ^A
Site 4 (5)	22.73±0.10 ^C	90.15±0.30 ^B
Control	22.55±0.39 ^C	92.33±0.48 ^A

Key: Values are mean ± SD. In Each column, means that do not share a superscript are significantly different at $\alpha < 0.05$. Site 1(5)- Average values of commercial processors of Nima, Site 2 (5)- Average values of commercial processors of Tulaku-Ashaiman, Site 3(5)- Average values of commercial processors of Amansaman and Site 4(5)- Average values of commercial processors of Koforidua.

The total phenolic content (TPC) of the commercial *zoom-koom* were in the range 22.73 to 29.92 mg/ml. The control also gave 22.55 mg/ml. Generally, the differences in the mean values of TCP were significant ($P < 0.05$), with *zoom-koom* from *Amansaman* giving the highest and those from Koforidua the lowest. The antioxidant activity which is expressed in % inhibition of DPPH ranged from 84.06% to 91.88% for the commercial *zoom-koom*, but the control gave the highest value of 92.33%. Agbor et al. (2011) reported strong correlation between the phenolic content and antioxidant activity. Similar trend can be found in this study) indicating that factors other than the

TPC content contributes to antioxidant activity. Antioxidants function as reducing agents, ultimately eliminating free radical intermediates and inhibiting further oxidation (Phang et al., 2013).

4.4 Influence of process variables on the physicochemical properties of laboratory-produced *zoom-koom*

The pH of the *zoom-koom* samples steeped for 2h ranged between 6.14 to 7.24, those steeped for 7 hrs ranged between 5.70 to 6.31 and for 12h also ranged between 5.77 to 6.87 (Table 10). This demonstrates the slightly acidic to slightly neutral nature of the beverage. This maybe as a result of the sodium benzoate added to the millet during the steeping process which was to some extent able to control the microorganisms that might initiate the fermentation process in order to increase the acidity of the beverage (Terna and Ayo, 2002). The regression model for pH could explain 91.66 % of the variability in the data (Figure 5). There were significant interaction effects between the blend ratio and the steeping time on pH of the samples. This means that the blend ratio (millet and spice) and how long they are steeped influences the pH of the final product. The contour plots generated using the model (Figure 5) shows that while increasing the blend ratio from 140 to 150 with respect to the spices and increase in steeping time from 2 to 2.5h increased pH. Also, increase in blend ratio with respect to the spices from 50 to 53 and increase in time 2 to 3h also resulted in an increase in the pH value of the *zoom-koom*. Finally increased steeping time from 11.5 to 12h and decrease in blend ratio from 50 to 52 with respect to the spices also resulted in a higher pH value. All these explanations are evidence in the darkest green colour (Figure 5).

Table 10: Physicochemical quality laboratory produced zoom-koom

Variants	TTA	pH	TSS	L *	a*	b*	ΔE
1	0.002±0.00 ^{BC}	7.24±0.03 ^A	0.80±0.17 ^{BC}	15.88±0.07 ^{FG}	1.50±0.10 ^K	7.20±0.07 ^H	17.66±0.06 ^G
2	0.004±0.00 ^B	7.18±0.02 ^A	0.90±0.17 ^A	14.32±0.10 ^I	2.82±0.10 ^G	7.35±0.15 ^{GH}	16.45±0.06 ^H
3	0.008±0.00 ^A	6.87±0.03 ^B	0.88±0.06 ^A	16.30±0.17 ^F	2.13±0.02 ^{IJ}	7.54±0.15 ^G	17.83±0.10 ^G
4	0.009±0.00 ^A	6.52±0.08 ^C	0.69±0.04 ^{ABCD}	17.60±0.10 ^D	2.40±0.10 ^{HI}	7.81±0.07 ^F	19.50±0.10 ^E
5	0.002±0.00 ^C	6.31±0.04 ^D	0.75±0.15 ^{ABCD}	20.39±0.21 ^B	2.15±0.10 ^{IJ}	7.44±0.10 ^G	21.70±0.06 ^A
6	0.008±0.00 ^A	6.14±0.01 ^E	0.59±0.06 ^{BCDE}	21.25±0.25 ^A	6.89±0.10 ^A	6.70±0.10 ^H	20.70±0.06 ^C
7	0.002±0.00 ^C	5.98±0.01 ^F	0.75±0.0 ^{ABCD}	13.60±0.10 ^J	1.86±0.10 ^J	8.12±0.10 ^F	15.81±0.10 ^I
8	0.002±0.00 ^C	6.23±0.04 ^{DE}	0.43±0.06 ^E	18.27±0.07 ^C	3.70±0.10 ^{DE}	9.95±0.05 ^B	21.20±0.06 ^B
9	0.002±0.00 ^C	6.23±0.03 ^{DE}	0.53±0.06 ^{DE}	16.15±0.10 ^{FG}	2.64±0.06 ^{GH}	7.38±0.06 ^{GH}	17.75±0.10 ^G
10	0.002±0.00 ^C	6.25±0.04 ^{DE}	0.82±0.10 ^{AB}	15.90±0.05 ^{FG}	3.42±0.10 ^{EF}	9.24±0.05 ^D	18.57±0.10 ^F
11	0.002±0.00 ^C	6.14±0.01 ^E	0.74±0.03 ^{ABCD}	18.37±0.10 ^C	3.25±0.06 ^F	9.54±0.06 ^C	20.85±0.10 ^C
12	0.009±0.00 ^A	5.77±0.08 ^G	0.55±0.02 ^{CDE}	16.74±0.10 ^E	3.84±0.10 ^D	9.17±0.02 ^D	19.67±0.10 ^E
13	0.002±0.00 ^C	6.01±0.02 ^F	0.86±0.03 ^A	15.38±0.10 ^H	3.33±0.20 ^F	9.68±0.10 ^C	18.46±0.10 ^F
14	0.001±0.00 ^D	6.27±0.02 ^D	0.51±0.06 ^{DE}	17.57±0.21 ^D	4.54±0.10 ^C	11.23±0.05 ^A	21.44±0.10 ^B
15	0.001±0.00 ^D	5.70±0.02 ^G	0.73±0.02 ^{ABCD}	15.78±0.21 ^{GH}	5.33±0.06 ^B	11.17±0.01 ^A	20.22±0.05 ^D

***SD-Standard deviation; TTA-Titratable acidity, pH-Hydrogen ion concentration, TSS-Total soluble solids, L*-Lightness, a*-(+) redness, b* (+) yellowness, ΔE -colour change; ***Means that do not share a letter in the same column are significantly different at $\alpha < 0.05$

The total titratable acidity (TTA) of the zoom-koom samples steeped for 2hrs ranged between 0.002 to 0.004(% lactic acid), those steeped for 7 h ranged between 0.002 to 0.008% and those steeped for 12h ranged between 0.002 to 0.009% (Table 10). In a study conducted by Soma et al. (2019) on zoom-koom produced with *Lactobacillus fermentum* starter culture reported that titratable acidity varied between 1.21 and 1.89 g of lactic acid / 100 g of zoom-koom product and Tapsoba et al., (2019) also between. The results of the present study (0.002 to 0.009 (% lactic acid)) are lower compared with the results reported by Soma et al. (2019) and that of the values obtained by Tapsoba et al., (2019) also varying from 0.25 to 0.39%. “In the two previous studies mentioned above, fermentation of the zoom-koom was done with starter culture of *Lactobacillus species* whiles in this study the millet went through spontaneous fermentation, and this could account for the differences in the results.

In many other studies of indigenous fermented beverages like *gowe*, *kunun-zaki*, *chicha de jora*, *champus* and *mahewu* reduced pH and increased TTA have been attributed to their fermented nature (Adinsi et al., 2017; Adinsi et al., 2015; Michodjehoun-Mestres et al., 2005; Oranusi et al., 2003; Elmahmood & Doughari, 2007; Oshoma et al., 2009, Aboh & Oladosu, 2014; Osorio-Cadavid et al., 2008, Gomes et al., 2009, Phiri et al., 2020, Basinskiene & Cizeikiene, 2020).

Fitting the data for TTA into a response surface regression model showed that all the effects (thus both interaction and quadratic effects of blend ratio (millet and spices) significantly influenced the model, with an R squared and adjusted R squared to be 78.37% and 39.43% respectively demonstrating that, the model was acceptable and precise to predict the responses. (Figure 6). The contour plot produced from the model (Figure 6) showed that increasing steeping time from 11.2 to 12h increased TTA with an increase in blend ratio from 50 to 60 with respect to the spices as seen in the darkest green region (Figure 6). Also, increased in blend ratio from 148 to 150 with respect to the spices and increase in steeping time from 2 to 2.4hrs also increased the TTA of the *zoom-koom*. Finally, an increase in blend ratio from 140 to 150 and its subsequent increase in steeping time from 11.5 to 12h also resulted in an increase in TTA as seen in the darkest green region of the contour plot (Figure 6)

Brix is the degree of the soluble solids content (simple sugars, dissolved proteins and other nitrogen compounds, etc) in the wort (Beulah et al., 2022). The brix of *zoom-koom* steeped for 2 hrs ranged between 0.53 °Brix to 0.74 °Brix, those steeped for 7 hrs ranged between 0.43 °Brix to 0.75 °Brix and for those steeped for 12h ranged between 0.55° Brix to 0.90° Brix (Table 10). The fitted regression model for °Brix (Figure 7), had an R-squared and an adjusted R-squared of 73.0% and 24.41% respectively shows that it was significantly influenced by the interaction effects of steeping temperature and blend ratio of steeped millet and spices (ginger, black pepper and gloves)

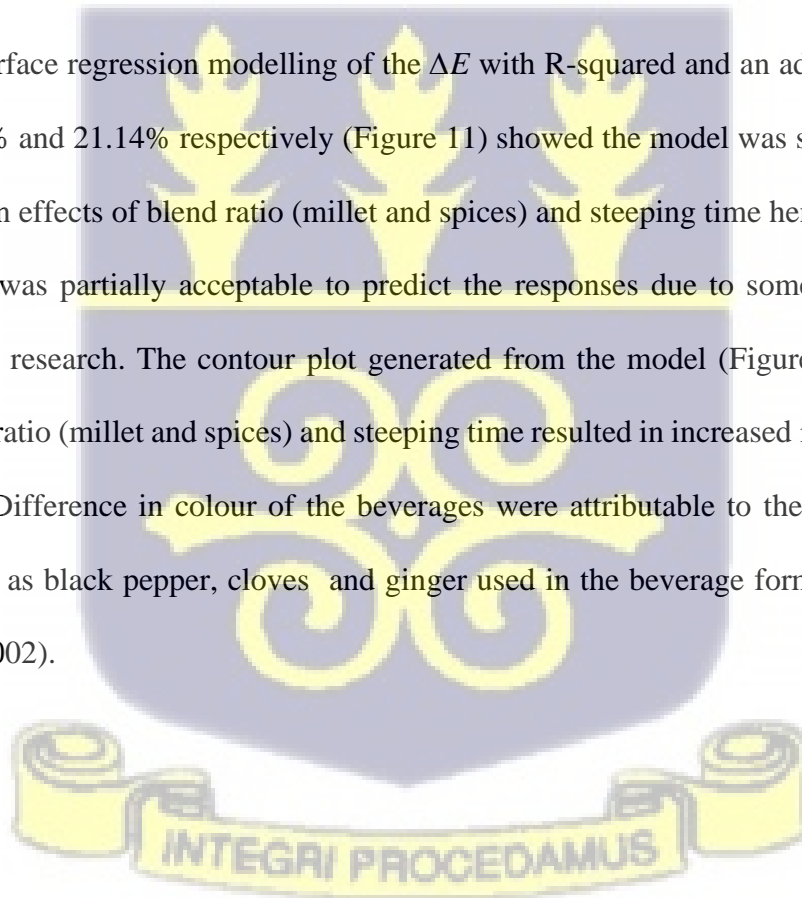
demonstrating that, the model was acceptable and precise to predict the responses. The contour plot (Figure 7) produced from the model confirms that brix was high at low blend ratio thus from 50 to 53 with respect to the spices and low and high steeping time from 2 to 2.4h and 11.2 to 12h, respectively. The brix values obtained from the laboratory processed *zoom-koom* compared to the values of some commercially processed *zoom-koom* may be as a result of the low sugar added to the wort. TSS of cereal based beverages reported by Akoma et al. (2014) and Danbaba et al. (2014) were much lower compared to the beverages in the present study because of the difference in ingredients used and the difference in processing parameters as fermentation reduced the TSS content.

The colour of the *zoom-koom*, measured as the lightness index L^* , ranged between 13.60 to 21.25, a^* index ranged from 1.5 to 5.33, b^* index ranged from 6.70 to 9.54 and the change in colour formation ranged from 15.81 to 21.70 (Table 10). *Zoom-koom* colour is a significant product property since it is one of the initial sensory parameters which people notice about the beverage and aids in product preference. The variation in unit operations, millet, blend ratio of the steeped millet, steeping time and temperature are important factors which influence the colour of the beverage.

Response surface regression modelling showed that the L^* value of the samples were not influenced by the quadratic effects of the blend ratio (millet and spices), with R-squared of 48.19% and adjusted R-squared also 17.21% respectively demonstrating less precision and hence the model cannot be fully relied upon in predicting the response (Figure 8). From the contour plot in Figure 8, increasing the blend ratio with respect to the millet and spices from 140 to 150 and increasing the steeping time from 6.2 to 12h resulted in a higher L value of the sample.

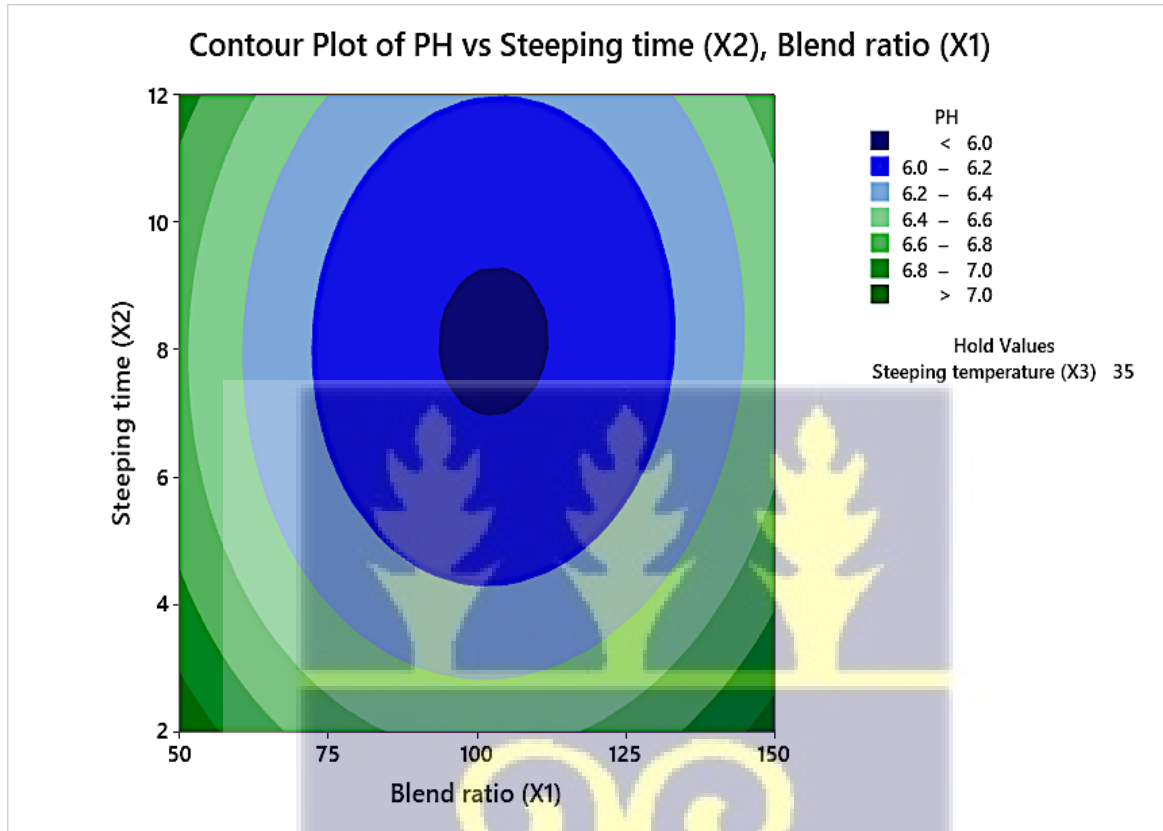
The Response surface regression modelling for the a^* and b^* with R-squared and adjusted R squared values of 71.03% and 18.89% (Figure 9), 70.95% and an adjusted R squared value of 18.67% (Figure 10), respectively. This showed that the models were significantly influenced by the interaction effects of blend ratio (millet and spices) and steeping time demonstrating that, the model was acceptable and precise to predict the responses. The contour plot generated from the model (Figure 9 & 10) showed that at a constant steeping temperature of 35°C, samples of *zoom-koom* steeped for 4 to 10.2 hrs at a blend ratio of 700: 85 to 700:150 with respect to the millet and the spices had higher a^* values whereas samples steeped for 5 to 9hrs at a blend ratio of 700: 110 to 700:150 with respect to the millet and the spices had higher b^* values.

The response surface regression modelling of the ΔE with R-squared and an adjusted R- squared values of 52.40% and 21.14% respectively (Figure 11) showed the model was slightly influenced by the interaction effects of blend ratio (millet and spices) and steeping time hence demonstrating that, the model was partially acceptable to predict the responses due to some sources of error encounter in the research. The contour plot generated from the model (Figure 11) showed that increased blend ratio (millet and spices) and steeping time resulted in increased in ΔE of the *zoom-koom* samples. Difference in colour of the beverages were attributable to the used of coloured ingredients such as black pepper, cloves and ginger used in the beverage formulations (Jayeola and Akinwale 2002).



4.4.1 Contour plots of the physicochemical properties of *zoom-koom*

Figure 5: Contour plots of pH of *zoom-koom* as a function of Blend ratio (X1) and Steeping time(X2).



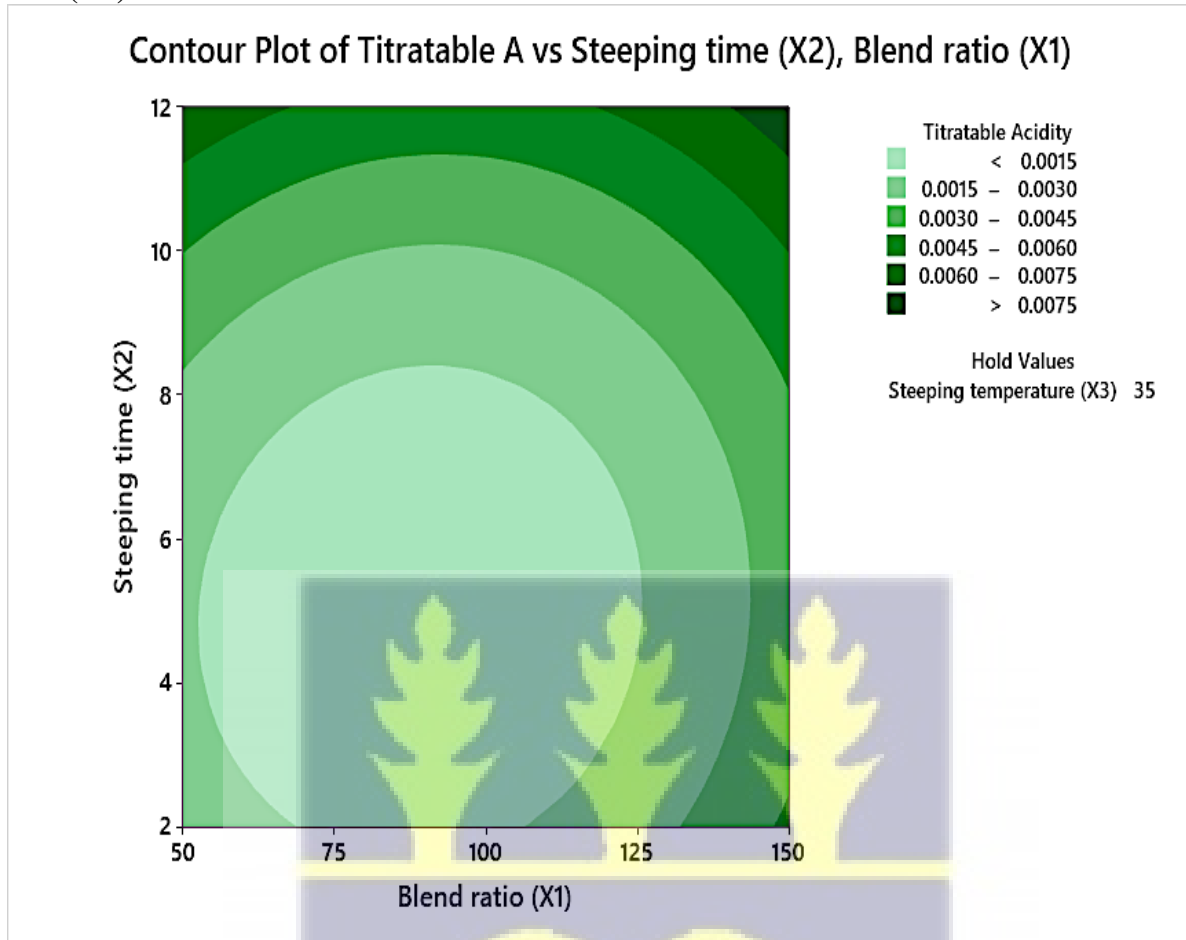
$$\text{pH} = 5.70 - 0.0438X_1 - 0.134X_2 + 0.2013X_3 + 0.000236X_1^2 + 0.01485X_2^2 - 0.00279X_3^2 - 0.000140X_1X_2 - 0.000100X_1X_3 - 0.00265X_2X_3$$

