

**EFFECT OF GREEN GRAM SUBSTITUTION ON THE
QUALITY OF PORK SAUSAGE**

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DECLARATION

I, **AGAKO Alod** hereby declare that the content of this dissertation is my original work and has never been presented/ submitted for the award of a degree in any higher institution of learning.

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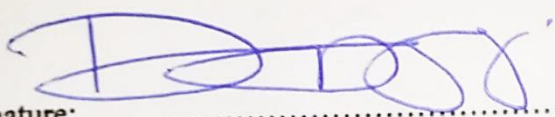
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APPROVAL

This dissertation has been submitted with our approval as supervisors:

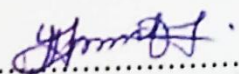
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LIST OF ACRONYMS

ACE	:	Angiotensin-Converting Enzyme
AGEs	:	Advanced Glycation End products
AOAC	:	Association of Analytical Chemists
FAMEs	:	Fatty acid methyl esters
FAO	:	Food and Agriculture Organization of the United Nations
GG	:	Green gram (<i>Vigna radiata</i>)
MUFA	:	Monounsaturated Fatty Acids
NCDs	:	Non-Communicable Diseases
PUFA	:	Polyunsaturated Fatty Acids
SFA	:	Saturated Fatty Acids
TAC	:	Total Antioxidant Capacity
UFA	:	Unsaturated Fatty Acids
WHC	:	Water Holding Capacity
WHO	:	World Health Organization

ABSTRACT

Substitution of meat-based food products with plant-based portions has been recommended by the World Health Organization (WHO) as one of the approaches to produce health-sensitive meat products that can provide both nutritional and health benefits. Despite this recommendation, the use of legumes to replace meat in conventional meat products, such as sausages, has not been adequately explored. Therefore, this study investigated the effect of green gram substitution on the quality of pork sausages. The study employed an experimental design where pork sausages were formulated by substituting pork with green gram flour at 0%, 25%, 50% or 75%. The formulated products were analyzed for nutritional quality, functional properties and sensory acceptability. The results showed that substituting pork ham with green gram flour positively impacted the nutritional quality of pork sausages. Specifically, crude fibre, crude protein, ash content, mineral (iron, calcium and zinc) content and antioxidant potential significantly ($p<0.05$) improved with increase in green gram substitution level. The moisture and crude fat contents decreased significantly with increase in green gram substitution level. However, there was a significant ($p<0.05$) inverse relationship between green gram substitution level and protein digestibility of products while mineral bioavailability was influenced differently depending on the individual mineral. There was also a significant ($p<0.05$) reduction in the content of total saturated fatty acids and monounsaturated fatty acids with increase in green gram substitution level. Conversely, a significant ($p<0.05$) increase in the content of polyunsaturated fatty acids was recorded. Regarding functional properties, the results showed that incorporation of green gram to sausages led to a significant increase ($p<0.05$) in water holding capacity and emulsion stability and a reduction ($p<0.05$) in cooking loss of the products. Generally, consumer sensory preference was largely negatively affected by green gram addition except at green gram substitution level of 25% where no significant difference ($p>0.05$) was observed with control. This study has illustrated that replacing pork with green gram enhances the nutritional quality and functional properties but negatively affects sensory acceptability of pork sausages. Consequently, there is need to improve the sensorial appeal at higher levels of green gram substitution which offer superior balance of nutritional and functional quality to enable consumers derive more nutritional and health benefits of green gram substituted pork sausages

Key words: *Green gram; pork sausage; substitution; nutritional quality; functional properties; antioxidant potential; mineral bioavailability; protein digestibility.*

CHAPTER ONE: INTRODUCTION

1.1 Background

Noncommunicable diseases (NCDs) have become a major cause of premature death, accounting for over 71% of all deaths globally, and 77% of all deaths that occur in low- and middle-income countries (World Health Organization (WHO), 2020). It was reported that in Sub-Saharan Africa, NCDs accounted for over 37% of total deaths in 2020. In Uganda, the latest health statistics indicated that deaths from NCDs accounted for over 33% of all deaths recorded in 2020 (WHO, 2020). The WHO identified cardiovascular diseases, diabetes, chronic respiratory diseases and cancer as the four major NCDs whose rapid rise has been attributed to risk factors such as an unhealthy diet, exposure to tobacco smoke, sedentary lifestyle and abuse of alcohol (Micha et al., 2017; WHO, 2020). Given the high burden of NCDs, it is critical that efforts are put in place to minimize the risks and hence reduce incidences of such diseases. Consequently, the WHO identified several indicators to show progress towards reducing the burden of NCDs. These include reducing consumption of tobacco, reducing harmful use of alcohol, reducing unhealthy diets and increasing public education and awareness campaigns on physical activity. In the context of diet, restricting the consumption of saturated fatty acids, salt and eradicating trans-fatty acids from the food chain has been emphasized (WHO, 2020). These can only be achieved by changing and adhering to proper dietary behaviour.

In keeping with the need to exercise healthy dietary behaviour, the past two decades have witnessed a rapid shift towards consumption of high-protein diets (Hajihashemi et al., 2021; Naghshi et al., 2020) ostensibly due to the associated health benefits such as cardiovascular

wellness and weight loss (Westerterp-Plantenga et al., 2012; Leidy et al., 2015). This shift in dietary behaviour was mainly towards animal-based protein sources (Boeckel et al., 2015) but limited with regard to plant protein foods. However, many studies have delineated the health consequences associated with over-consumption of animal-source foods. In particular, consumption of red meat, has been linked to increased risks of cancer (Aykan 2015; Budhathoki et al., 2019; Huxley et al., 2009; Kitada et al., 2019) and other chronic diseases such as obesity, depression and diabetes (Hertzler et al., 2020; Machado et al., 2022; Wolk, 2017). These have been attributed to the high content of saturated fats, low content of unsaturated fats, low or no fibres, low content of bioactive compounds in meat and meat-based products. However, prospective cohort studies conducted over the last decades revealed that substitution of plant protein source for animal protein source foods is strongly associated with positive health outcomes which include cardiovascular wellness, low risk of cancer development and progression, low risk of development type 2 diabetes and longevity (Budhathoki et al., 2019; Ferrari et al., 2022; Itkonen et al., 2021; Naghshi et al., 2020; Song et al., 2016).

Integrating plant protein source foods into the diet offers several mechanisms through which consumers can derive better health outcomes. For instance, the amino acid arginine linked with improved metabolism, and cysteine linked with reduced insulin resistance and oxidative stress, are more abundant in plant-protein source foods (Banerjee et al., 2020; Jobgen et al., 2009; Liang et al., 2018; Zaloga et al., 2004). Plant protein source foods also offer great protective functions against oxidative stress (Bradford & Awad 2007; Craig 2010; Narasinga Rao 2003; Prakash et al., 2012). This is attributable to good antioxidant properties (Kothari, Lee, & Kim, 2020), the existence of phytonutrients (Ganesan & Xu 2018; Singh et al., 2021), high fiber content (Yang et al., 2020), lower saturated fats and cholesterol, and the high nutrient density (Grosso et al., 2016;

Marventano et al., 2017; Tirosh et al., 2016). In light of the better health outcomes associated with the consumption of plant protein source foods compared to animal-based source foods highlighted above, a recommendation to increase the consumption of plant protein source foods (Spencer & Guinard, 2018; McClements & Grossmann, 2021) has been put forward. Accompanying this recommendation, a surge in demand for plant-based protein foods in order to take advantage of its associated cardiovascular wellness, reduced cancer risk and overall longevity has been observed (Ahnen et al., 2019; Azzini et al., 2022; Huang et al., 2020; Naghshi et al., 2020; Päivärinta et al., 2020; Qin, Wang & Luo, 2022b). Such an increase in demand has prompted the need to substitute products that are primarily animal source-based with plant-based protein sources (Budhathoki et al., 2019).

Moreover, eating habits across the world have been changing rapidly. For instance, the consumption of animal protein source foods have increased over the last decade while consumption of plant-based foods have been decreasing (Ismail et al., 2020; Messina et al., 2023). With rapid urbanization and largely youthful working populations dominating such urban areas, consumption of snack foods is also on the rise (Amour et al., 2020; Casari et al., 2022; Habbab & Bhutta 2020; Popkin & Ng, 2022; Vaida, 2013). One of the major snacks widely consumed by these consumers of meat-based product is sausages, whose consumption is known to be most dominant compared to non-meat sausages (Kigaru et al., 2015). In Africa and Uganda particularly, meat sausages are largely consumed by the populace of urban and peri-urban areas such as cities and major towns (Mutumba & Bieliaiev, 2020). Its consumption has also been reported among the time-constrained working class in urban areas and youths (Escoto et al., 2012). Therefore, to achieve the strategy of lowering the consumption of animal-based proteins and saturated fats, animal-based sausages need to be directly targeted for nutritional modification.