$$R^2 = 91.66\%, \text{ adjusted } R^2 = 76.65\%$$

X1-blend ratio, X2- steeping time, X3- steeping temperature

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Figure 6: Contour plots of TTA of *zoom-koom* as a function of Blend ratio (X1) and Steeping time(X2)



$$TTA = 0.0098 - 0.000051 X_1 - 0.00221 X_2 - 0.000076 X_3 + 0.000001 X_1^2 + 0.000104 X_2^2 + 0.000002 X_3^2 - 0.000001 X_1 X_2 - 0.000003 X_1 X_3 + 0.000036 X_2 X_3$$

$$R^2 = 78.37\%, \text{ adjusted } R^2 = 39.43\%$$

X1- blend ratio, X2- steeping time, X3- steeping temperature

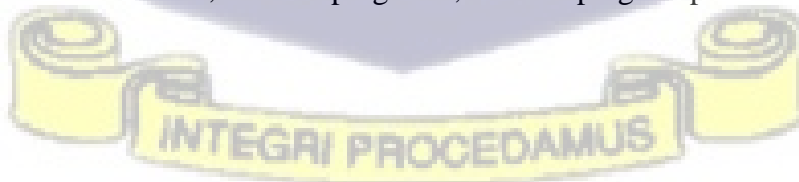
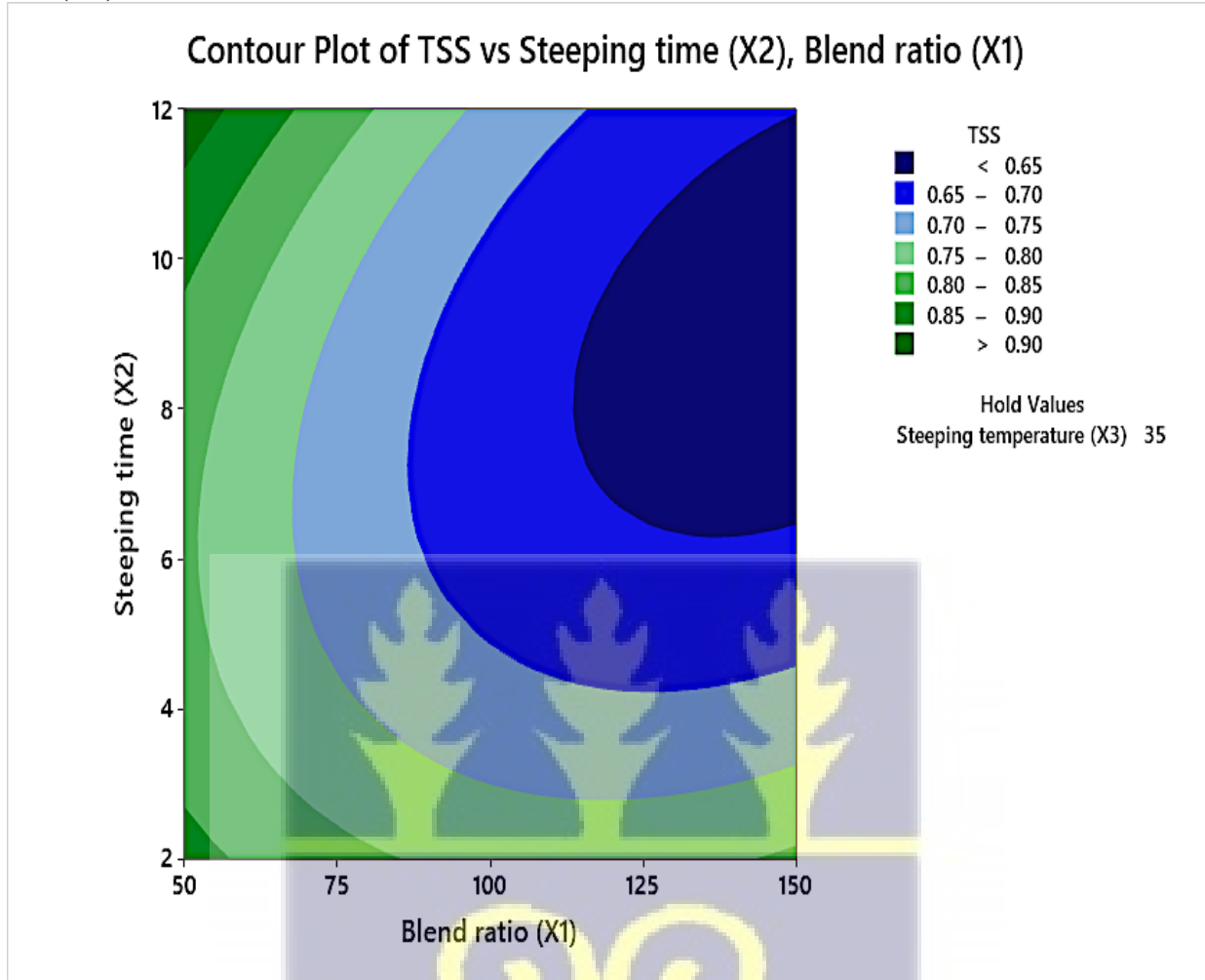


Figure 7: Contour plots of TSS of *zoom-koom* as a function of Blend ratio (X1) and Steeping time(X2).



$$\text{TSS} = 1.56 + 0.00092X_1 + 0.0458X_2 + 0.1219X_3 + 0.000021X_1^2 + 0.00358X_2^2 - 0.001304X_3^2 - 0.00220X_1X_2 - 0.000150X_1X_3 - 0.00225X_2X_3$$

$R^2 = 73.00\%$, adjusted $R^2 = 24.41\%$

X1- blend ratio, X2- steeping time, X3- steeping temperature

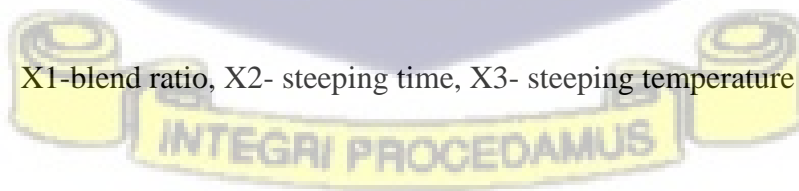
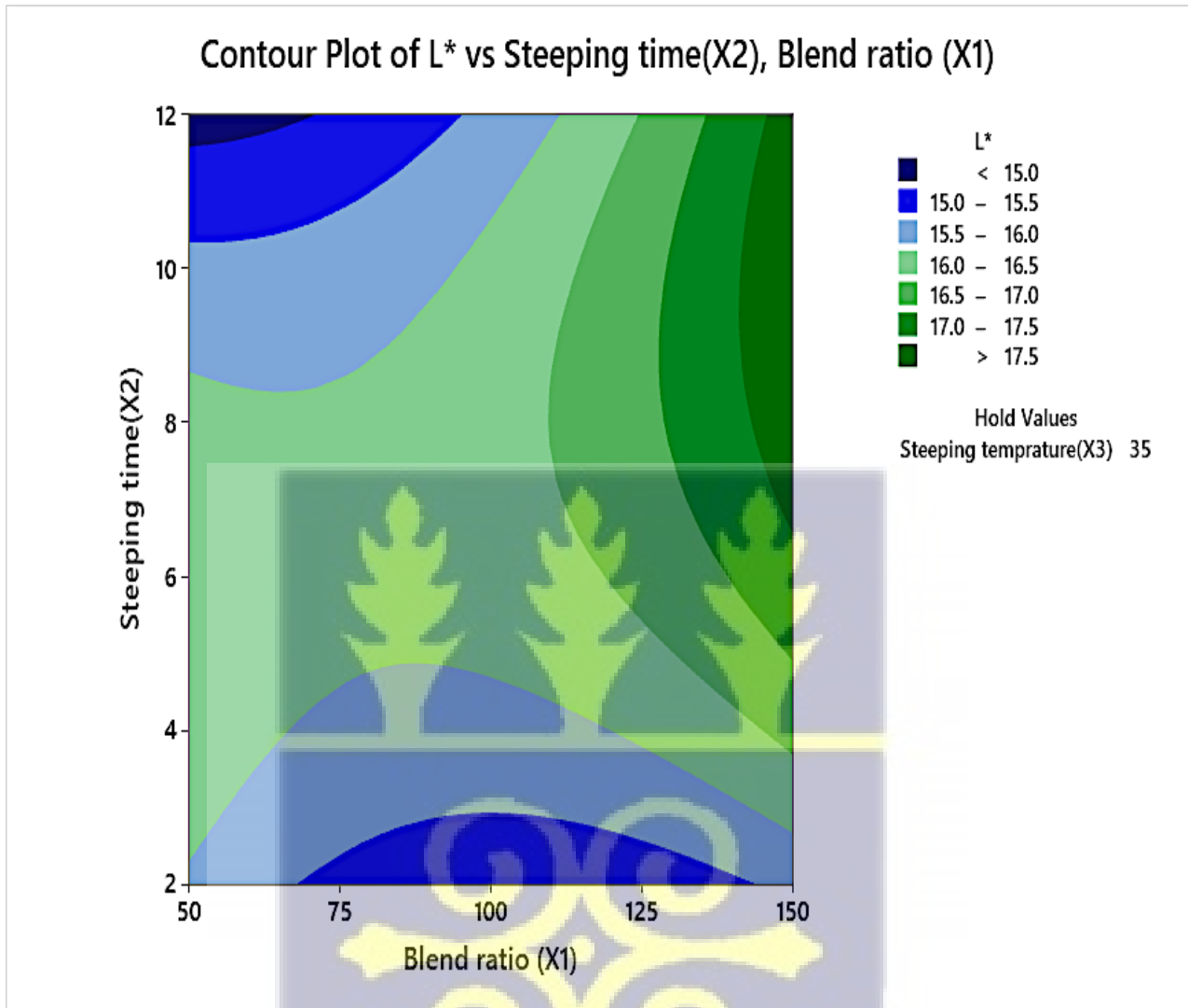


Figure 8: Contour plots of L* of *zoom-koom* as a function of Blend ratio (X1) and Steeping time(X2).

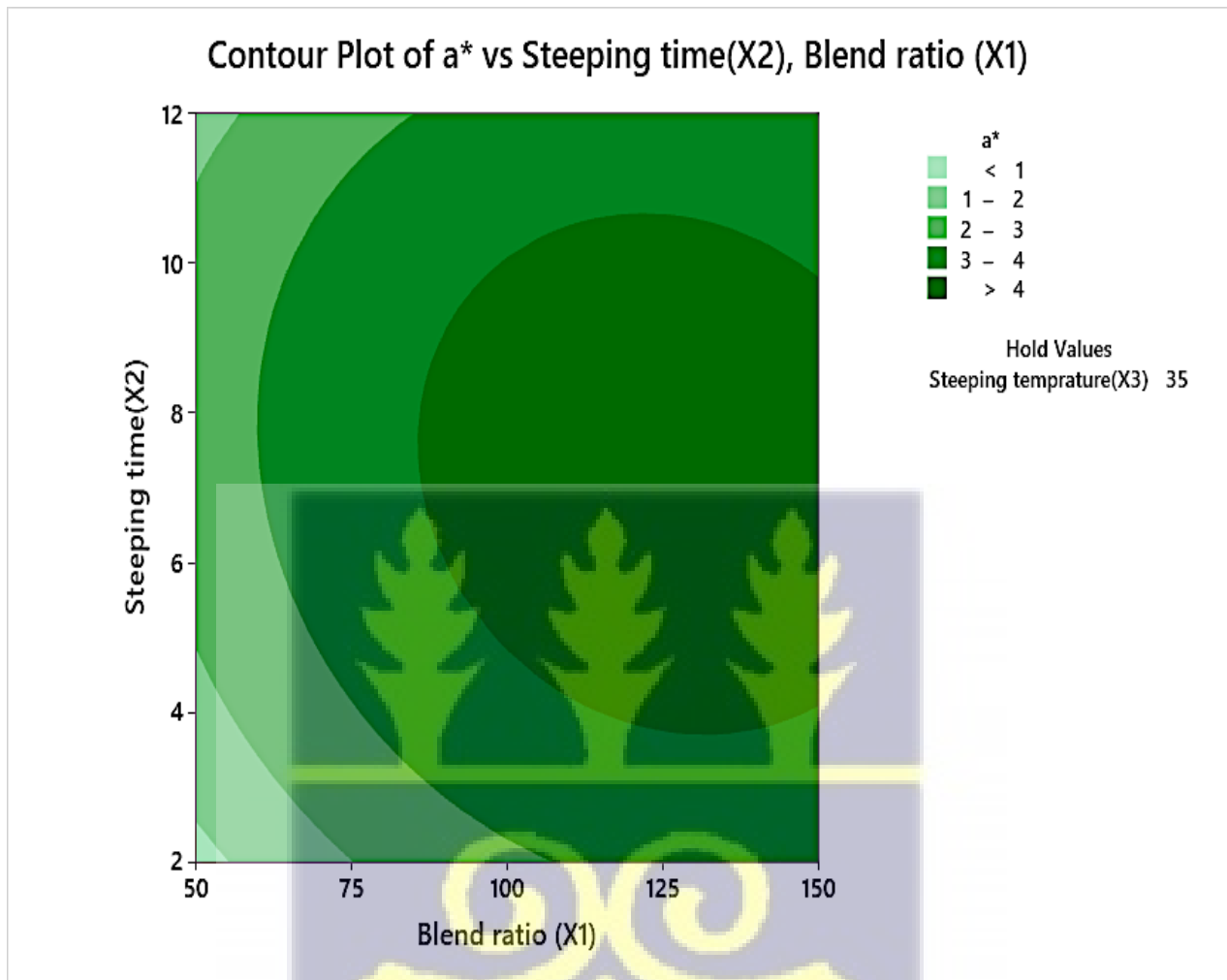


$$L^* = 42.7 - 0.131 X_1 + 0.46 X_2 - 1.195 X_3 + 0.000255 X_1^2 - 0.0368 X_2^2 + 0.0137 X_3^2 + 0.00323 X_1 X_2 + 0.00201 X_1 X_3 - 0.0061 X_2 X_3$$

$$R^2 = 48.19\%, \text{ adjusted } R^2 = 17.21\%$$

X1-blend ratio, X2- steeping time, X3- steeping temperature

Figure 9: Contour plots of a^* of *zoom-koom* as a function of Blend ratio (X1) and Steeping time(X2).

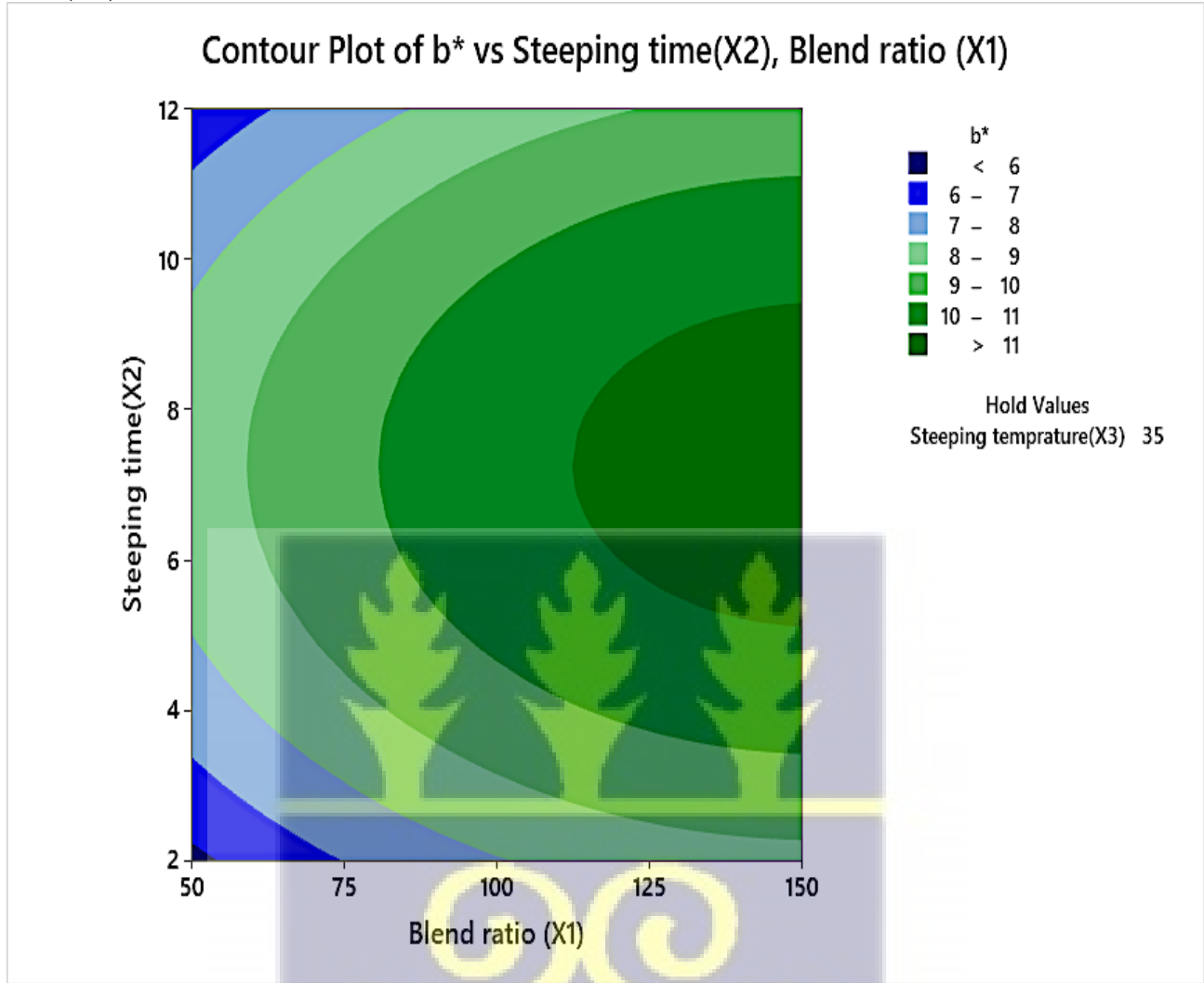


$$a^* = -6.3 + 0.1526X_1 + 0.899X_2 - 0.042X_3 - 0.000365X_1^2 - 0.0508X_2^2 + 0.00245X_3^2 - 0.00103X_1X_2 + 0.00151X_1X_3 - 0.0011X_2X_3$$

$$R^2 = 71.03\%, \text{ adjusted } R^2 = 18.89\%$$

X1-blend ratio, X2- steeping time, X3- steeping temperature

Figure 10: Contour plots of b^* of *zoom-koom* as a function of Blend ratio (X_1) and Steeping time(X_2).