One strategy is targeting high-quality plant protein sources from locally consumed legumes such as green gram for incorporation into pork sausage production. Addition of plant-based protein food sources to traditional animal-based products is believed to ameliorate the negative health effects associated with animal protein source foods and thus confer beneficial health effects on meat-based products (Bryant 2022). Plant-based protein sources are primarily legumes, including soybeans, beans, and green gram, among others, chiefly made of globular protein (Day et al., 2022). Substitution of plant protein sources in animal-based products has largely been conducted with soybeans (Kurek et al., 2022; Messina et al., 2022; Qin, Wang & Luo 2022a). Considering the allergic characteristics of soy (Herian, Taylor, & Bush 1990; Kerezsi, Jacsquet, & Blecker 2022; Savage et al., 2010), there is a need to generate empirical evidence to support the use of other legumes such as green gram.

Green gram (*Vigna radiata*), also known as mung beans, is one of the most important leguminous crops in the world today (Zia-Ul-Haq, Ahmad, & Iqbal 2008). It has been documented that green gram provides several nutritional and health benefits (Pandiyan et al., 2012; Sudhakaran & Bukkan 2021). Nutritionally, green gram is high in protein, non-starch carbohydrates, vitamins, and minerals content, low in fat content amongst others as presented in Table 1. In the context of health, green gram is believed to offer additional health benefits such as anti-inflammatory, anti-cancerous, antioxidant and hypolipidemic activities (Bhosale et al., 2021; Kang et al., 2015; Sudhakaran & Bukkan 2021). In addition, it is also believed to confer prebiotic properties (Sudhakaran & Bukkan 2021). This is because previous studies showed that the husks of green gram contain non-digestible constituents that support the growth of gut beneficial microbiomes (Swaroop et al., 2021).

Table 1: Nutritional composition of green gram and pork ham

Nutrients per 100g	green gram, raw	pork, ham
Moisture (g)	9.95±0.42	61.94±0.39
Protein (g)	22.53±0.43	18.83±0.59
Ash (g)	3.22±0.04	0.62 ± 0.0 2
Total fat (g)	1.14±0.17	18.55±0.78
Total dietary fibre (g)	17.04±0.38	—
Carbohydrates (g)	46.13±0.64	218± 20
Energy (KJ)	1229±10	1006 ± 20
Calcium (mg)	92.43±10.6	7.40±2.16
Iron (mg)	4.89 ± 0.46	1.27 ± 0.48
Potassium (mg)	177±74.3	239±34.6
Phosphorus (mg)	353±33.6	140±26.6
Sodium (mg)	12.48±0.07	40.44±9.89
Magnesium (mg)	198±39.2	17.05±3.66
Zinc (mg)	2.67 ± 0.13	1.76 ± 0.41
Thiamine (B1) (mg)	0.45±0.027	0.24±0.02
Riboflavin (B2) (mg)	0.27±0.011	0.10±0.01
Niacin (B3) (mg)	2.16±0.13	4.59±0.68
Total Folate (B9) (µg)	145±5.4	1.51±0.21
Vitamin A (µg)	—	2.98±0.30
Total polyphenols (mg)	90.74±5.49	—
Palmitoleic (C16:1) (mg)	—	466±108
Oleic (C18:1n9) (mg)	27.22±5.09	7600±769
Linoleic (C18:2n6) (mg)	430±10.8	1598±387
α-Linolenic (C18:3n3) (mg)	180±6.7	163±107

Source: Longvah *et al.* (2017) , Jiménez-Colmenero *et al.* (2010) and Ivanovic *et al.* (2016)

Despite the nutritional and nutraceutical properties indicated above, green gram is among the underutilized legumes in improving the healthiness of animal protein source food products such as pork sausages (Popoola *et al.*, 2012). On the other hand, previous studies have shown that

combining different ingredients in food formulations may affect its quality parameters like nutritional quality, functional properties, sensory properties, and overall acceptability (Li et al., 2022). Functional and sensory properties are key quality parameters that affect the acceptability and usability of food (Laaksonen et al., 2016). In the context of meat products, functional properties such as cooking loss, water holding capacity (WHC), emulsion stability need to be known. This is important because, WHC and fat retention affects juiciness, cooking loss and WHC impacts on water content which in turn affects storage stability. In the case of sensory properties, they are the most important because they are key drivers of food acceptability (Kim et al., 2010; Melovic et al., 2020). Therefore, to ascertain the potential of green gram in production of health-sensitive pork sausages, the influence of green gram substitution on these parameters need to be understood.

1.2 Problem statement

Noncommunicable diseases (NCDs) have increasingly become a significant global public health concern. This is illustrated by the fact that deaths from NCDs accounted for over 33% of all deaths in 2020 with a 22% probability of premature deaths (WHO, 2020). Among other factors, high consumption of animal protein source foods has been associated with the occurrence and progression of NCDs (Machado et al., 2022). This challenge has necessitated research on nutritional strategies to modify diets and ensure healthy consumption of animal protein source foods (Boer and Aiking, 2019). Pork sausages is one of the animal protein source foods whose consumption is increasing in Uganda (Roesel et al., 2019). It is therefore a potential source of increased consumer exposure to high intake of animal-based foods, contributing to the escalation of occurrence and progression of NCDs in the country. This situation necessitates innovation in the use of plant protein source foods to improve the healthiness of pork sausages. Green gram is one of the candidate legume plants that can be used owing to its good nutritional properties such

as high mineral content, high unsaturated fatty acids, in addition to conferring anti-inflammatory, antioxidant and prebiotic properties (Sudhakaran & Bukkan, 2021). Despite the potential health benefits associated with green gram, limited empirical information exists on how green gram can be utilized as a substitute ingredient in the production of pork sausages. This is because substitution may result in the alteration of nutritional quality (Day et al., 2022), sensory acceptability (Cordelle, Redl & Schlich, 2022) and functional properties (Li et al., 2022); key factors that affect acceptance and usability of foods.

1.3 Objectives of the Study

1.3.1 General objective

To investigate the effect of green gram substitution on the quality of pork sausages.

1.3.2 Specific objectives

Specific objectives of the study were:

- i. To determine the effect of green gram substitution on nutritional quality of pork sausages;
- ii. To determine the effect of green gram substitution on functional properties of pork sausages; and
- iii. To determine the effect of green gram substitution on sensory acceptability of pork sausages.

1.4.1 Research Questions

- i. What is the effect of green gram substitution on nutritional quality of pork sausages?
- ii. What is the effect of green gram substitution on functional properties of pork sausages?

- iii. What is the effect of green gram substitution on sensory acceptability of pork sausages?

1.5 Significance of the study

The results of this study are expected to contribute to the global efforts on diet modifications necessary for better noncommunicable diseases outcomes. The results will also be of use to nutritionists in promoting healthy food consumption towards better noncommunicable diseases outcomes. The results can also be used to inform policy on strategic approaches for reducing animal-based source foods in favour of plant protein source foods using locally sourced legume foods.

CHAPTER TWO: LITERATURE REVIEW

2.1 Nutritional quality of foods and influencing factors

Research on nutritional quality of food has been largely directed to nutritional elements but with limited emphasis on the health significance (Gul et al., 2015; Maestre, Poole & Henson, 2017; Wallace, Murray & Zelman, 2016). This is against the fact that consumers globally are increasingly demanding foods that do not only offer nutritional benefits but also improve health, and are sustainable and ethically acceptable on account of increased nutritional and dietetics knowledge (Reisch et al., 2013). In this review, nutritional quality is viewed in terms of health promotion. The negative health consequences associated with the consumption of red meat and meat products (Godfray et al., 2018; He et al., 2018; Poore & Nemecek, 2018; Ritchie & Roser, 2020) have prompted both the scientific research and food industry to explore new products that can meet consumers demand, majorly health benefits and sustainability as synthesized by McClements and Grossmann (2021). The shift in demand and innovations has been leaning toward more plant-based food materials compared to meat products (Lynch et al., 2018; Qin et al., 2022b; Tso & Forde, 2021).