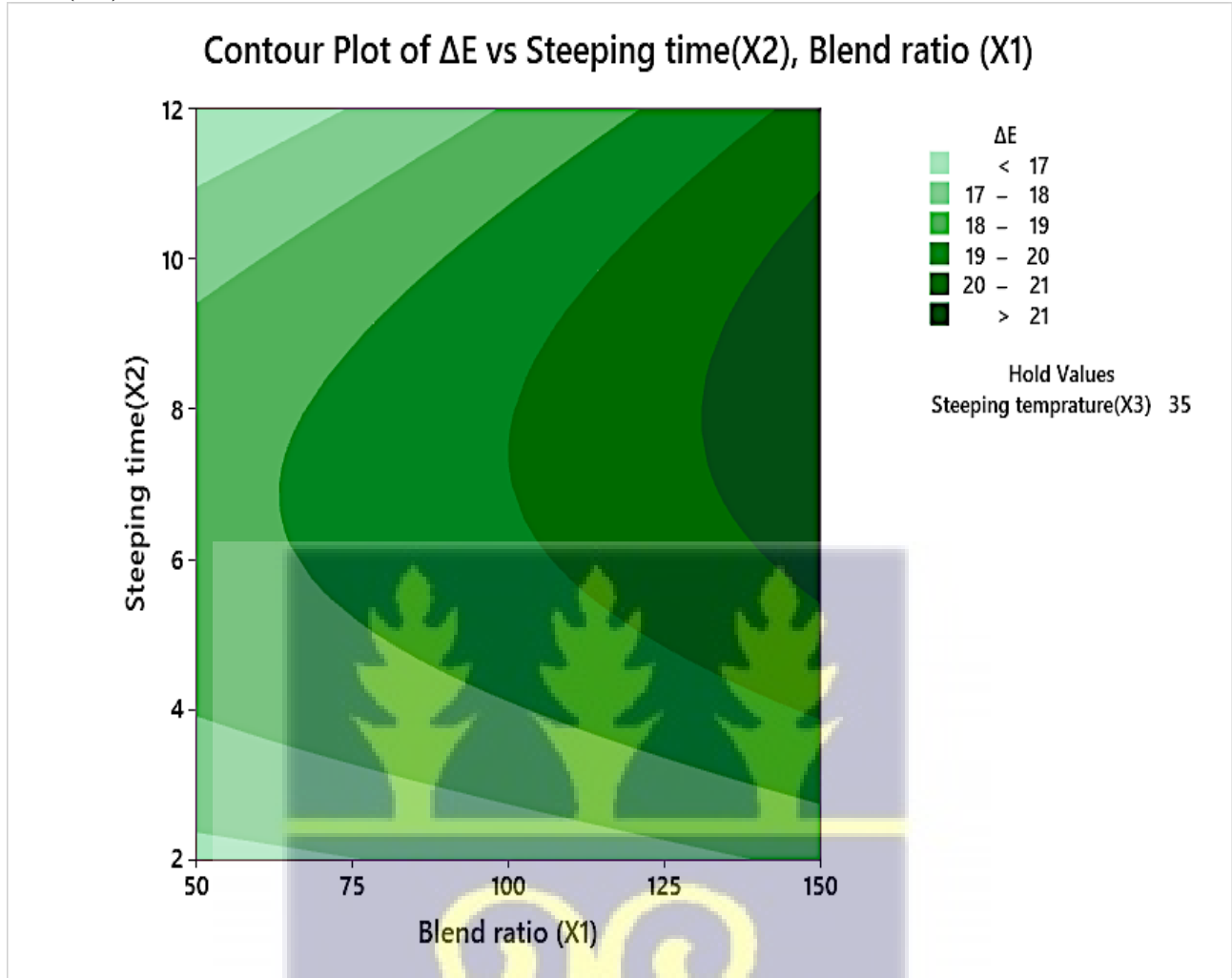


$$b^* = -6.8 + 0.215 X_1 + 1.82 X_2 - 0.021X_3 + 0.000280 X_1^2 - 0.0985 X_2^2 + 0.0059 X_3^2 - 0.00002 X_1X_2 - 0.00369 X_1X_3 - 0.0111 X_2X_3$$

$$R^2=70.95\%, \text{ adjusted } R^2=18.67\%$$

X_1 -blend ratio, X_2 - steeping time, X_3 - steeping temperature

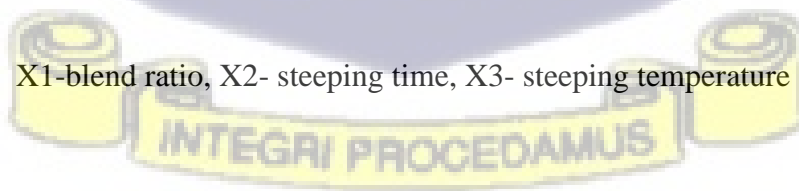
Figure 11: Contour plots of ΔE * of *zoom-koom* as a function of Blend ratio (X1) and Steeping time(X2).



$$\Delta E = 33.3 + 0.011X_1 + 1.45X_2 - 1.07X_3 + 0.000056X_1^2 - 0.0913X_2^2 + 0.0153X_3^2 + 0.00276X_1X_2 - 0.00037X_1X_3 - 0.0105X_2X_3$$

$$R^2 = 52.40\%, \text{ adjusted } R^2 = 21.14\%$$

X1- blend ratio, X2- steeping time, X3- steeping temperature



4.5 Influence of process variables on the microbiological quality of laboratory-produced *zoom-koom*.

Aerobic Plate count for *zoom-koom* samples from steeping for 2 h, ranged between 2.83 to 3.45 log cfu/ml, whilst those steeped for 7 h ranged 1.30 to 3.22 log cfu/ml and those steeped for 12hrs ranged between 1.70 to 3.18 log cfu/ml (Table 11). Yeasts and moulds count ranged between 0.66 to 2.11 log cfu/ml for those steeped for 2 h whilst those steeped for 7 h ranged between 1.01 to 2.42 log cfu/ml and those steeped for 12 h also ranged 1.18 to 3.45 log cfu/ml (Table 14). *S. aureus* count also ranged between 2.15 to 2.18 log cfu/ml for those steeped for 2 h whilst those steeped for 7 hrs ranged between 0.66 to 2.49 log cfu/ml and those steeped for 12 h ranged 2.32 to 3.24 log cfu/ml. Yeast and moulds, *S. aureus* was not detected in some of the *zoom-koom* variants (Table 11). It has been observed that the main microorganism in other indigenous beverages such as in gowe, kunun-zaki, chicha de jora, mahewu are yeasts and moulds (Adinsi et al. 2017, Adinsi et al. 2015, Michodjehoun-Mestres et al. 2005). Yeasts convert sugars into ethanol, carbon dioxide and other useful metabolites. They also produce volatile organic compounds such as alcohols, esters, aldehydes and ketones which may influence the sensory properties of the fermented cereal product and also help to decrease mould growth and spore formation (Fredlund et al., 2004).

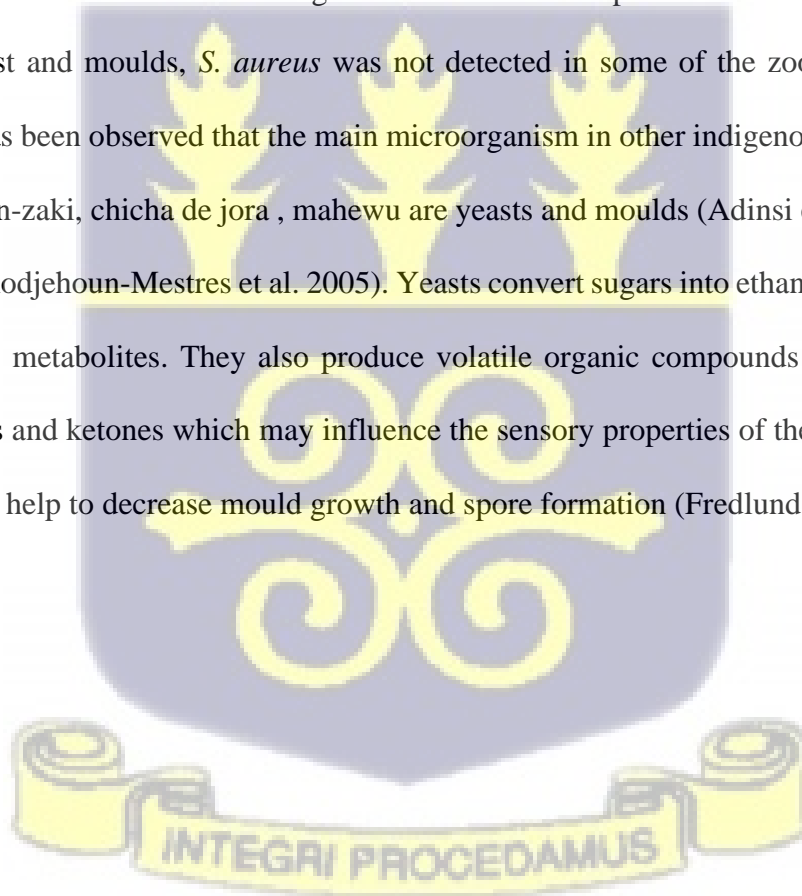


Table 11: Microbiological quality of laboratory produced zoom-koom

Variants	TPC	Yeast & Moulds	<i>S. aureus</i>	Total Coliform	<i>E. coli</i>
1	2.83±0.34 ^{BC}	2.11±0.03 ^{CD}	2.18±0.05 ^E	N. D	N. D
2	3.17±0.05 ^{AB}	N. D	2.15±0.02 ^E	N. D	N. D
3	3.18±0.05 ^{AB}	2.08±0.10 ^{CD}	3.24±0.1 ^A	N. D	N. D
4	2.73±0.08 ^C	1.91±0.02 ^{DE}	N. D	N. D	N. D
5	3.22±0.10 ^A	2.14±0.10 ^C	2.49±0.10 ^{BC}	N. D	N. D
6	2.30±0.10 ^D	1.78±0.10 ^E	N. D	N. D	N. D
7	2.14±0.10 ^D	1.80±0.06 ^E	N. D	N. D	N. D
8	2.31±0.10 ^D	1.75±0.10 ^E	N. D	N. D	N. D
9	3.18±0.07 ^{AB}	2.22±0.05 ^{BC}	2.59±0.1 ^B	N. D	N. D
10	3.12±0.10 ^{ABC}	1.80±0.10 ^E	2.42±0.10 ^{BCD}	N. D	N. D
11	3.45±0.05 ^A	0.66±0.05 ^G	N. D	N. D	N. D
12	1.70±0.05 ^{EF}	3.48±0.10 ^A	2.32±0.1 ^{CDE}	N. D	N. D
13	1.68±0.02 ^{EF}	2.42±0.10 ^B	N. D	N. D	N. D
14	1.30±0.06 ^F	1.01±0.01 ^F	N. D	N. D	N. D
15	1.99±0.06 ^{DE}	1.83±0.01 ^E	2.23±0.1 ^{DE}	N. D	N. D

***SD-Standard deviation; ND- Not detected, TPC-Total plate count; ***Means that do not share a letter in the same column are significantly different at $\alpha < 0.05$

Total coliform and *E. coli* were also not detected in all the fifteen (15) laboratory produced zoom-koom which are within the satisfactory limit of 4 log cfu/ml and 4 log cfu/ml respectively according to the Ghana standard authority (GSA) microbiological standard for ready to eat foods. Non-detection of total coliform and *E. coli* and the absence of pathogenic bacteria such as *S. aureus* may be attributed to the strict hygienic measures observed during processing.

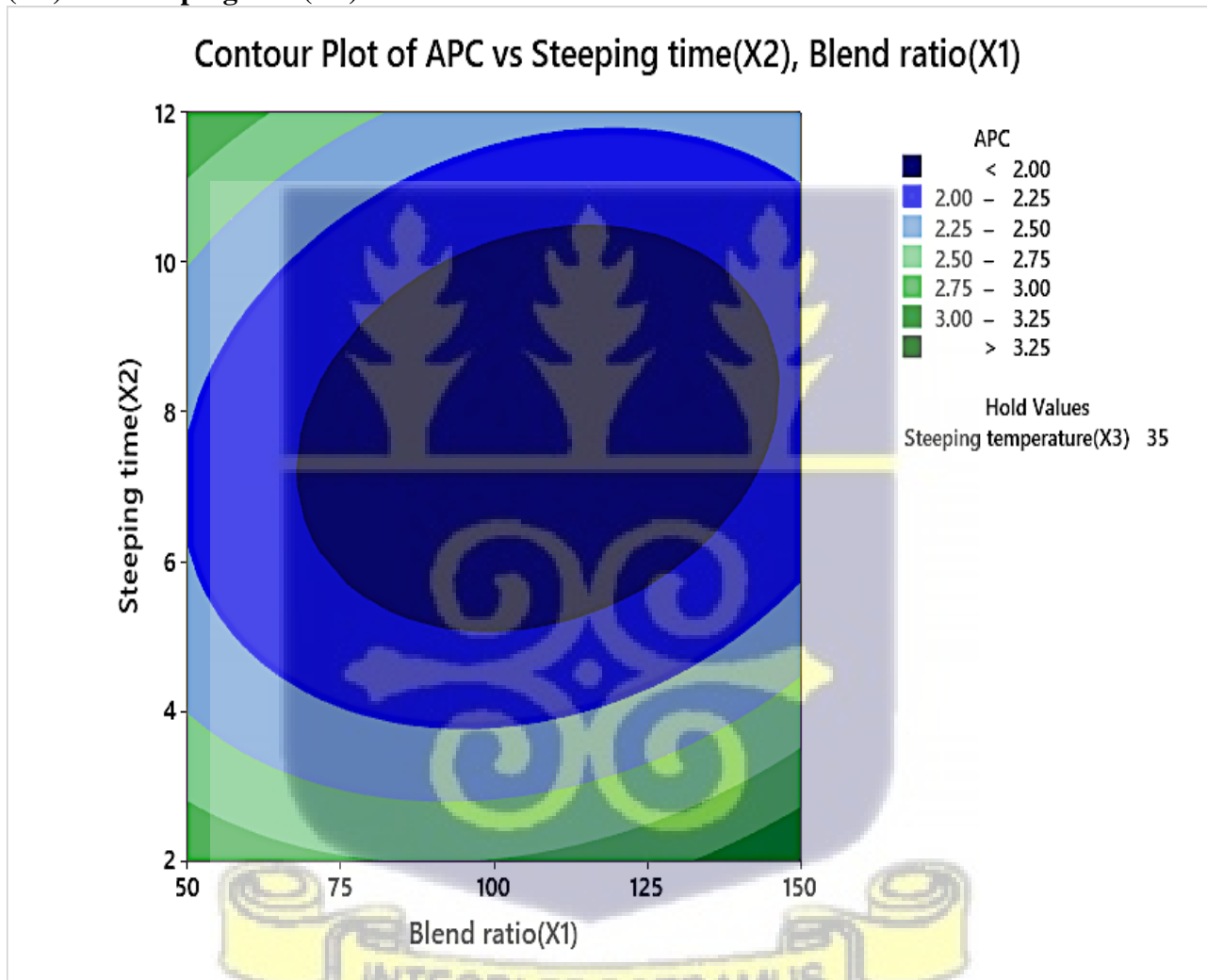
A regression, model output was attained to display the influence of process variables on the microbiological quality of laboratory-produced as presented in Figure 13 and 14. There was no regression output for *E. coli*, Total coliform and *S. aureus* since all the fifteen (15) zoom-koom experimental samples mostly had no counts (0).

The interaction effects of the blend ratio & steeping time as well as the quadratic effects of the steeping temperature and blend ratio (millet and spices) significantly influenced the model.

The process variables significantly influenced Aerobic plate count of *zoom-koom* and the model was able to explain 82.15% and 50.03% for R- square and adjusted R- squared values, demonstrating that, the model was acceptable and precise to predict the responses. Figure 12. As shown in the contour plot in Figure 12, increasing the blend ratio with respect to the spices from 130 to 150 at a steeping time of 2 to 2.6hrs, decreased the Aerobic plate counts. Consequently, at high blend ratio with respect to the spice, increasing the steeping time had marginal effects on the Aerobic plate counts. The process variables also significantly influenced the *Staphylococcus aureus* and yeast and moulds count since their models were able to explain 79.96% and 80.98%, respectively of their variation (Figure 13 and 14).

The models of the *S. aureus* count and yeast and moulds counts were used to generate contour plots (Figure 13 and 14) which explains that decreasing blend ratio with respect to the spices from 50 to 55 and increase in steeping time from 11.2 to 12h significantly decreased the *S. aureus* count. Also, an increased in blend ratio with respect to the spices from 55 to 150 and subsequent increase in steeping time resulted in decreased yeast and moulds counts. The interaction effects of blend ratio and steeping time as well as the quadratic effects of steeping temperature and blend ratio significantly influenced the model considering the R-square and adjusted R-square values of 79.96% and 43.89% for *S. aureus* count and also a R-square and adjusted R-square values of 80.98% and 46.73% respectively and in instance demonstrating that, the model was acceptable and precise to predict the responses.

Figure 12: Contour plots of Aerobic plate Count of *zoom-koom* as a function of Blend ratio (X1) and Steeping time(X2).

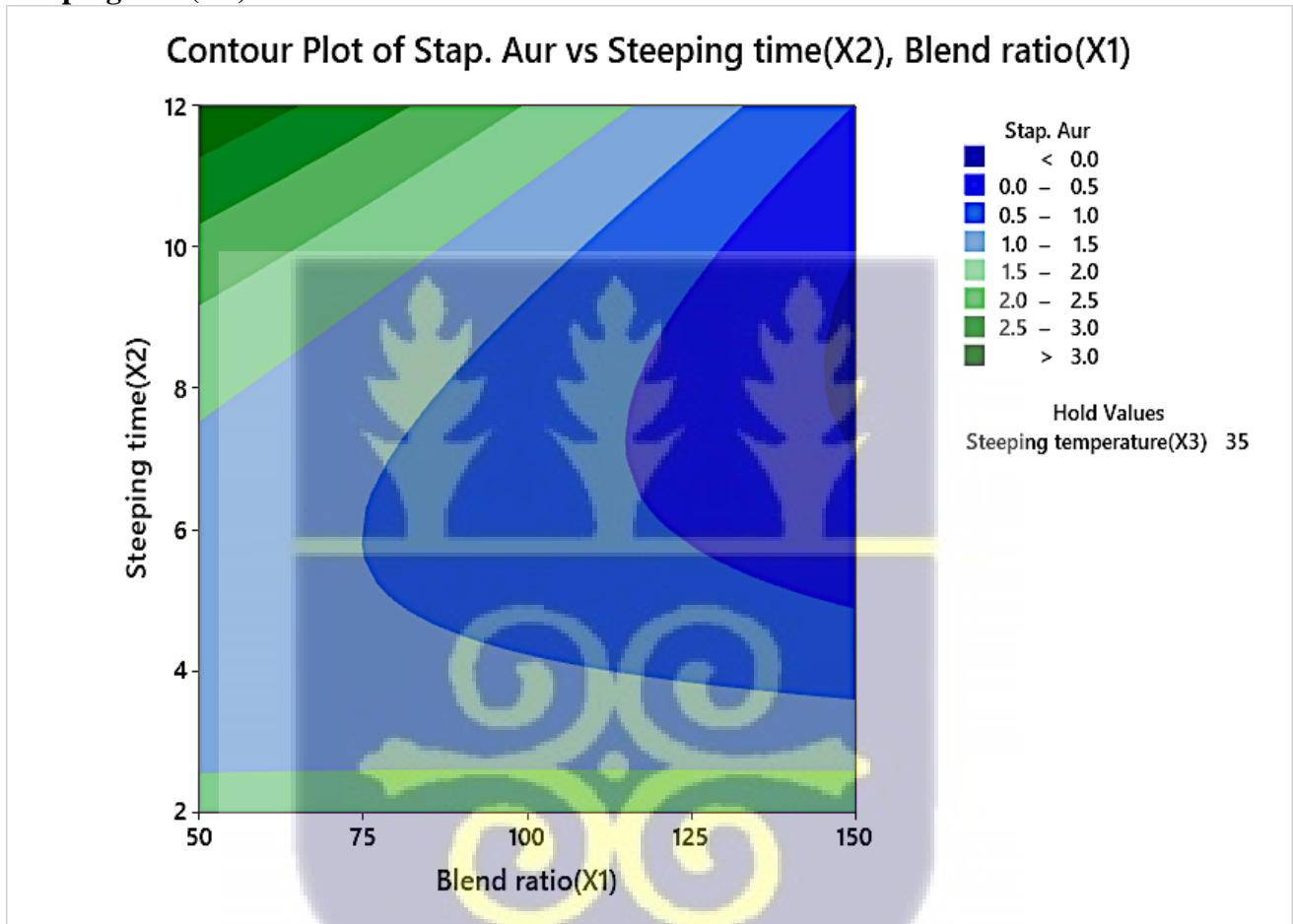


$$APC = 8.23 - 0.0415X_1 - 0.061X_2 - 0.196X_3 + 0.000142X_1^2 + 0.02984X_2^2 + 0.00256X_3^2 - 0.000857X_1X_2 + 0.000505X_1X_3 - 0.00891X_2X_3$$

$R^2=82.15\%$, adjusted $R^2=50.03\%$

X1-blend ratio, X2- steeping time, X3- steeping temperature

Figure 13: Contour plots of *S. aureus* of zoom-koom as a function of Blend ratio (X1) and Steeping time(X2).