The preference for plant-based sources has been attributed to specific nutritional benefits associated with plant-based foods such as high anti-oxidant contents and relatively high composition of unsaturated fatty acids, dietary fiber, bioactive compounds and low contents of saturated fats compared to traditional animal-based products like sausages (Ambika & Hu, 2018; McMacken & Shah, 2017; Budhathoki et al., 2019). These properties have been associated with improved cardiovascular wellness reduced progression of cancers, reduced incidence of obesity, hypertension, and stroke and improved general longevity (Kahleova et al., 2018; Tosti et al., 2018; Satija & Hu, 2018; Zheng et al., 2022). To obtain the above benefits associated with plant-based foods, there is a need to substitute

animal-based diets with plant-based food sources in commonly consumed meat products such as sausages (Zheng et al., 2019; Päivärinta et al., 2020). As such, high quality plant-based protein sources could play a critical role in providing high-quality, healthier, safer and more sustainable proteins to consumers. Essentially these can be obtained by exploiting leguminous crops such as soybeans, pigeon peas, and green grams among others (Lemken et al., 2019; Munialo & Vriesekoop, 2023; Rööös et al., 2022).

Green gram (*Vigna radiata*), also known as mung beans, is a leguminous plant grown worldwide (Zia-Ul-Haq et al., 2008). However, its consumption (utilization) has remained low despite its high production yield (Bandula & Nath, 2020; Kilimo, 2017; Popoola et al., 2012). Nair et al. (2013) reported that green gram contains significant amounts of carbohydrates (630 grams/kg), proteins (240 grams/kg) and micronutrients. The same report also stated that protein and carbohydrates are easily digestible and contribute less to flatulence than other legumes. Other nutritional constituents of green gram are presented in Table 1 above.

Several studies have shown that green gram has several health benefits. For instance, Lee et al. (2011) found that green gram ethanol extract has high anti-inflammatory effects, especially on pro-inflammatory cytokines. Another study by Peng et al. (2008) investigating the inhibitory effect of green gram extract on the advanced glycation end products (AGEs) formation found that green gram showed the highest (80.4%) inhibitory effect against AGEs formation compared to soybean (70.1%). These authors also attributed the inhibitory activity to a green gram's major phenolic components: isovitexin and vitexin (Kang et al., 2015). Advanced Glycation End-products (AGEs) are complex compounds developed as a result of the elevated-temperature processing of protein- and carbohydrate-rich foods (Sharma et al., 2015). Accumulation of AGEs has a detrimental effect on humans, including neuropathy, retinopathy, nephropathy, and Alzheimer's disease (Poulsen et al., 2013). Legumes have

long been recommended for diabetic diets because of their high fibre and protein content (Tura et al., 2018). Furthermore, a study by Peng et al. (2008) has affirmed the claims that legumes prevent the pathogenesis of various complications in diabetics by the activity of phenolic components of legumes, especially green gram. Green gram has also been reported to exhibit prebiotic and nutraceutical properties (Sudhakaran & Bukkan, 2021).

Zhong et al. (2021) reported that substituting nuts, whole grains and legumes, including green gram for animal-based food products especially red meat, prevent cardiovascular diseases and premature death. Salomé et al. (2021) also reported that using legumes as plant-based substitutes for animal products appears more nutritionally adequate than other substitutes. Moreover, green gram protein has been identified as a safe novel food in accordance with regulation (EU) 2015/2283 (Turck et al., 2021). Furthermore, green gram is helpful for its nutritional content, angiotensin-converting enzyme (ACE) inhibition, and anti-fungal activities offering additional health benefits and medical application (Yi-Shen et al., 2018). Mehta et al. (2021) concluded in a review of the metabolites and pharmaceutical potential of green gram that the crop can be used as an efficient food additive in the preparation of different types of food, such as dietary supplements. Moreover, Bhat et al. (2013) found that the use of green gram as a non-meat extender conferred several nutritional benefits to chicken seekh-kababs. Despite the above observations, it has been reported that the use of green gram as a meat substitute has limitedly been explored compared to non-meat products such as composite flour, cookies and others (Bello et al., 2022; Rajiv et al., 2012). Thus, the benefits of green gram consumption, are less harnessed in meat products.