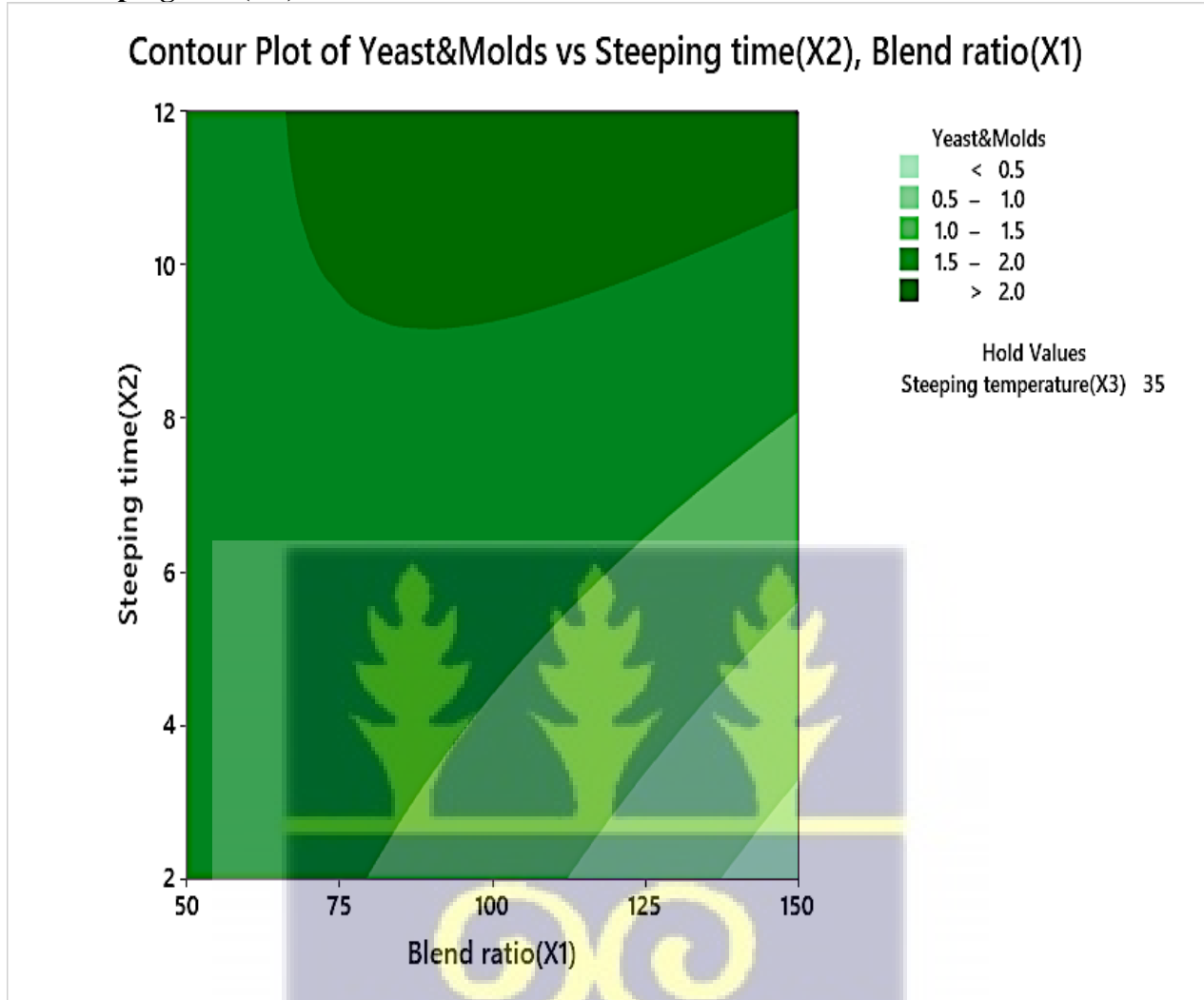


$$S. \text{ aureus} = 9.98 - 0.0328X_1 - 0.725X_2 - 0.194X_3 - 0.000004X_1^2 + 0.0460X_2^2 - 0.00102X_3^2 - 0.00314X_1X_2 + 0.001195X_1X_3 + 0.01195X_2X_3$$

$$R^2=79.96\%, \text{ adjusted } R^2=43.89\%$$

X1-blend ratio, X2- steeping time, X3- steeping temperature

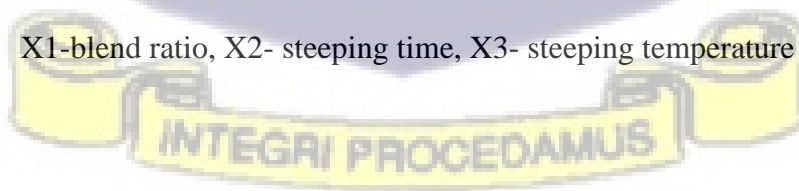
Figure 14: Contour plots of Yeast & moulds of *zoom-koom* as a function of Blend ratio (X1) and Steeping time(X2).



$$\text{Yeast\& Molds} = 9.94 - 0.0100X_1 - 0.612X_2 - 0.319X_3 - 0.000080X_1^2 - 0.0027X_2^2 + 0.00272X_3 + 0.00198X_1X_2 + 0.000174X_1X_3 + 0.01580X_2X_3$$

$$R^2=80.98\%, \text{ adjusted } R^2=46.73\%$$

X1-blend ratio, X2- steeping time, X3- steeping temperature



4.6 Influence of process variables on bioactive properties of laboratory-produced *zoom-koom*.

Total phenolic compounds for *zoom-koom* steeped for 2 h, ranged between 11.54 to 15.36mg/ml, whilst those steeped for 7 h ranged amongst 12.83 to 15.52mg/ml, and those steeped for 12hrs also ranged between 11.79 to 21.03mg/ml (Table 12). Antioxidant activity (%inhibition) ranged between 82.36 to 96.01%, for those steeped for 2 hrs whilst those steeped for 7 hrs ranged between 85.91 to 97.54%, and those steeped for 12 hrs ranged 88.32 to 96.82% (Table 12). Antioxidants are substances that neutralize the harmful free radicals in our bodies. Antioxidants act as “free radical scavengers” and hence prevent or slow the damage done by these free radicals. Antioxidants function as reducing agents, ultimately eliminating free radical intermediates and inhibiting further oxidation (Phang et al., 2013). Some phenolic compounds are known to exhibit some antioxidant properties. As anticipated, the results showed increases in quantities of phenolic compounds and their subsequent increase in antioxidant activity. The addition of the spices (ginger, cloves and black pepper) also resulted in an increase in phenolic compounds of the *zoom-koom* samples. A surface regression model output was used to display the influence of process variables on the bioactive quality of laboratory-produced *zoom-koom* in (Figure 15 and 16). The interaction effects of the blend ratio and steeping time as well as the quadratic effects of the steeping time and blend ratio (spices) significantly influenced the model.



Table 12: Bioactivity of fifteen (15) zoom-koom experimental samples

Variants	TPC (mg/ml)	A.A (% inhibition)
1	15.36±0.13 ^{BC}	82.36±1.00 ^H
2	14.47±0.10 ^{BCD}	84.46±0.15 ^{GH}
3	15.31±0.10 ^{BC}	94.38±1.00 ^{BCD}
4	21.03±0.58 ^A	88.32±1.00 ^F
5	15.20±0.58 ^{BC}	95.37±0.58 ^{ABCD}
6	14.02±0.58 ^{CDE}	85.91±1.00 ^G
7	15.52±0.58 ^B	94.06±0.58 ^{CD}
8	12.88±0.58 ^{EFG}	90.79±1.16 ^{EF}
9	13.11±0.58 ^{DEF}	90.37±0.58 ^E
10	12.83±0.58 ^{EFG}	93.52± 0.28 ^D
11	11.54±0.58 ^G	96.01±0.58 ^{ABC}
12	11.79±0.58 ^{FG}	96.82±1.00 ^A
13	12.83±0.58 ^{EFG}	96.41±0.03 ^{AB}
14	14.77±0.46 ^{BC}	96.20±0.06 ^{ABC}
15	13.93±0.58 ^{CDE}	97.54±0.58 ^A

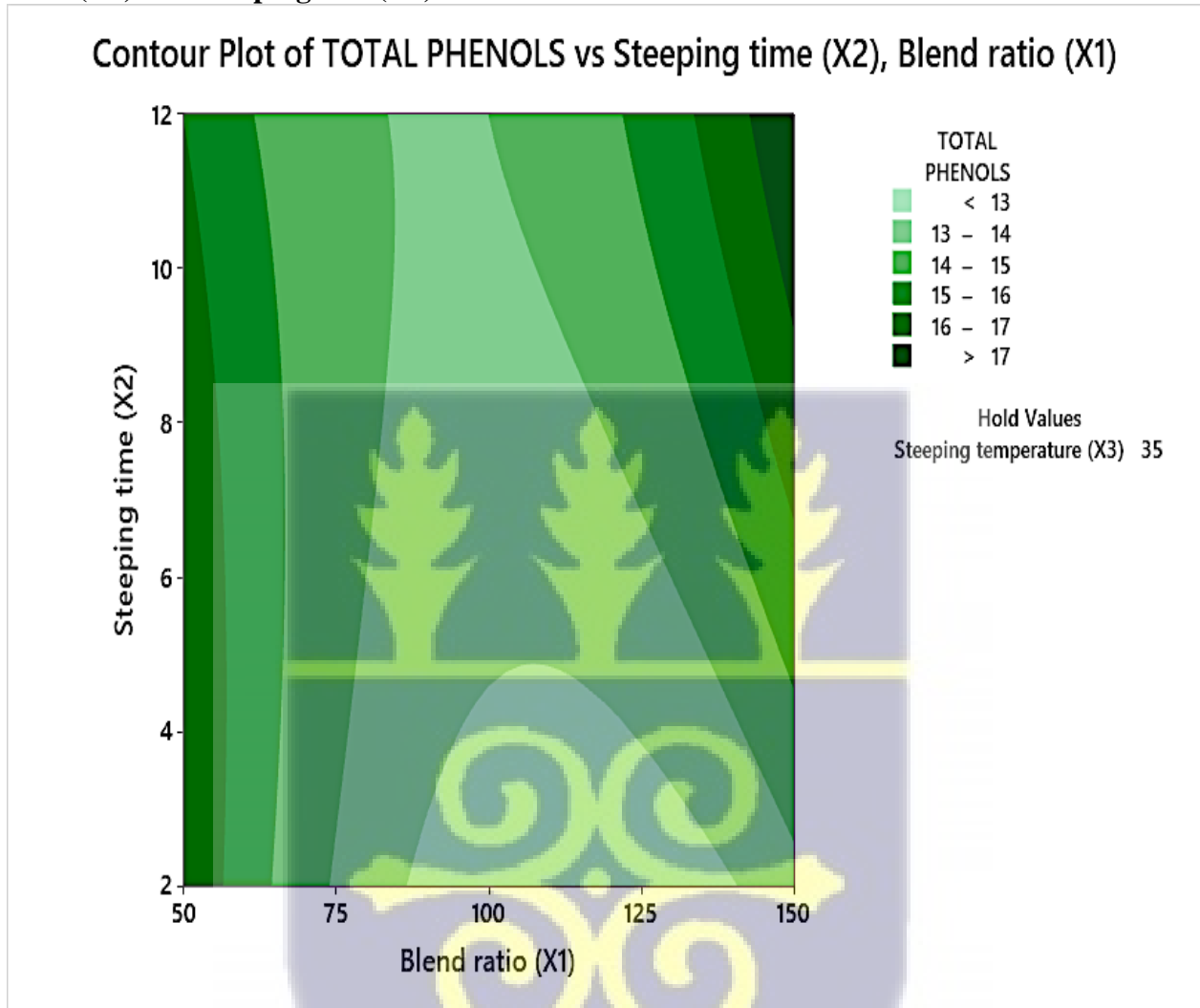
***SD-Standard deviation; Total phenolic compounds, A.A-Antioxidant activity; ***Means that do not share a letter in the same column are significantly different at $\alpha < 0$.

The process variables significantly influenced total phenolic compounds of the zoom-koom by explaining 85.70% and 59.97% for both R-square and adjusted R-squared values demonstrating that, the model was acceptable and precise to predict the responses. of the variations in Figure 15. From the contour plot in Figure 16, increasing blend ratio with respect to the spices from 140 to 150 at a steeping time of 9 to 12 h increased the total phenolic compounds.

The process variables also significantly influenced the antioxidant activity of the zoom-koom samples since their model was able to explain 89.80% and 21.44% variation with respect to both R-square and adjusted R-squared demonstrating that, the model was acceptable and precise to predict the responses. (figure 4.14). The model of the antioxidant activity was used to generate contour plots (figure 4.14) which explains that increasing the blend ratio with respect to the spices

from 55 to 150 and increase in steeping time from 6 to 12h significantly increased the antioxidant activity of the samples from 85% to 95%.

Figure 15: Contour plots of Total phenolic compounds of *zoom-koom* as a function of Blend ratio (X1) and Steeping time(X2).

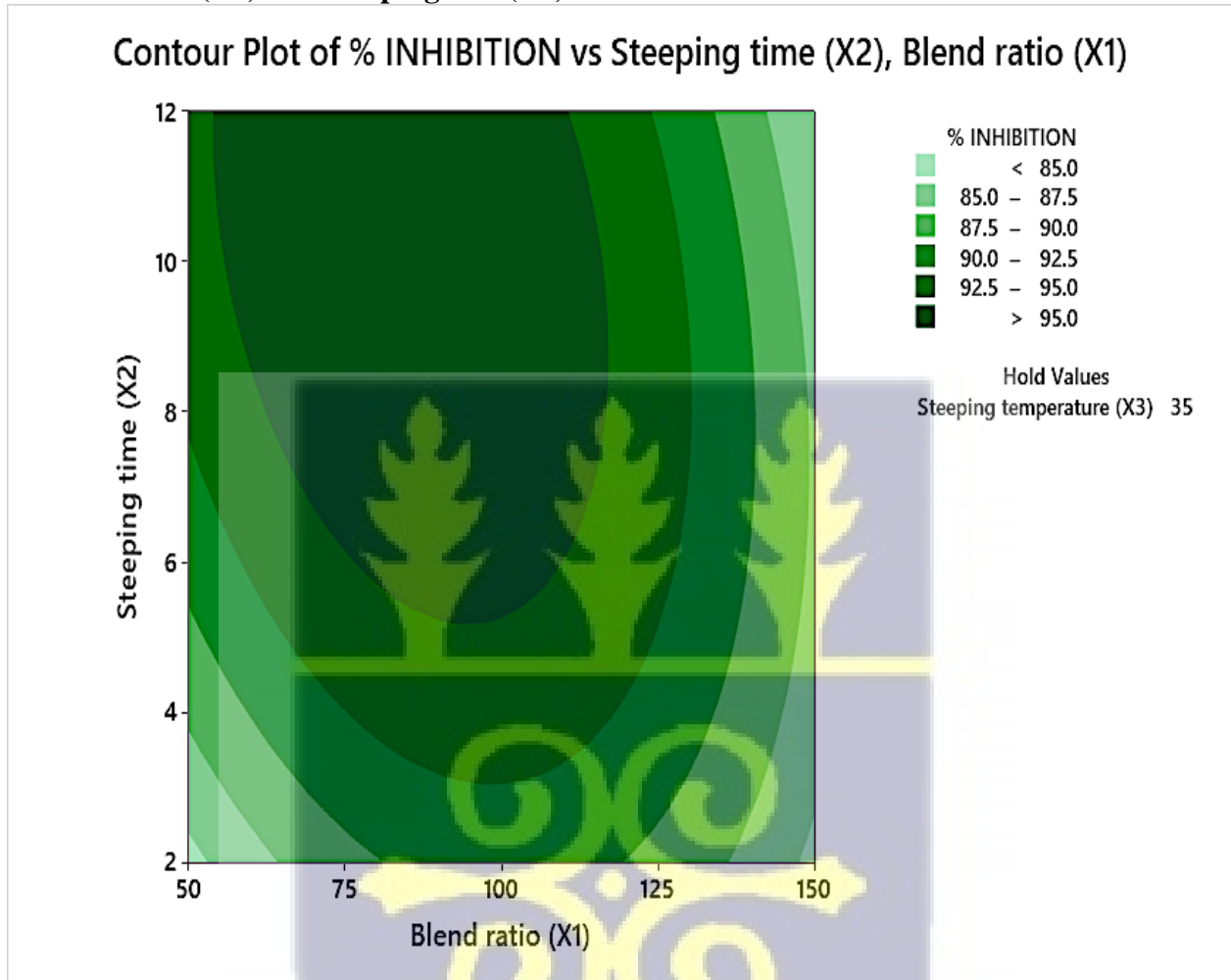


$$\text{TPC} = 1.5 - 0.2485X_1 - 0.590X_2 + 1.509 X_3 + 0.001183X_1^2 - 0.0116X_2^2 - 0.02161X_3^2 + 0.00519X_1X_2 - 0.00088X_1X_3 + 0.0114X_2X_3$$

$$R^2=85.70\%, \text{ adjusted } R^2=59.97\%$$

X1-blend ratio, X2- steeping time, X3- steeping temperature

Figure 16: Contour plots of Antioxidant activity (%Inhibition) of *zoom-koom* as a function of Blend ratio (X1) and Steeping time(X2).



$$\% \text{Inhibition} = 65.6 + 0.451X_1 + 3.13 X_2 - 0.23X_3 - 0.002672X_1^2 - 0.1044X_2^2 + 0.0009X_3^2 - 0.00960X_1 X_2 + 0.00297 X_1X_3 - 0.0050X_2X_3$$

$$R^2=89.80\%, \text{ adjusted } R^2=21.44\%$$

X1-blend ratio, X2- steeping time, X3- steeping temperature

4.7 Consumer acceptability of laboratory produced zoom-koom

Generally, the *zoom-koom* samples were scored using a 9-point hedonic scale by a panellist of 105 consumers recruited from the University of Ghana community. For *zoom-koom*, the most relevant sensory attributes were appearance, taste, aroma, flavour, mouthfeel, aftertaste, overall acceptability. From the results in Table 16, the overall likeness ranged between 7 and 8. The scores for appearance ranged from 7 to 7, and the scores for aroma ranged from 7 to 8. Equally, the scores for flavour varied from 6 to 7, the mouthfeel and aftertaste ranged from 6 to 7 and 6 to 7, respectively. The overall liking also ranged from 7 to 8 all on a 9-point hedonic scale. The score ranges of all the attributes from the consumer's assessment of the *zoom-koom* showed it was moderately liked. The influence of process variables on the attribute liking scores of the samples were modelled using response surface regression and the coefficients of the explanatory variables with equivalent R-squared values are presented in Table 14.

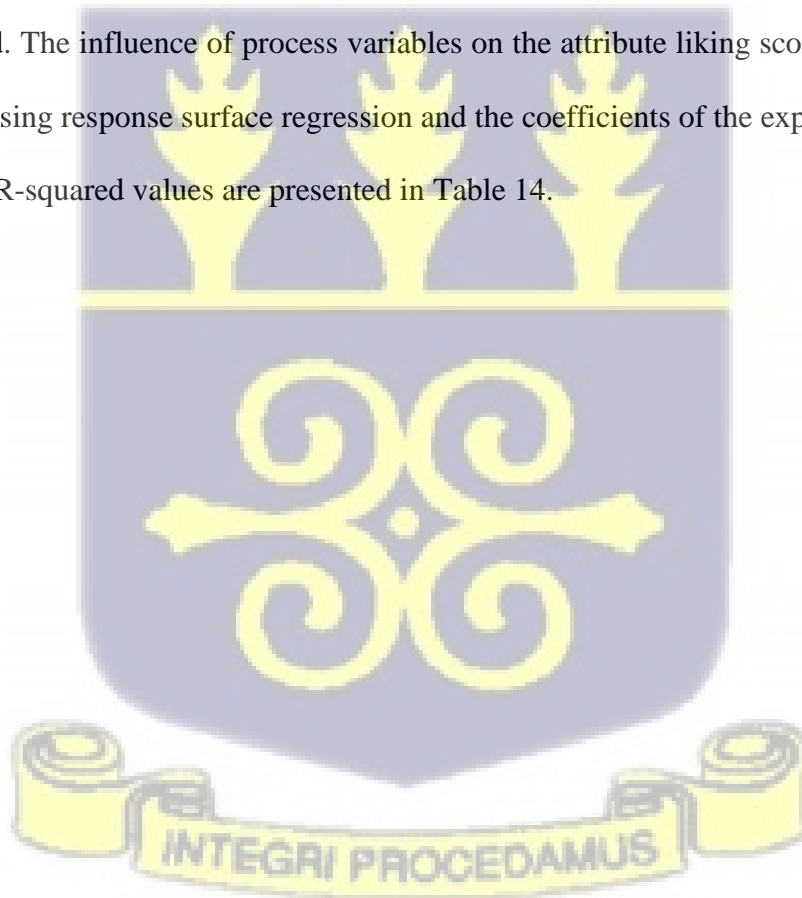


Table 13: Attribute liking scores of laboratory produced *zoom-koom*

Variant	Appearance	Aroma	Flavour	Mouthfeel	Aftertaste	Overall Acceptability
1	7	7	7	7	7	8
2	7	7	6	7	6	7
3	7	8	6	7	6	8
4	7	7	6	7	6	7
5	7	7	7	6	6	7
6	7	8	7	7	7	7
7	7	7	7	7	7	7
8	7	7	6	7	6	7
9	7	7	7	7	6	7
10	7	7	7	7	7	7
11	7	7	7	7	7	8
12	7	7	7	7	7	7
13	7	7	7	6	6	7
14	7	8	6	6	6	7
15	7	8	7	6	6	7

***1=dislike extremely; 2= dislike very much; 3= dislike moderately; 4= dislike slightly; 5= either like nor dislike; 6= like slightly; 7= like moderately; 8= like very much; 9= like extremely.

***Means scores that do not share a letter in the same column are significantly different at $\alpha < 0.05$.

4.8: Modelling of the sensory attributes of laboratory produced *zoom-koom*

Response surface regression models were used to fit the data for the appearance, aroma, flavour, mouthfeel, aftertaste, and overall acceptability scores (Table 14). Analysis of variance of the regression models revealed that the lack of fit, which measures the suitability of the selected model, was significant for the sensory attributes of flavour, mouthfeel, aftertaste and overall acceptability (Table 14). This indicated that the models were useful for predicting those responses, considering the high R- squared and adjusted R- squared values demonstrating that, the models generated were acceptable and precise to predict the responses. Some responses (flavour, mouthfeel, aftertaste, and overall liking) had high coefficients of determination (R^2), which means that a significant

amount of variability was explained by the data. The low r-square values for some of the attributes (appearance and taste) were low signalling that their model cannot be very reliable under this condition and hence the model cannot be reliable and precise in predicting the responses. The models for the various sensory attributes were used to generate contour plots (Figure 17– 22).

Table 14: Regression parameters of the model

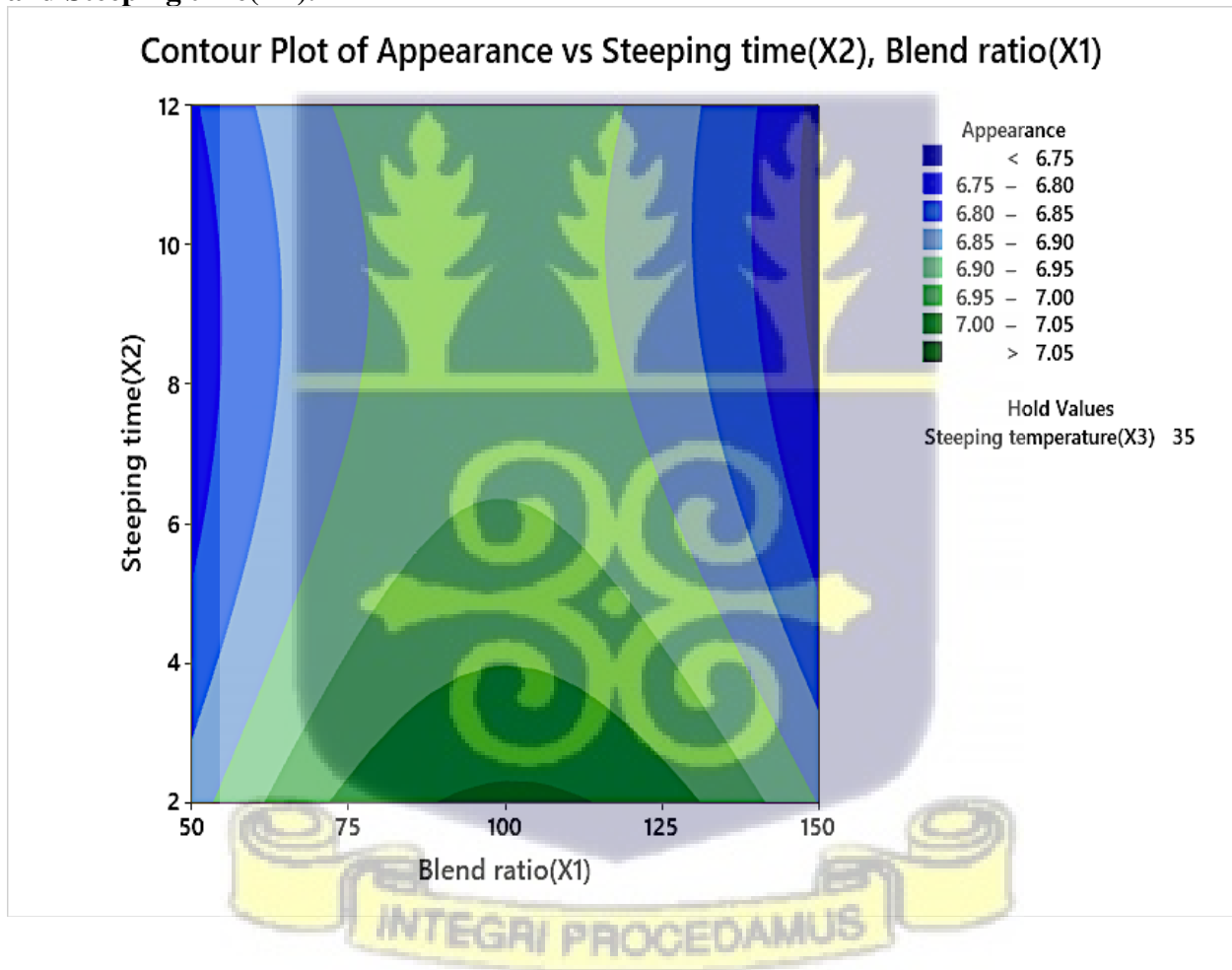
Variables/factors	Appearance	Aroma	Flavour	Mouthfeel	Aftertaste	Overall Acceptability
Constant	6.48	5.12	8.09	7.91	8.59	8.26
X ₁	12.3×10 ⁻³	0.033	0.025	0.025	0.009	0.020
X ₂	-0.019	-0.029	0.021	0.016	0.008	-0.003
X ₃	-0.004	0.027	-0.147	-0.171	-0.155	-0.134
X ₁ X ₂	-8.1×10 ⁻⁵	-2.8×10 ⁻⁴	3.5×10 ⁻⁴	-2.40×10 ⁻⁴	2.0×10 ⁻⁴	4.7.0×10 ⁻⁴
X ₁ X ₃	5.6×10 ⁻³	-4.3×10 ⁻⁴	-3.6×10 ⁻⁴	-3.85×10 ⁻⁴	-3.95×10 ⁻⁴	-2.95×10 ⁻⁴
X ₂ X ₃	5.0×10 ⁻⁴	1.4×10 ⁻³	-6.0×10 ⁻⁴	0	-2.03×10 ⁻³	-1.05×10 ⁻³
X ₁ ²	6.9×10 ⁻⁵	-8.3×10 ⁻⁵	-7.8×10 ⁻⁵	-4.7×10 ⁻⁵	1.5×10 ⁻⁵	-7.1×10 ⁻⁵
X ₂ ²	2.3×10 ⁻³	7.0×10 ⁻⁴	-4.02×10 ⁻³	8.5×10 ⁻⁴	3.07×10 ⁻³	-1.52×10 ⁻³
X ₃ ²	6.0×10 ⁻⁵	7.0×10 ⁻⁵	2.61×10 ⁻³	0.003	2.94×10 ⁻³	0.003
R ²	26.87%	44.96%	83.20%	74.58%	73.44%	79.20%
R ² (adjusted)	14.12%	26.12%	52.97%	28.83%	25.62%	34.14%

X₁: Blend ratio, X₂: Steeping time (hours), X₃- steeping temperature (°C)

4.8.1 Appearance

The contour plots show that increasing the blend ratio with respect to the spices from 85 to 110 and steeping time from 2 to 2.2h increases the appearance quality parameter of the *zoom-koom* samples from 6.75 to 7.05 (Figure 17). The low R-squared and adjusted R squared values (Table 14) for the sensory quality parameter “appearance” demonstrates that the model is not precise and unreliable under this condition.

Figure 17: Contour plots for the appearance of *zoom-koom* as a function of Blend ratio (X1) and Steeping time(X2).

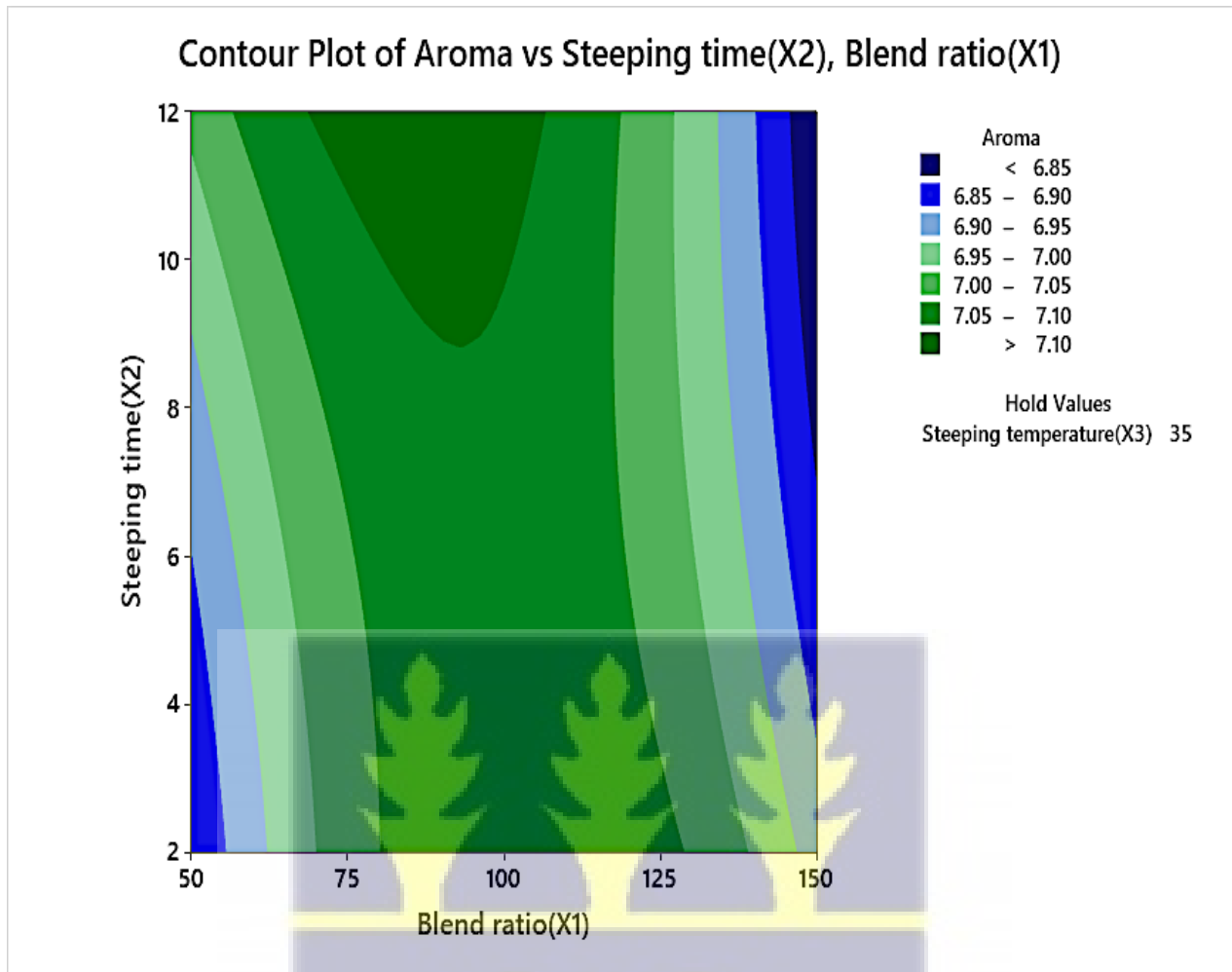


4.8.2 Aroma

The contour plots for aroma show that increasing the blend ratio with respect to the spices from 76 to 120 and an increase in steeping time from 8.2 to 12h increases the aroma of the *zoom-koom* samples from 6.85 to 7.10 (Figure 18). The low R-squared and adjusted R squared values (Table 14) for the sensory quality parameter “aroma” demonstrates that the model is not precise and unreliable under this condition.

Figure 18: Contour plots for the aroma of *zoom-koom* as a function of Blend ratio (X1) and Steeping time(X2).

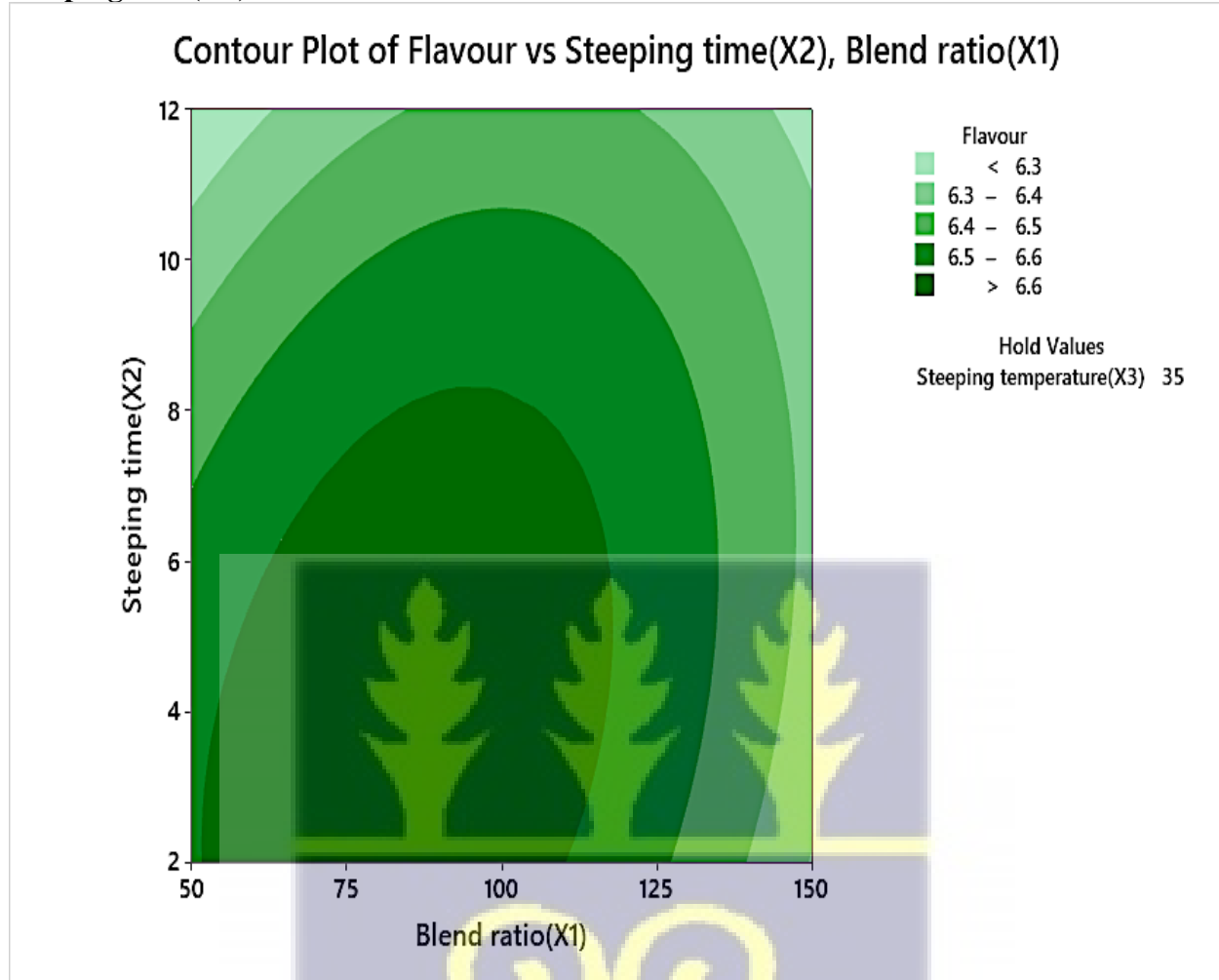




4.8.3 Flavour

The contour plots for flavour showed that increasing the blend ratio with respect to the spices from 52 to 110 and an increase in steeping time from 2 to 8hrs increased the likeness for the flavour of the *zoom-koom* samples from 6.3 to 6.8 (Figure 19). The high R-squared and adjusted R-square values (Table 14) for the “flavour” quality sensory parameter is an indication that the model can be reliable under this condition due to its precision.

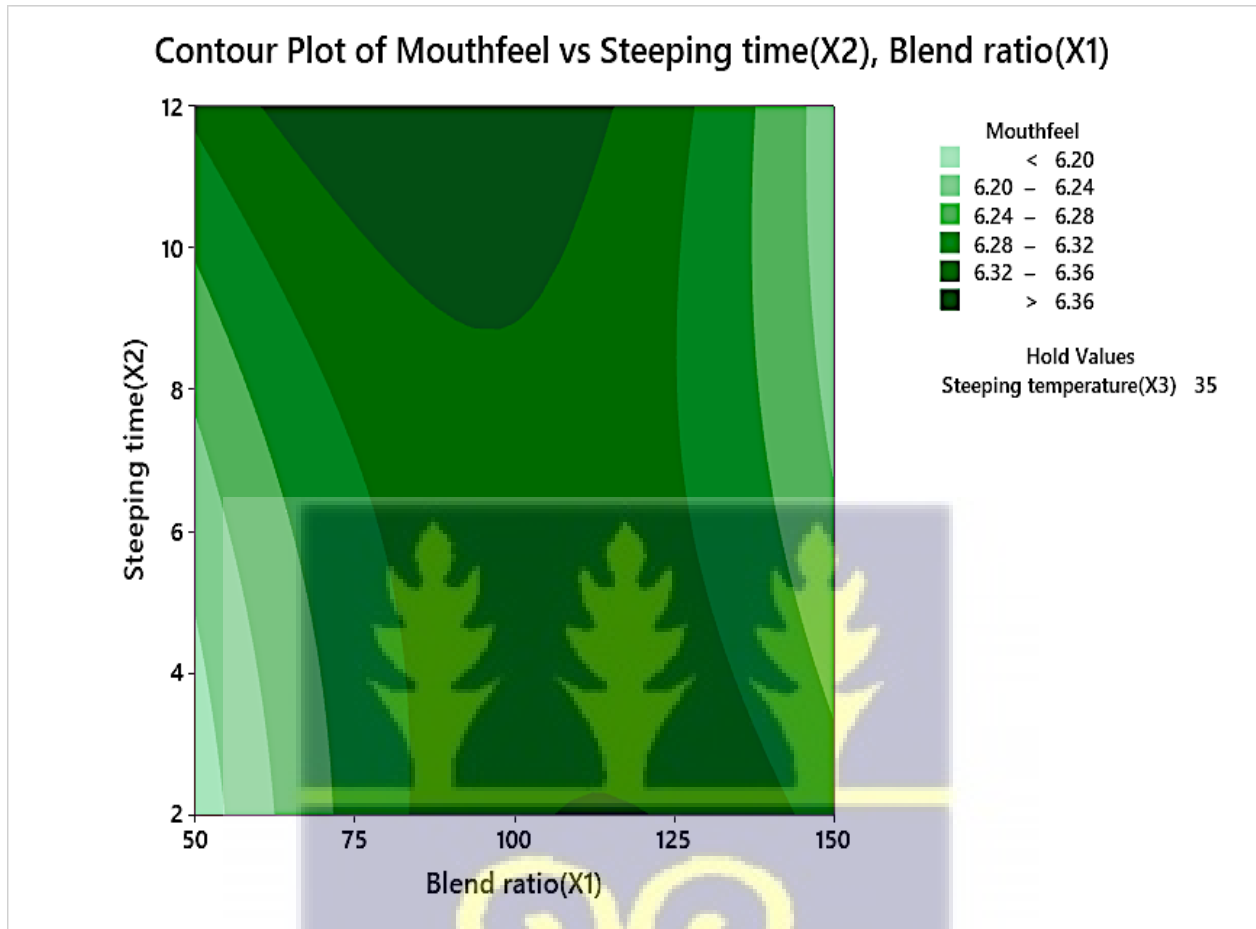
Figure 19: Contour plots for the flavour of *zoom-koom* as a function of Blend ratio (X1) and Steeping time(X2).



4.8.4 Mouthfeel

The contour plots for mouthfeel show that increasing the blend ratio with respect to the spices from 115 to 120 and a slightly increased in steeping time from 2 to 2.4h increases the mouthfeel of the *zoom-koom* samples from 6.20 to 6.36 (Figure 20). Also, an increase in the blend ratio with respect to the spices from 70 to 120 and an increased in steeping time from 9 to 12h increased the flavour of the *zoom-koom* samples. The high R-squared and adjusted R-square values (Table 14) for the “mouthfeel” quality sensory parameter is an indication that the model can be reliable under this condition due to its precision.

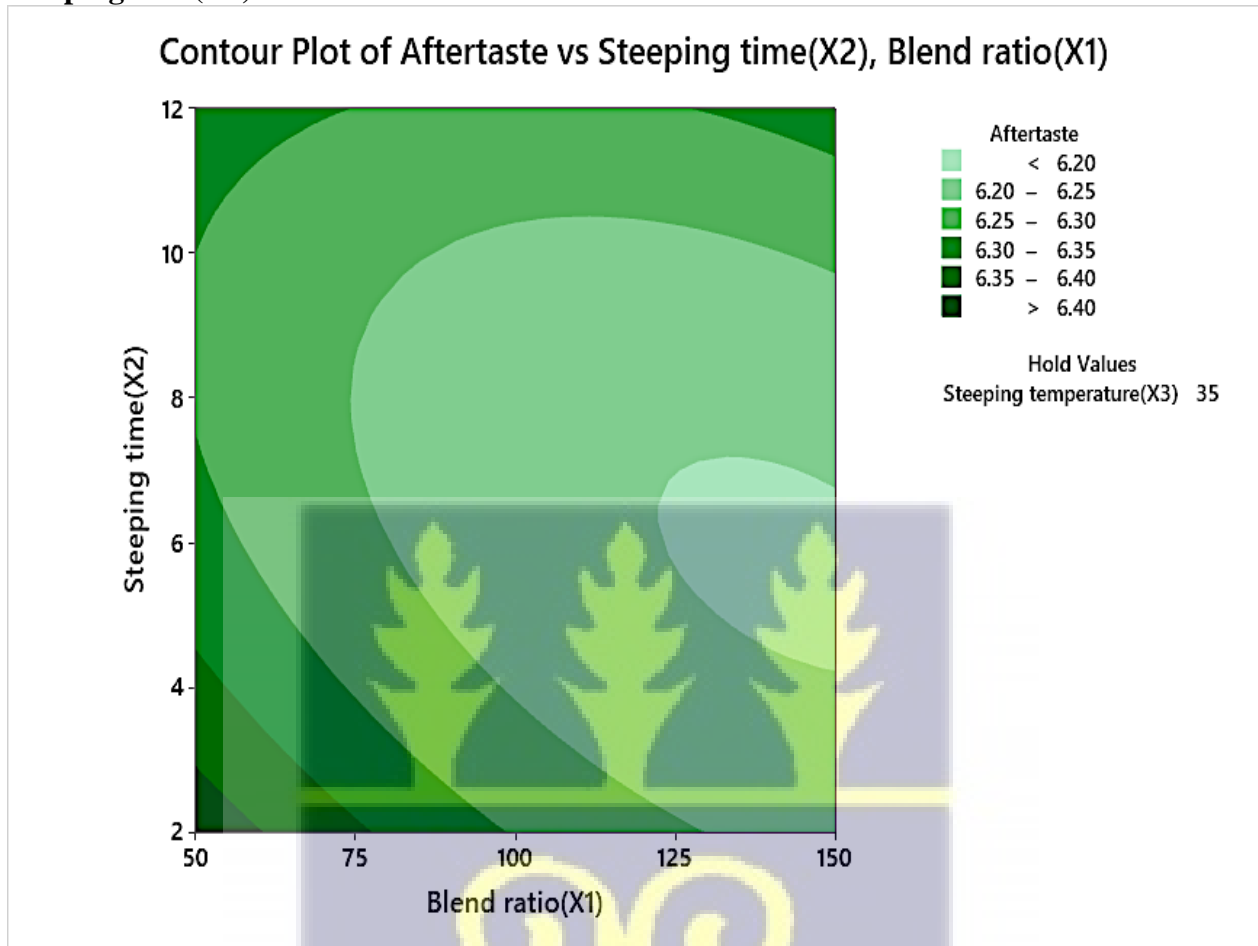
Figure 20: Contour plots for the mouthfeel of *zoom-koom* as a function of Blend ratio (X1) and Steeping time(X2).



4.8.5 Aftertaste

The contour plots for aftertaste show that slightly increasing the blend ratio with respect to the spices from 50 to 60 and slightly increasing in steeping time from 2 to 2.8h increased the aftertaste of the *zoom-koom* samples from 6.20 to 6.40 (Figure 21). The high R-squared and adjusted R-square values (Table 14) for the “aftertaste” quality sensory parameter is an indication that the model can be reliable under this condition due to its precision.

Figure 21: Contour plots for the aftertaste *zoom-koom* as a function of Blend ratio (X1) and Steeping time(X2).

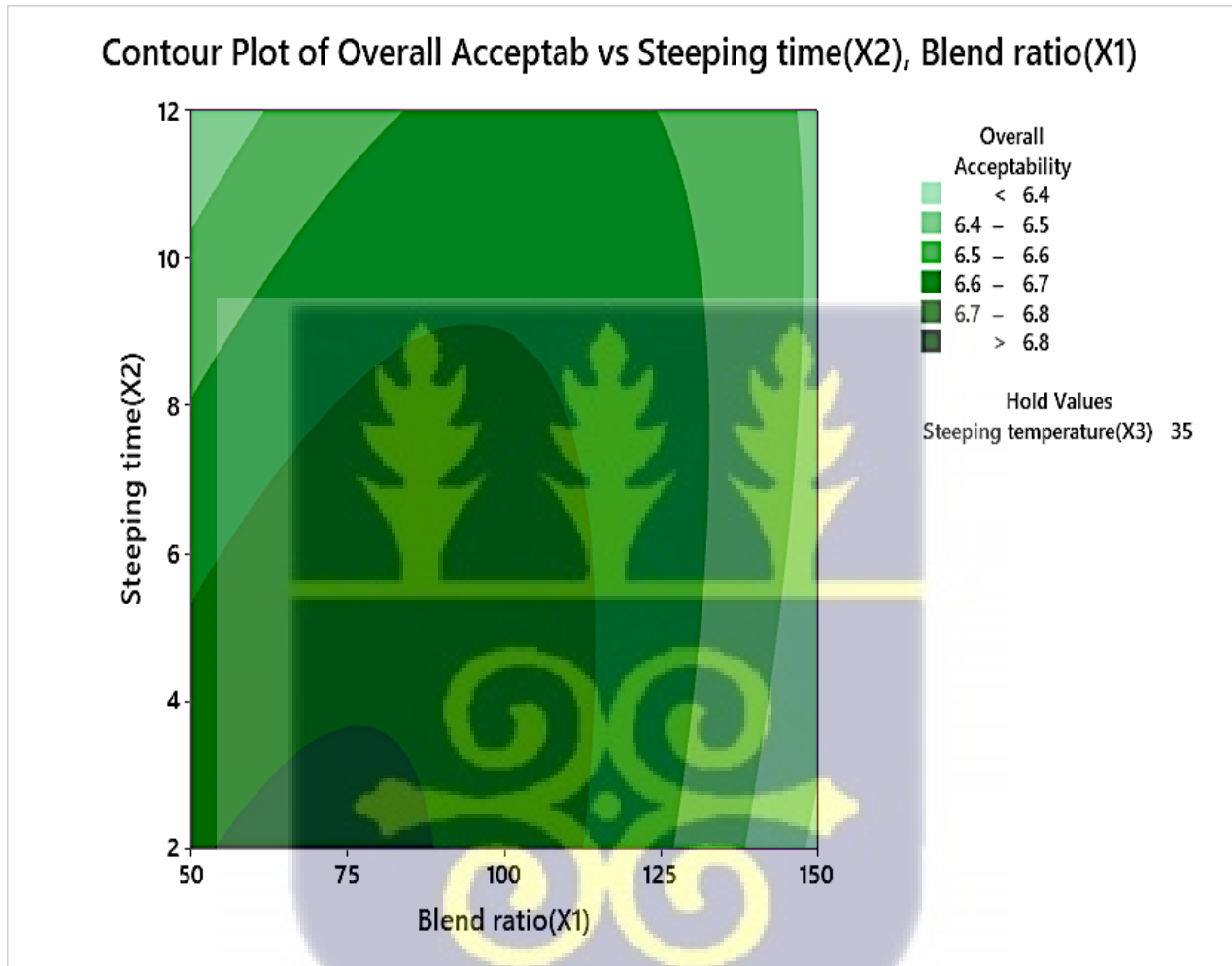


4.8.6 Overall Acceptability

The contour plots for overall acceptability show that decreasing the blend ratio with respect to the spices from 55 to 80 and slightly increasing the steeping time from 2 to 4h increased the overall acceptability of the *zoom-koom* samples from 6.40 to 6.80 (Figure 22). The high R-squared and

adjusted R-square values (Table 14) for the “overall acceptability” quality sensory parameter demonstrates that, the model was acceptable and precise to predict the responses.

Figure 22: Contour plots for the overall acceptability of *zoom-koom* as a function of Blend ratio (X1) and Steeping time(X2).



4.9 Selection of optimum conditions

The contour plots of all the sensory attributes for *zoom-koom* were overlaid on the same axis, using Minitab (version 20, Minitab Inc., UK). The optimum region of the process treatment variables was where all the criteria of the sensory attributes were satisfied. The sensory criteria used were

those founded on the mean scores that suggested very acceptable sensory attributes which ranged from like moderately to like extremely. Accordingly, the constraints for the acceptable criteria for the sensory attributes ranged from scores of 7 (liked moderately) to 9 (liked extremely). The overlaid contour plot for all the sensory attributes is illustrated in Figure 23. From the plot, the process treatment combinations that provided optimum Using Minitab (version 20, Minitab Inc. UK), all of the sensory qualities for *zoom-koom's* contour plots were superimposed on the same axis. Where all of the sensory attribute criteria were met was the optimal location for the process treatment variables. The sensory criteria used were those founded on the mean scores that suggested very acceptable sensory attributes which ranged from like moderately to like extremely. Accordingly, the constraints for the acceptable criteria for the sensory attributes ranged from scores of 7 (liked moderately) to 9 (liked extremely). The overlaid contour plot for all the sensory attributes is illustrated in figure 4.20. From the plot, the process treatment combinations that provided optimum product sensory characteristics were the optimum blend ratio 700:50 to 700:100, steeping time of 2 to 6h and steeping temperature of 35°C. The location of the optimum region in the experimental space recommends that good quality *zoom-koom* may be obtained when the blend ratio of millet is 700:50 to 700:100, steeped for 2 to 6h over at ambient temperature 35°.

um product sensory characteristics were the optimum blend ratio 700:50 to 700:100, steeping time of 2 to 6h and steeping temperature of 35°C. The location of the optimum region in the experimental space recommends that good quality *zoom-koom* may be obtained when the blend ratio of millet is 700:50 to 700:100, steeped for 2 to 6h over at temperature of 35°C using Minitab software (version 20, Minitab Inc. UK), all of the sensory qualities for *zoom-koom's* contour plots were superimposed on the same axis. Where all of the sensory attribute criteria were met was the

optimal location for the process treatment variables. The sensory criteria used were the mean scores that suggest very acceptable sensory attributes ranging from like moderately to like extremely. Accordingly, the constraints for the acceptable criteria for the sensory attributes ranged from scores of 7 (liked moderately) to 9 (liked extremely). The overlaid contour plot for all the sensory attributes is illustrated in figure 23. From the plot, the process treatment combinations that provided optimum product sensory characteristics were the optimum blend ratio 700:50 to 700:100, steeping time of 2 to 6h and steeping temperature of 35°C. This was defined by overlaying the respective sensory quality parameters together.

The location of the optimum region in the experimental space recommends that good quality *zoom-koom* may be obtained when the blend ratio of millet is 700:50 to 700:100, steeped for 2 to 6h at ambient temperature of 35°C.

4.10 Verification of optimized conditions

To verify that the predictive models obtained were adequate for determining consumer acceptability of *zoom-koom*, two process treatment combinations were selected from within the optimum region and two from outside the region (Table 15). *Zoom-koom* made from the four treatments were subjected to sensory evaluation. Results from the verification study (Table 15) showed that the ratings for appearance, taste, aroma, mouth taste, aftertaste and acceptability compared well with the predicted ratings based on statistics. The verification results indicated a good agreement between the observed and predicted ratings.

Figure 23: Overlaid contour plot of appearance, aroma, flavour, mouthfeel, aftertaste and overall acceptability of *zoom-koom*.

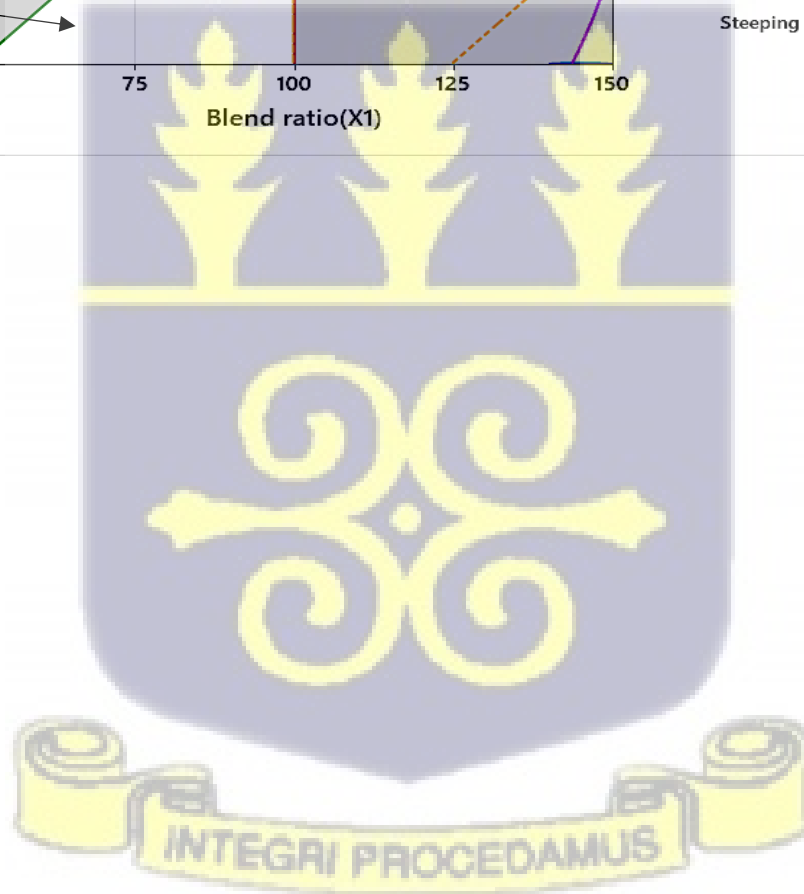
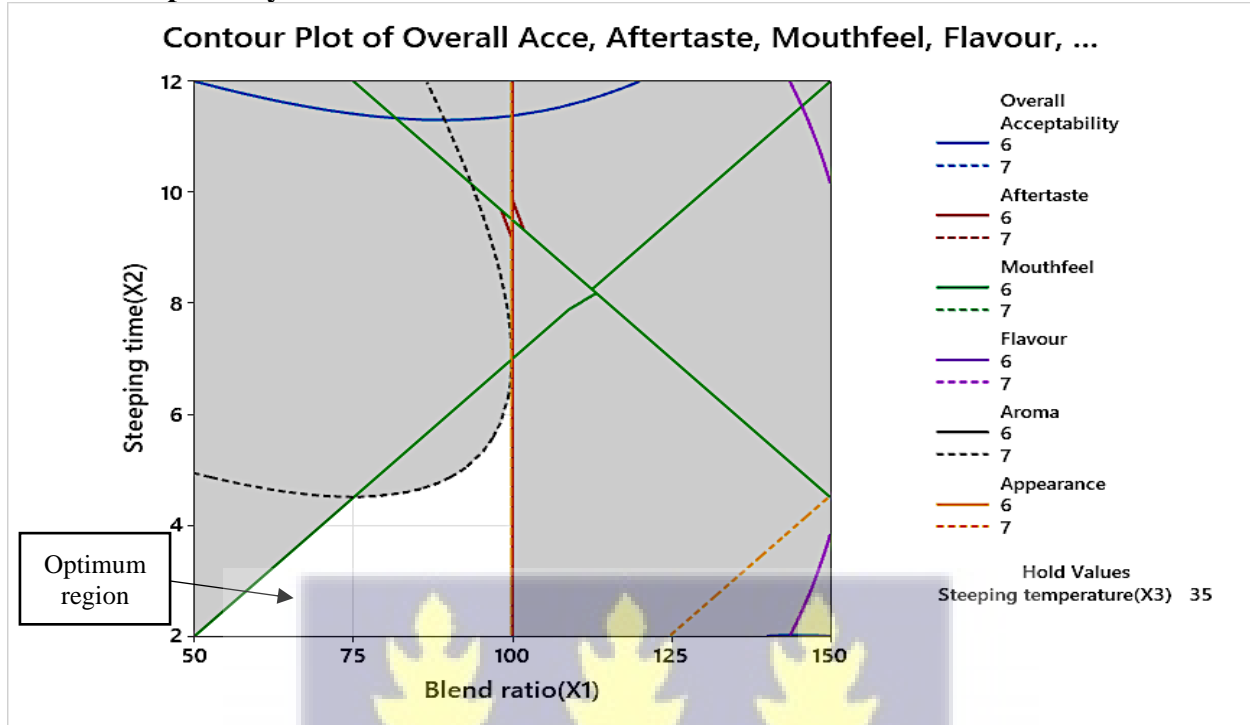


Table 15: Process combinations for verification of optimum region

	Blend ratio	Steeping time (hrs)	Steeping temperature (°C)
T1	700:50	2	35
T2	700:100	6	35
T3	700:125	7	35
T4	700:150	7	35

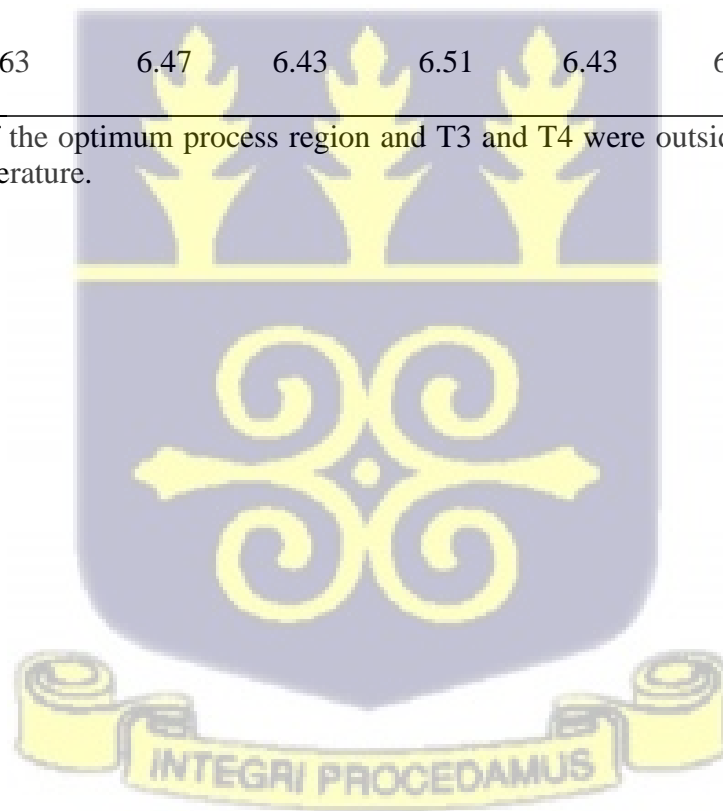
T1 and T2 are treatment conditions within optimum region, T3 and T4 are treatment conditions outside the optimum region



Table 16: Predicted and validated ratings for sensory attributes in the optimum region

Sample	Appearance		Aroma		Flavour		Mouthfeel		After taste		Overall acceptability	
	<i>observed</i>	<i>predicted</i>	<i>observed</i>	<i>predicted</i>	<i>observed</i>	<i>predicted</i>	<i>observed</i>	<i>predicted</i>	<i>observed</i>	<i>predicted</i>	<i>observed</i>	<i>predicted</i>
T1	6.84	6.88	6.87	6.85	6.69	6.60	6.31	6.17	6.64	6.44	6.91	6.79
T2	6.80	7.02	7.05	7.19	6.85	6.90	6.54	6.59	6.48	6.52	6.86	6.96
T3	6.85	6.94	6.75	7.09	6.73	6.63	6.30	6.35	6.13	6.22	6.69	6.73
T4	6.86	6.82	6.51	6.63	6.47	6.43	6.51	6.43	6.43	6.27	6.57	6.55

Treatments T1 and T2 were within of the optimum process region and T3 and T4 were outside the optimum process region of blend ratio, steeping time and steeping temperature.



4.11. Microbiological, Physicochemical and Bioactive properties of commercially processed *zoom-koom* and optimised (laboratory processed) *zoom-koom*.

Aerobic bacteria and yeasts and moulds were the most predominant microorganisms in both the commercial samples and optimized samples. This was in line with other findings signifying aerobic bacteria and yeasts and moulds are the most prevalent microorganisms in African traditional beverages (Adesulu & Awojobi, 2014; De Vuyst et al., 2016, Adinsi et al. 2017, Adinsi et al. 2015.). Aerobic bacterial counts for the commercial *zoom-koom* were significantly higher than that of the optimized samples except for Nima. There was also significant difference between yeasts and mould counts of the commercial and optimized samples (Table 18) with the commercial samples recording a higher value compared to the latter one. There was also a significant difference in the *E. coli*, total coliform and *S. aureus* counts of the commercial and optimized samples with the optimized samples recording no count for *E. coli*, total coliform and *S. aureus* respectively (Table 18). The no count was a result of the sodium benzoate that completely reduced and, in some instances, completely eliminates all the microorganisms from the *zoom-koom* samples. These microbes are usually associated with poor hygiene and their presence could show the potential existence of pathogenic organisms and mean a possible health risk (Nyambane et al., 2014). Indicator organisms, such as coliforms and its generic *Escherichia coli*, are often employed to detect the potential presence of pathogenic organisms, as well as sanitation failures (Eden, 2014). Safety-wise, the optimized samples are safer for consumption than the commercial samples. Numerous studies have shown the presence of pathogenic bacteria in traditional beverages. For example, a study done by Aboh and Oladosu (2014), where samples of *kunun-zaki* were assessed for microbiological quality showed the presence of pathogenic microbes such as *Staphylococcus aureus*, *E. coli*, *Salmonella typhi* and *Shigella spp* ranging from 0 to 8.3 log CFU/ml in a pH range

of 2.64 to 5.0. Also, in a study by Oshoma et al. (2009) on the growth and survival of *E. coli* in *kunun-zaki* during storage at different temperatures, it was shown that *E. coli* could survive during storage at different temperatures and at low pH although survival rate decreased with time. Elmahmood and Doughari (2007), also determined the microbiological quality of *kunun-zaki* from Adamawa state in Nigeria revealing the presence of pathogenic microorganisms such as *Staphylococcus aureus* and indicators such as *E. coli* with the pH of the samples ranging between 3.44 to 4.34. Lastly a study conducted by Adinsi et al., (2017) showed *E. coli* were detected in some *gowe* samples from a market in Nigeria.

The pH, TTA, TSS and L^* , a^* , b^* and ΔE colour indices of the optimized samples were significantly different from the commercial samples with the optimized samples being whitish than the commercial samples (Table 17). The difference was as a result of the varieties of millet, different dilution factors and the variation in production process used in the *zoom-koom* production compared to the optimum samples.

The results of the bioactivity *zoom-koom* with respect to the TPC and % inhibition showed significant difference between the commercially processed samples compared to the optimized sample with the latter exhibiting higher values (Table 19).

The high polyphenols contents of *zoom-koom* are related to the use of millet grain and spices as raw materials for the production of *zoom-koom* beverage. Pearl millet has been identified as one of the main contributors of polyphenols in fermented African cereal beverages. Millet grain contains a unique phenolic profile due to the high proportion of phenolic acids (such as gallic, vanillic, protocatechuic, cinnamic, p-coumaric, p-hydroxybenzoic, syringic, ferulic and caffeic acids) (Xiong et al.,2019), known as powerful bioactive compounds with antioxidant and free radical scavenging properties (Sroka et al.,2003).

Table 17: Physicochemical properties of zoom-koom (commercial vs optimised)

Sample	pH	Acidity (% lactic acid).	TSS (°Brix)	<i>L</i> *	<i>a</i> *	<i>b</i> *	ΔE
Site 1 (5)	3.08±0.01 ^C	0.09±0.01 ^A	8.65±0.02 ^B	26.49 ± 0.09 ^A	0.53± 0.00 ^A	11.45 ± 0.11 ^A	11
Site 2 (5)	3.14±0.01 ^C	0.06± 0.01 ^{AB}	6.80±0.010 ^D	21.29 ±0.03 ^C	4.27 ± 0.01 ^B	10.19 ± 0.08 ^B	6
Site 3 (5)	3.59±0.07 ^B	0.04±0.01 ^{BC}	9.63±0.04 ^A	21.27 ±0.02 ^C	4.29 ± 0.03 ^B	10.39 ± 0.13 ^B	7
Site 4 (5)	3.56±0.02 ^B	0.05±0.04 ^{ABC}	6.99±0.01 ^C	22.05 ±0.02 ^B	3.95 ± 0.05 ^C	3.78 ± 0.28 ^C	5
Optimized 1	7.25±0.04 ^A	0.0024±0.00 ^D	10.55±0.04 ^A	27.38±0.16 ^A	6.89±0.10 ^A	6.70±0.10 ^H	21
Optimized 2	6.07±0.07 ^B	0.0018±0.00 ^D	10.11±0.02 ^B	28.25±0.65 ^B	3.25±0.06 ^F	9.54±0.06 ^C	21

Key: Values are Means SD. In Each column, means that do not share a superscript are significantly different at $\alpha < 0.05$. Site 1(5)- Average values of commercial processors of Nima, Site 2 (5)- Average values of commercial processors of Tulaku-Ashaiman, Site 3(5)- Average values of commercial processors of Amansaman and Site 4(5)- Average values of commercial processors of Koforidua.



Table 18: Microbiological quality of zoom-koom (commercial vs optimised)

Sample	Aerobic plate count	Yeast & Moulds	Total coliform	<i>E. coli</i>	<i>S. aureus</i>
Site 1 (5)	2.73±0.01 ^D	3.16±0.01 ^A	1.32±0.03 ^D	1.06±0.01 ^C	2.04±0.02 ^D
Site 2 (5)	3.05±0.05 ^A	3.13±0.03 ^{AB}	1.18±0.01 ^E	0.77±0.03 ^D	2.37±0.05 ^C
Site 3 (5)	2.97±0.02 ^B	3.07±0.01 ^B	2.76±0.01 ^A	2.12±0.07 ^A	2.81±0.01 ^B
Site 4 (5)	2.83±0.02 ^C	2.14±0.01 ^C	2.14±0.00 ^B	1.93±0.00 ^B	3.27±0.01 ^A
Optimized 1	2.66±0.02 ^D	2.16±0.04 ^D	N.D.	N.D.	N.D.
Optimized 2	2.41±0.02 ^E	0.64±0.4 ^E	N. D.	N.D.	N.D.

Key: Values are mean Log CFU /ML ± SD. N.D-Not detected In Each column, means that do not share a superscript are significantly different at $\alpha < 0.05$. Site 1(5)- Average values of commercial processors of Nima, Site 2 (5)- Average values of commercial processors of Tulaku-Ashaiman, Site 3(5)- Average values of commercial processors of Amansaman and Site 4(5)- Average values of commercial processors of Koforidua.

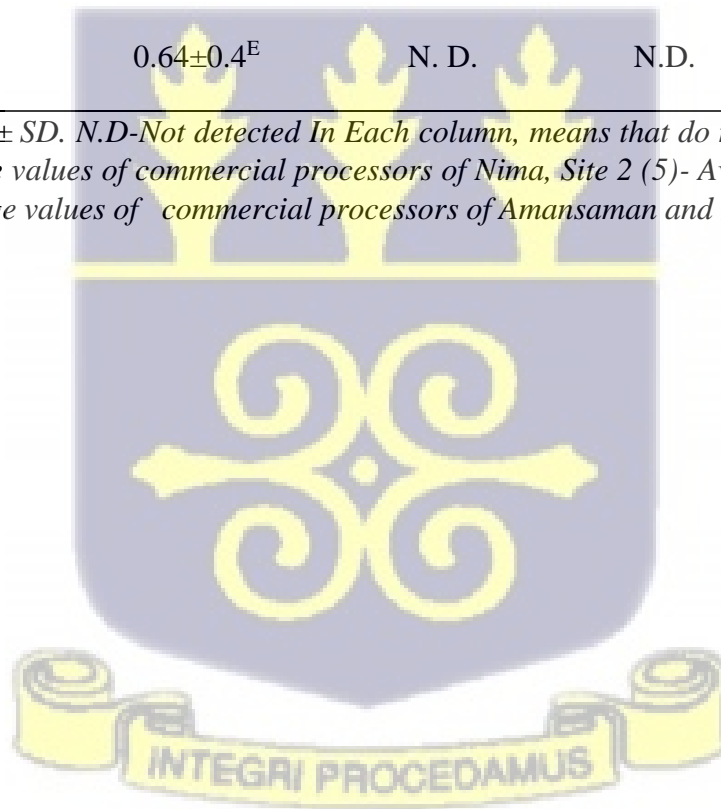
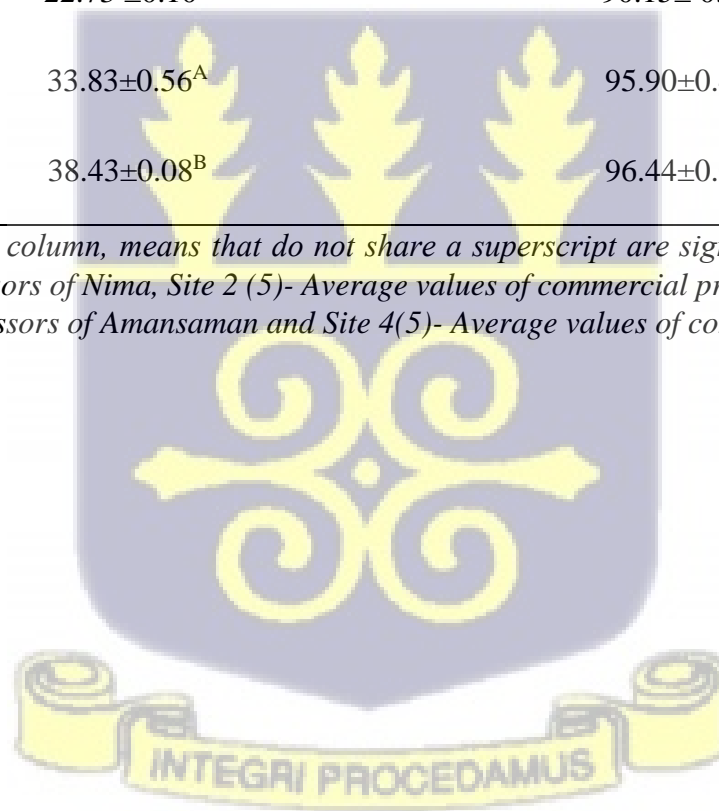


Table 19: Bioactivity of zoom-koom (commercial vs optimised)

Sample	Total Phenolic Compound(mg/ml)	Antioxidant Activity (%Inhibition)
Site 1 (5)	29.92 ±0.03 ^A	84.06 ± 0.15 ^D
Site 2 (5)	24.06± 0.02 ^B	89.17± 0.06 ^C
Site 3 (5)	30.10 ±0.90 ^A	91.88± 0.10 ^A
Site 4 (5)	22.73 ±0.10 ^C	90.15± 0.30 ^B
Optimized 1	33.83±0.56 ^A	95.90±0.48 ^A
Optimized 2	38.43±0.08 ^B	96.44±0.05 ^A

Key: Values are mean ± SD. In Each column, means that do not share a superscript are significantly different at $\alpha < 0.05$. Site 1(5)- Average values of commercial processors of Nima, Site 2 (5)- Average values of commercial processors of Tulaku-Ashaiman, Site 3(5)- Average values of commercial processors of Amansaman and Site 4(5)- Average values of commercial processors of Koforidua.



4.12 Proximate composition of optimized *zoom-koom*

Proximate analysis is an important tool for the assessment of the composition of food and food products (Ajiboye et al., 2014). The moisture content of food goes a long way in signifying the shelf life of the produce. Table 4.12 shows the composition of the optimized *zoom-koom*. The result of the moisture was high for both the optimized and control samples. This was as a result of the dilution factor (water to steeped millet and spices ratio) during the various *zoom-koom* production. Thus, the high moisture content means the *zoom-koom* beverage may be more susceptible to microbial attack particularly fungi and mould (Ihekoronye and Ngoddy, 1985). The result of this study showed moisture content was similar to *Kunu-zaki*, another cereal non-alcoholic beverage which was found to be in the range of $82.0\pm 0.15\%$ to $90.70\pm 0.15\%$ (Ajiboye et al., 2014). The high moisture content is characteristic of a liquid-based beverage.

The crude protein of the control and the optimized *zoom-koom* ranged between 3.33% and 3.73% (Table 4.12). There was no significance difference in the protein content of the control and optimized sample 1. Ofudje et al. (2016) reported that the protein content of *Kunu-zaki*, a non-alcoholic local beverage was in the range of 2.18 to 8.40% and are comparable to the present results.

The percentage of fat in the *zoom-koom* varied from 1.12 to 1.35% (Table 19). These values were are higher than 0.39% obtained by Ajiboye et al. (2014) for millet beverage but lower than 5.5% obtained by Ekanem et al. (2018).

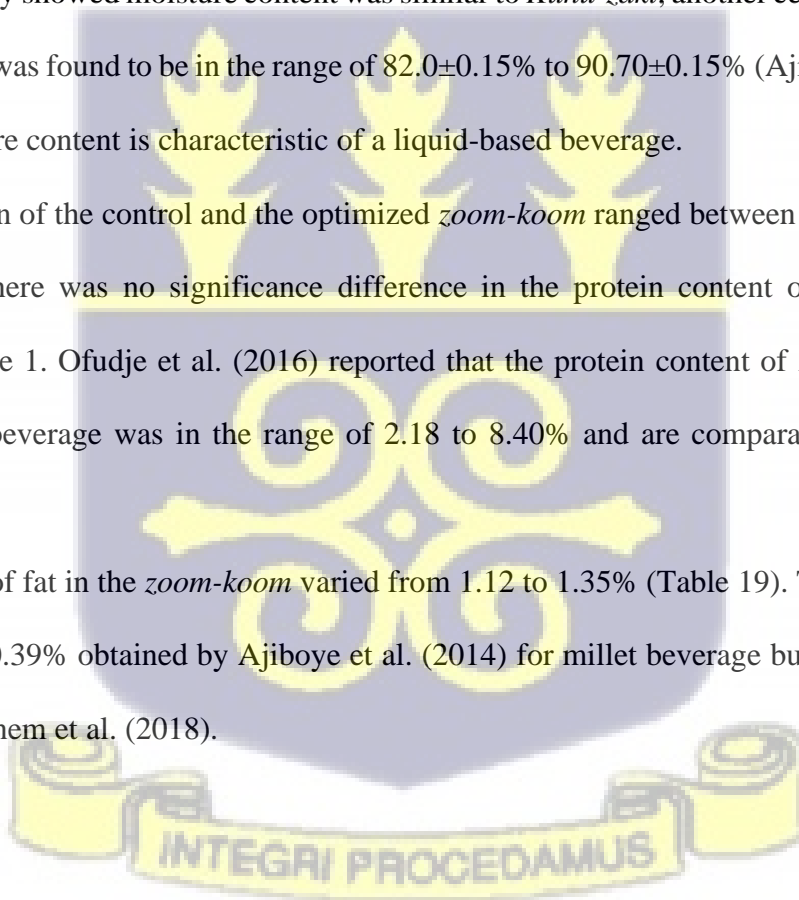


Table 20: Proximate composition of optimized *zoom-koom*

Sample/Parameters	Control	Optimized 1	Optimized 2
Moisture (%)	92.04±0.02 ^A	90.04±0.04 ^A	89.22±0.07 ^B
Ash (%)	2.40±0.01 ^B	2.49±0.04 ^B	3.06±0.04 ^A
Crude Fat (%)	1.12±0.20 ^B	1.18±0.11 ^B	1.35±0.01 ^A
Crude Fibre (%)	0.61±0.04 ^B	0.64±0.02 ^B	0.82±0.00 ^A
Crude Protein (%)	3.33±0.03 ^B	3.29±0.07 ^B	3.73±0.03 ^A
Total Solids (%)	9.90±1.11 ^B	9.93±1.12 ^B	10.31±0.02 ^A
Carbohydrate (%)	2.41±0.10 ^A	2.46±0.12 ^A	1.54±0.12 ^B
Energy (KJ)	28.39±0.4 ^B	28.44±0.45 ^B	32.88±0.17 ^A

Key: In each row, means that do not share a superscript are significantly different at $\alpha < 0.05$. Optimized 1 = 700:50, 2Hrs, 35°C, Optimized 2 = 700:100, 2Hrs, 35°C.

Ash content represent the total amount of minerals present in the sample. The ash value for the *zoom-koom* ranged from 2.40% to 3.06% (Table 20). These values were similar to the 2.40% obtained by Ajiboye et al. (2014). However, they were lower than what was obtained by Ekanem et al. (2018) who worked on *Kunu*, a non-alcoholic cereal beverage. Crude fibre and total solids content ranged from 0.6% to 0.81% and 9.9% to 10.31%, respectively (Table 20). The carbohydrate and energy value for the *zoom-koom* ranged from 1.54% to 2.41% and 28.39 to 32.88, respectively (Table 20). The main carbohydrate source in cereal beverages is starch which contributes significantly to the calories that can be obtained from the beverage (Chaves-Lopez et al., 2014). The amount of accessible carbohydrate in the beverage could serve as a source of energy for consumers (Ajiboye et al., 2014).

CHAPTER 5

5.0 CONCLUSION AND RECOMMENDATION

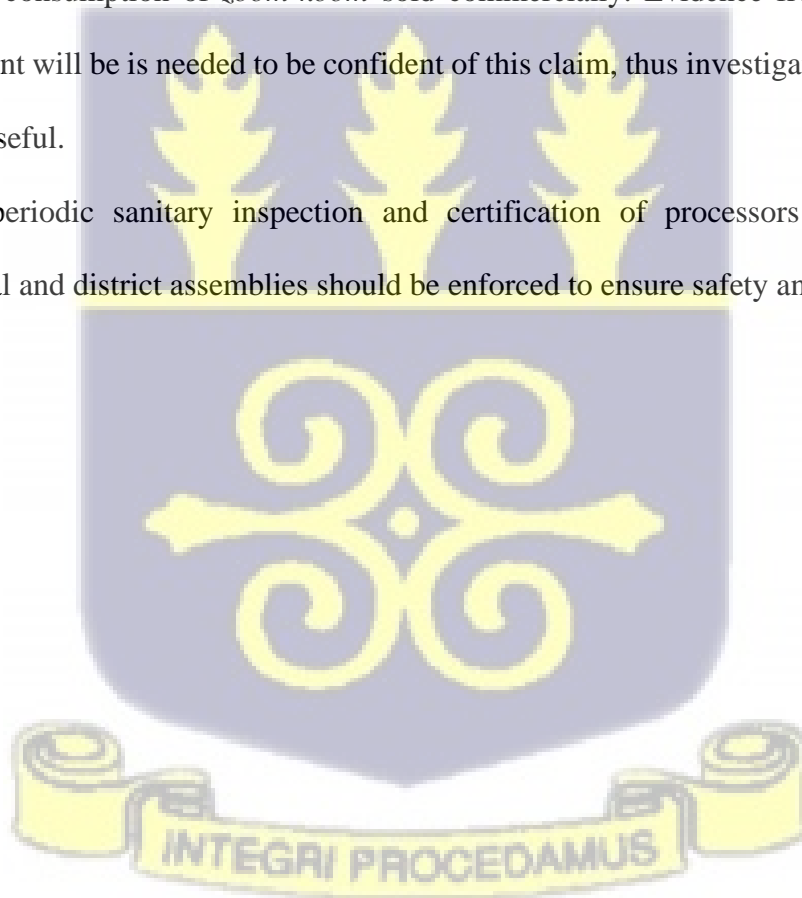
5.1 Conclusion

In this study, statistically based experimental designs (Box-Behnken design) was used to characterize and optimize the *zoom-koom* production process. The design has been proven to be a valuable tool for optimizing medium to produce the *zoom-koom* beverages. This study showed *zoom-koom* produced using different variants with respect to their different processing variables exhibited high antioxidant activity, total phenolic compounds, and high microbial quality suitable for consumption. The effect of steeping temperature, steeping time and blend ratio of the millet and the spices on the bioactivity, physicochemical, microbial quality and sensory was investigated. Results obtained indicated that all the independent variables' treatment temperatures had the highest effect on all the response variables. The desirability index obtained in this work with respect to the independent variables considered ranged from 0.7 to 1.000. The prediction of the desirability model based on 95% confidence in the range of the independent variable with respect to the sensory quality that gave optimal conditions of a blend ratio 700:50 to 700:100, steeping time of 2 to 6hrs and steeping temperature of 35°C. Proximate analysis of optimized *zoom-koom* and control showed their compositions were similar. The mathematical models developed could be used to predict the outcome of the response variables to a high degree of accuracy.



5.2 Recommendations

1. Shelf-life studies should be conducted on the optimized *zoom-koom* samples.
2. Different packaging materials (transparent, translucent, opaque etc) should be explored to determine effect on quality and shelf life.
3. Other processing techniques such as such a microwave, radiofrequency, infrared, ohmic heating, high-pressure processing, ultrasound, pulsed electric fields, pulsed light and ultraviolet ionizing should be explored to compare it with the conventional method.
4. According to the evidence gathered this far, there is a possible public health risk associated with the consumption of *zoom-koom* sold commercially. Evidence from microbial risk assessment will be is needed to be confident of this claim, thus investigating in this respect will be useful.
5. Lastly, periodic sanitary inspection and certification of processors and vendors by municipal and district assemblies should be enforced to ensure safety and quality.



APPENDICES

APPENDIX 1: PHYSICOCHEMICAL PROPERTIES OF ZOOM-KOOM SAMPLES

Table 21: Physicochemical quality of fifteen (15) zoom-koom experimental samples

Variants	Blend ratio	Steeping time (hrs)	Steeping temperature (°C)	TTA	pH	TSS	L *	a*	b*	ΔE
1	700:50	2	35	0.002±0.00 ^{BC}	7.24±0.03 ^A	0.80±0.17 ^{BC}	15.88±0.07 ^{FG}	1.50±0.10 ^K	7.20±0.07 ^H	17.66±0.06 ^G
2	700:150	2	35	0.004±0.00 ^B	7.18±0.02 ^A	0.90±0.17 ^A	14.32±0.10 ^I	2.82±0.10 ^G	7.35±0.15 ^{GH}	16.45±0.06 ^H
3	700:50	12	35	0.008±0.00 ^A	6.87±0.03 ^B	0.88±0.06 ^A	16.30±0.17 ^F	2.13±0.02 ^{IJ}	7.54±0.15 ^G	17.83±0.10 ^G
4	700:150	12	35	0.009±0.00 ^A	6.52±0.08 ^C	0.69±0.04 ^{ABCD}	17.60±0.10 ^D	2.40±0.10 ^{HI}	7.81±0.07 ^F	19.50±0.10 ^E
5	700:50	7	25	0.002±0.00 ^C	6.31±0.04 ^D	0.75±0.15 ^{ABCD}	20.39±0.21 ^B	2.15±0.10 ^{IJ}	7.44±0.10 ^G	21.70±0.06 ^A
6	700:150	7	25	0.008±0.00 ^A	6.14±0.01 ^E	0.59±0.06 ^{BCDE}	21.25±0.25 ^A	6.89±0.10 ^A	6.70±0.10 ^H	20.70±0.06 ^C
7	700:50	7	45	0.002±0.00 ^C	5.98±0.01 ^F	0.75±0.0 ^{ABCD}	13.60±0.10 ^J	1.86±0.10 ^J	8.12±0.10 ^F	15.81±0.10 ^I
8	700:150	7	45	0.002±0.00 ^C	6.23±0.04 ^{DE}	0.43±0.06 ^E	18.27±0.07 ^C	3.70±0.10 ^{DE}	9.95±0.05 ^B	21.20±0.06 ^B
9	700:100	2	25	0.002±0.00 ^C	6.23±0.03 ^{DE}	0.53±0.06 ^{DE}	16.15±0.10 ^{FG}	2.64±0.06 ^{GH}	7.38±0.06 ^{GH}	17.75±0.10 ^G
10	700:100	12	25	0.002±0.00 ^C	6.25±0.04 ^{DE}	0.82±0.10 ^{AB}	15.90±0.05 ^{FG}	3.42±0.10 ^{EF}	9.24±0.05 ^D	18.57±0.10 ^F
11	700:100	2	45	0.002±0.00 ^C	6.14±0.01 ^E	0.74±0.03 ^{ABCD}	18.37±0.10 ^C	3.25±0.06 ^F	9.54±0.06 ^C	20.85±0.10 ^C
12	700:100	12	45	0.009±0.00 ^A	5.77±0.08 ^G	0.55±0.02 ^{CDE}	16.74±0.10 ^E	3.84±0.10 ^D	9.17±0.02 ^D	19.67±0.10 ^E
13	700:125	7	35	0.002±0.00 ^C	6.01±0.02 ^F	0.86±0.03 ^A	15.38±0.10 ^H	3.33±0.20 ^F	9.68±0.10 ^C	18.46±0.10 ^F
14	700:125	7	35	0.001±0.00 ^D	6.27±0.02 ^D	0.51±0.06 ^{DE}	17.57±0.21 ^D	4.54±0.10 ^C	11.23±0.05 ^A	21.44±0.10 ^B
15	700:125	7	35	0.001±0.00 ^D	5.70±0.02 ^G	0.73±0.02 ^{ABCD}	15.78±0.21 ^{GH}	5.33±0.06 ^B	11.17±0.01 ^A	20.22±0.05 ^D

***SD-Standard deviation; TTA-Titratable acidity, Ph-Hydrogen ion concentration, TSS-Total soluble solids, L*-Lightness, a*-(+) redness, b* (+) yellowness, ΔE-colour change; ***Means that do not share a letter in the same column are significantly different at $\alpha < 0.05$



APPENDIX 2: MICROBIOLOGICAL QUALITY OF ZOOM-KOOM SAMPLES

Table 22: Microbiological quality of fifteen (15) zoom-koom experimental samples

Variants	Blend ratio	Steeping time (hrs)	Steeping temperature (°C)	TPC	Yeast & Moulds	<i>S. aureus</i>	Total Coliform	<i>E. coli</i>
1	700:50	2	35	2.83±0.34 ^{BC}	2.11±0.03 ^{CD}	2.18±0.05 ^E	N. D	N. D
2	700:150	2	35	3.17±0.05 ^{AB}	N. D	2.15±0.02 ^E	N. D	N. D
3	700:50	12	35	3.18±0.05 ^{AB}	2.08±0.10 ^{CD}	3.24±0.1 ^A	N. D	N. D
4	700:150	12	35	2.73±0.08 ^C	1.91±0.02 ^{DE}	N. D	N. D	N. D
5	700:50	7	25	3.22±0.10 ^A	2.14±0.10 ^C	2.49±0.10 ^{BC}	N. D	N. D
6	700:150	7	25	2.30±0.10 ^D	1.78±0.10 ^E	N. D	N. D	N. D
7	700:50	7	45	2.14±0.10 ^D	1.80±0.06 ^E	N. D	N. D	N. D
8	700:150	7	45	2.31±0.10 ^D	1.75±0.10 ^E	N. D	N. D	N. D
9	700:100	2	25	3.18±0.07 ^{AB}	2.22±0.05 ^{BC}	2.59±0.1 ^B	N. D	N. D
10	700:100	12	25	3.12±0.10 ^{ABC}	1.80±0.10 ^E	2.42±0.10 ^{BCD}	N. D	N. D
11	700:100	2	45	3.45±0.05 ^A	0.66±0.05 ^G	N. D	N. D	N. D
12	700:100	12	45	1.70±0.05 ^{EF}	3.48±0.10 ^A	2.32±0.1 ^{CDE}	N. D	N. D
13	700:125	7	35	1.68±0.02 ^{EF}	2.42±0.10 ^B	N. D	N. D	N. D
14	700:125	7	35	1.30±0.06 ^F	1.01±0.01 ^F	N. D	N. D	N. D
15	700:125	7	35	1.99±0.06 ^{DE}	1.83±0.01 ^E	2.23±0.1 ^{DE}	N. D	N. D

***SD-Standard deviation; ND- Not detected, TPC-Total plate count; ***Means that do not share a letter in the same column are significantly different at $\alpha < 0.05$

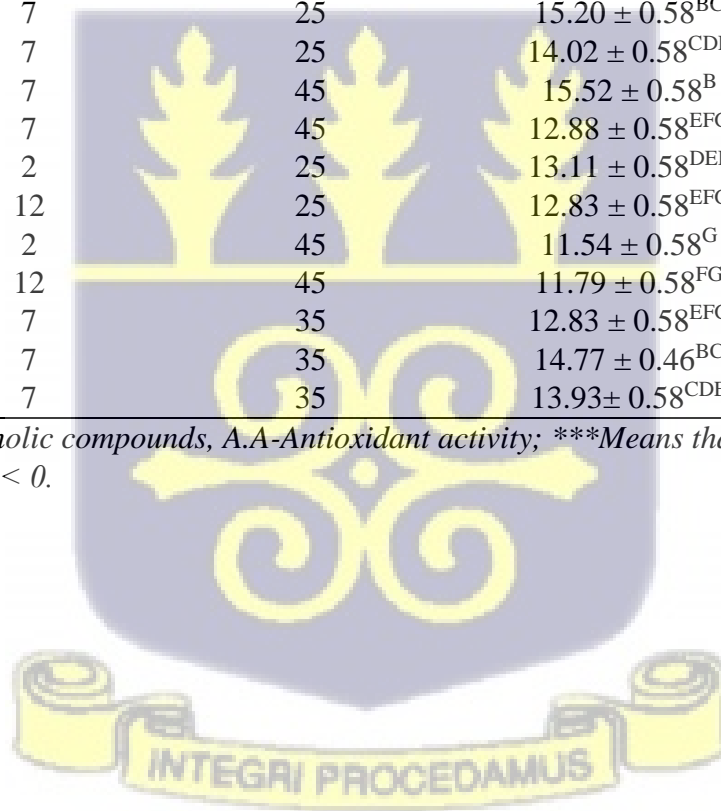


APPENDIX 3: BIOACTIVITY OF ZOOM-KOOM SAMPLES

Table 23: Bioactivity of fifteen (15) zoom-koom experimental samples

Variants	Blend ratio	Steeping time (hrs)	Steeping temperature (°C)	TPC (mg/ml)	A.A (% inhibition)
1	700:50	2	35	15.36 ± 0.13 ^{BC}	82.36± 1.00 ^H
2	700:150	2	35	14.47 ± 0.10 ^{BCD}	84.46±0.15 ^{GH}
3	700:50	12	35	15.31 ± 0.10 ^{BC}	94.38±1.00 ^{BCD}
4	700:150	12	35	21.03 ± 0.58 ^A	88.32±1.00 ^F
5	700:50	7	25	15.20 ± 0.58 ^{BC}	95.37±0.58 ^{ABCD}
6	700:150	7	25	14.02 ± 0.58 ^{CDE}	85.91±1.00 ^G
7	700:50	7	45	15.52 ± 0.58 ^B	94.06±0.58 ^{CD}
8	700:150	7	45	12.88 ± 0.58 ^{EFG}	90.79±1.16 ^{EF}
9	700:100	2	25	13.11 ± 0.58 ^{DEF}	90.37±0.58 ^E
10	700:100	12	25	12.83 ± 0.58 ^{EFG}	93.52± 0.28 ^D
11	700:100	2	45	11.54 ± 0.58 ^G	96.01±0.58 ^{ABC}
12	700:100	12	45	11.79 ± 0.58 ^{FG}	96.82±1.00 ^A
13	700:125	7	35	12.83 ± 0.58 ^{EFG}	96.41±0.03 ^{AB}
14	700:125	7	35	14.77 ± 0.46 ^{BC}	96.20± 0.06 ^{ABC}
15	700:125	7	35	13.93± 0.58 ^{CDE}	97.54± 0.58 ^A

***SD-Standard deviation; Total phenolic compounds, A.A-Antioxidant activity; ***Means that do not share a letter in the same column are significantly different at $\alpha < 0$.



APPENDIX 4: SENSORY EVALUATION SCORES OF FIFTEEN (15) ZOOM-KOOM EXPERIMENTAL SAMPLES

Table 24: Attribute liking scores of fifteen (15) zoom-koom experimental samples

Variant	Appearance	Aroma	Flavour	Mouthfeel	Aftertaste	Overall Acceptability
1	7	7	7	7	7	8
2	7	7	6	7	6	7
3	7	8	6	7	6	8
4	7	7	6	7	6	7
5	7	7	7	6	6	7
6	7	8	7	7	7	7
7	7	7	7	7	7	7
8	7	7	6	7	6	7
9	7	7	7	7	6	7
10	7	7	7	7	7	7
11	7	7	7	7	7	8
12	7	7	7	7	7	7
13	7	7	7	6	6	7
14	7	8	6	6	6	7
15	7	8	7	6	6	7

***1=dislike extremely; 2= dislike very much; 3= dislike moderately; 4= dislike slightly; 5= either like nor dislike; 6= like slightly; 7= like moderately; 8= like very much; 9= like extremely.

***Means scores that do not share a letter in the same column are significantly different at $\alpha < 0.05$



APPENDIX 5: CONSENT FOR SENSORY EVALUATION

Project Title: Process Optimization of *Zoom-koom*

Supervisor:

Dr. Bennett Dzandu

Dr. Idolo Ifie

Prof. John Owusu

Sensory Supervisor:

Dr. Maame Yaakwaah Blay Adjei

Investigator:

Emmanuel Tei-Mensah

Address:

Sensory Evaluation Laboratory, Department of Nutrition and Food Science. School of Biological Sciences, College of Basic and Applied Science, University of Ghana, Legon, Accra.

General Information about Research

You have been invited to participate in a sensory evaluation involving *zoom-koom*. Sensory evaluation involves the use of all your basic senses to evaluate and assess a food's characteristics. You will be presented with seven of the *zoom-koom* products and tell us how much you like or dislike them. Food taste test is an individual work activity and will involve no discussion with other participants on how you feel about the foods we show you. In any instance, the researcher will provide you with further details on the test you are to perform and the assessment protocol you should use.

Possible Risks and Discomforts

In general, this consumer acceptance test is non-invasive and should not be a source of risk to your health or person. The products you have to taste are all normal foods or the ingredients used to make normal foods. Unless you react adversely *name allergens* or have any allergies to any kinds

of fruits and vegetables, this test should not pose a risk to you. If you feel uncomfortable at any point, please call the attention of the researcher who will be able to help you.

Possible Benefits

By participating in the food taste test, you are contributing immensely to the development of *zoom-koom*. This is a huge emotional benefit to you as you will have contributed significantly to improved food security in Ghana.

Confidentiality

The data you provide to us will be kept confidential by the research team. You will never be personally identified in any work published as a result of your participation in any taste test without your prior consent. We will protect your personal information and not hand this to any third party. Unless you give us permission to contact you again for any sensory work we carry out at the Department of Nutrition and Food Science, we will not keep your contact information after the end of the research project. If you allow us to contact you again, we will only keep your contact details for the purpose of contacting you for sensory studies only and will not give your contact information to any third party. Your details will be kept in a secure file with the sensory research team.

Compensation

At the end of the study, you will be given a small token to show our appreciation for your time spent on the project. You should understand that there is no economic benefit to you for participating in a sensory study, only the emotional benefit of knowing that you have contributed significantly to the development and improvement in the quality of our local foods. This benefit cannot be overlooked.

Additional Cost

There is no additional cost to you for participating in a sensory study organized by the Department of Nutrition and Food Science.

Voluntary Participation and Right to Leave the Research

Although we would like you to complete the study, you should know that your participation in is purely voluntary and you have the right to withdraw from the study without giving us any explanation and without any penalty to you. Your withdrawal from the study will not negatively affect your personal relationship with the investigator, the department or the university as a whole.

Termination of Participation by the Researcher

It is possible that for some tests you sign up to participate in, some exclusion criteria will exempt you from participating. You will be notified of such studies at the onset. If in the middle of a test the investigator realizes that you are not capable of completing a test the investigator may ask you to discontinue the test. This does not have any negative consequence on your relationship with the investigator, the department or the university. You should understand that such decisions are made purely on the basis of preserving the scientific quality of the data we collect from our volunteering participants and have no personal bias to you.

Notification of Significant New Findings

To preserve the scientific quality of the data we collect in sensory testing, we are unable to disclose too much information about the products we test at the onset of the project. However, if your interest in the product is raised through your participation in the project, we can provide additional information about the product to you at the end of the project. You will have to leave your details with the investigator to share such information about the product with you at the end of the study.

Contacts for Additional Information

For information and questions about this study and general sensory tests and protocols at the Department of Nutrition and Food Science at the University of Ghana, please contact:

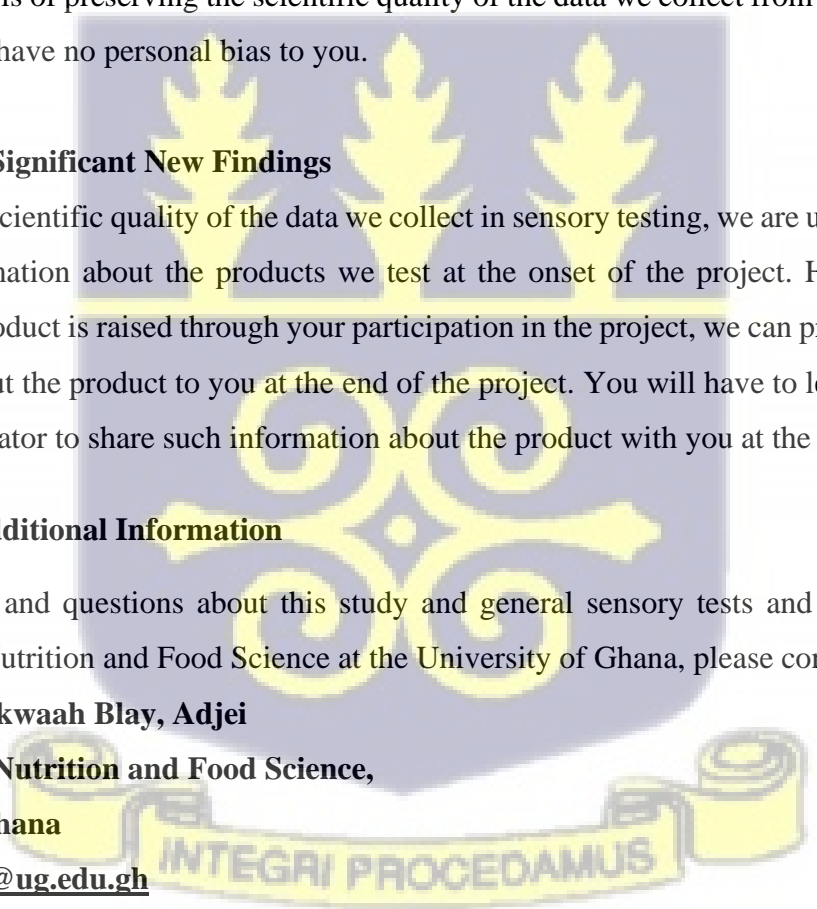
Dr Maame Yaakwaah Blay, Adjei

Department of Nutrition and Food Science,

University of Ghana

Email: myblay@ug.edu.gh

Tel: 0545525974



Your rights as a Participant

This research has been reviewed and approved by the Ethics Committee for Basic and Applied Science (ECBAS). If you have any questions about your rights as a research participant, you can contact the ECBAS Office through the address below

Administrator, Ethics Committee for Basic and Applied Sciences

College of Basic and Applied Sciences

University of Ghana

P. O. Box LG 68

Legon – Accra

Tel: +233242759315

Email: janoku@ug.edu.gh

VOLUNTEER AGREEMENT

The above document describing the benefits, risks and procedures for the sensory evaluation of foods has been read and explained to me. I have been given an opportunity to have any questions about the research answered to my satisfaction. I agree to participate as a volunteer.

Date

Name and signature or mark of volunteer

If volunteers cannot read the form themselves, a witness must sign here:

I was present while the benefits, risks and procedures were read to the volunteer. All questions were answered and the volunteer has agreed to take part in the research.

Date

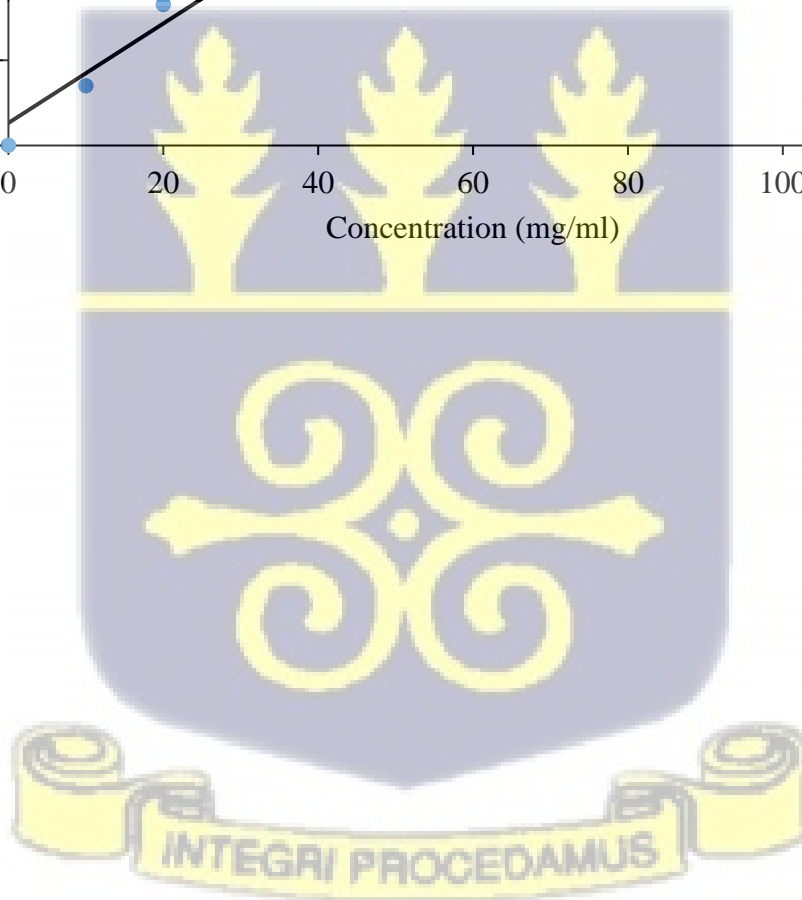
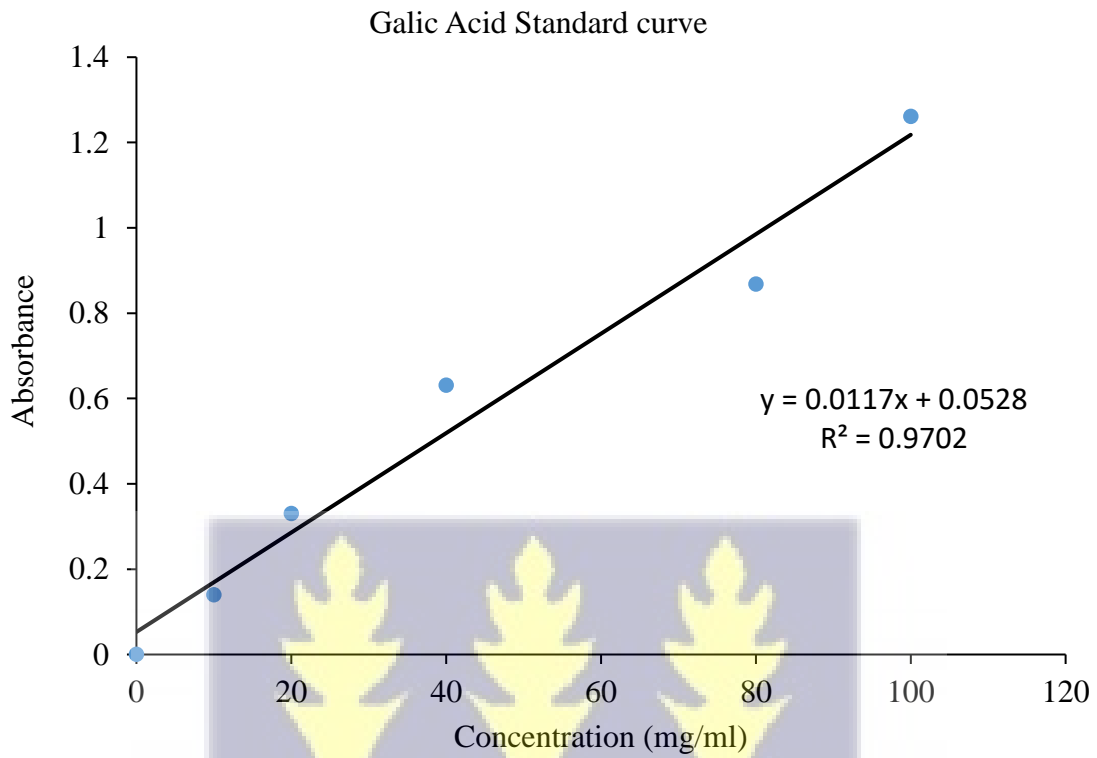
Name and signature of witness

I certify that the nature and purpose, the potential benefits, and possible risks associated with participating in this research have been explained to the above individual.

Date

Name & Signature of Person Who Obtained Consent

APPENDIX 6: GALIC ACID STANDARD CURVE FOR TOTAL PHENOLICS



APPENDIX 7: COMMERCIAL PROCESSING OF ZOOM-KOOM



Figure 24: Steeping of Millet



Figure 25: Cleaning of ginger



Figure 26: Cutting of spices (ginger)

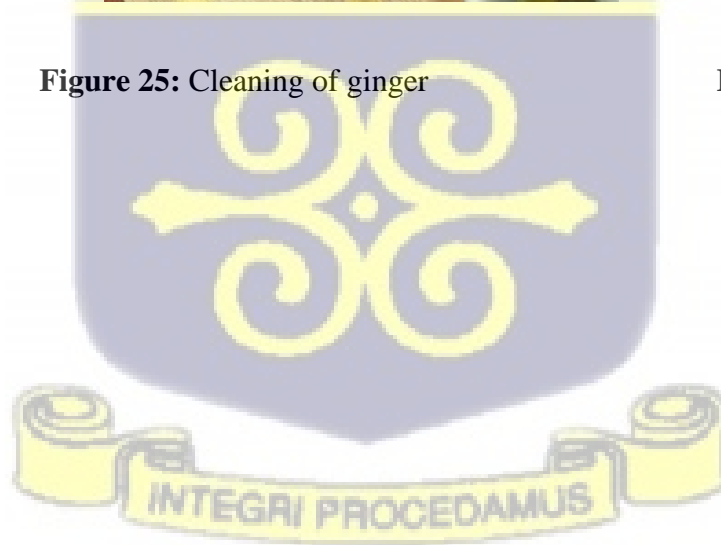




Figure 27: Mixed steeped millet and spices



Figure 28: Blending process



Figure 29: Filtration to obtain millet wort



Figure 30: Addition of sugar to the wort

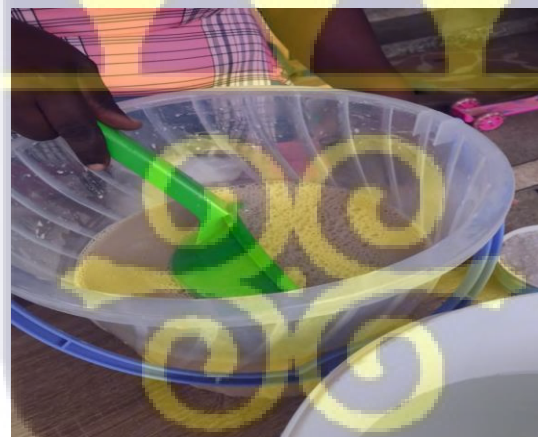
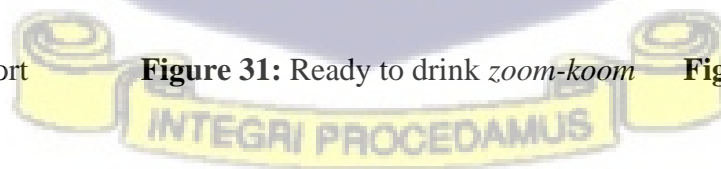


Figure 31: Ready to drink zoom-koom



Figure 32: Packaged zoom-koom.



APPENDIX 8: FIFTEEN (15) VARIANTS OF ZOOM-KOOM PRODUCED IN THE LABORATORY



Figure 33: Experimental *zoom-koom* produced in the laboratory



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