On the other hand, challenges have been reported in relation to the replacement of animal-based proteins with plant proteins in food products. For instance, alongside impacting the proximate composition of foods, partial replacement of plant protein foods for animal protein foods has been

reported by Itkonen et al. (2020) to reduce born mineralization. The probable reason is that plant-based protein sources are limited in the amount of vitamin D and Ca (Itkonen et al., 2021; Tucker, 2014). Another challenge presented by substituting animal-source foods with plant sources is the effects on protein digestibility and nutrient bioavailability. Protein structure, the presence of anti-nutritional factors, and the methods and conditions used during processing all influence the digestibility and bioavailability of nutrients (Gu et al., 2022; Silva et al., 2022). Moreover, the inherent differences between animal proteins and plant protein's structure imply that direct and or complete replacement of plants for animal proteins may not be the most scientifically beneficial option regarding health and nutrition outcomes. For instance, animal proteins are known to supply essential amino acids more effectively than plant proteins (Leroy et al., 2022), and the low digestibility of plant proteins may result in less bioavailability, especially for infants (Marette et al., 2021). However, it has been observed that when a mix of animal protein and plant proteins is taken into a single product, there can be a synergistic advantage of improved nutrient bioavailability from the products consumed (Sá, et al., 2019). Therefore, these observations further strengthen the argument for promoting plant-based nutrient substitution in meat products as a vehicle to deliver more health-sensitive meat products such as pork sausages and beef sausages.

Furthermore, despite the potential health benefits of plant-based meats as a viable alternative to traditional meats, food safety concerns with respect to allergens, antinutrients, and potential carcinogens must be considered. Plants, especially legumes contain several allergens that can pose serious ill health to consumers (Hadi & Brightwell, 2021; Misra et al., 2011; Verma et al., 2013). There has been evidence that a variety of processing methods like roasting, fermentation, dehulling, germination, boiling, and crushing may reduce the negative effects of plant materials on human health. (Ghavidel and Prakash, 2007; Verma et al., 2012). This study, therefore, used green gram to substitute

for pork ham in processing pork sausages. Pork ham provides a rich source of high-quality protein and essential nutrients, which are essential for muscle repair and growth (Halagarda et al., 2017; Ivanovic et al., 2016; Lucarini et al., 2013; Sen et al., 2017). Pork ham plays a significant role in sausage making. This is due to its flavour, balanced fat content, texture, natural binding abilities and versatility in various types of sausages (Tarte, 2008). Above all, it has a good nutritional profile as listed in Table 1 above. However, the effect of incorporation of green gram into pork ham sausages is unknown and thus had to be explored in this study.

2.2 Functional properties of foods and influencing factors

The functional properties of food products are critical determinants of their quality, including the sensorial quality, processing quality and overall useability of the product (Zhang et al., 2023). The major functional properties of meat products include the water holding capacity, oil absorption capacity, cooking yield, and emulsion stability (total fluid released, water released and fat released) (Jridi et al., 2015; Talukder, 2015a). These properties affect the processing and processing outcomes of meat and ultimately the product by influencing the yield, economic value, and eating quality (Cheng and Sun 2008a). For instance, water holding capacity is a crucial attribute that determines products' juiciness and subsequent texture. While, water holding capacity is a crucial factor affecting food products' texture, colour and sensory properties (Cheng and Sun, 2008b; Warner, 2023).

The functional properties of meat and meat products is largely determined by its protein structure (Lonergan et al., 2019). Essentially, the interaction between water and protein is considered one of the most important factors determining protein functionality in foods. Specifically, the water-protein interactions lead to water retention, a vital attribute in food formulation. Myofibrillar

protein is responsible for water absorption in meat and meat products (Lonergan et al., 2019). These are responsible for water binding and encapsulating fats, which significantly determine the texture, juiciness, taste, flavour and overall acceptability of meat products (Xiong, 2018). Generally, sausage processing methods affect the ability of the myofibrillar protein to bind to water. For example, "hot processing"; that is, deboning pre-rigour pork carcass immediately after slaughter and before chilling the carcass, allows myofibrillar protein in the muscle to retain additional water resulting in a juicier sausage (Lonergan et al., 2019). In addition, pre-rigour pork, as an ingredient in pork sausages, has a high PH; this gives the product high WHC, fat-binding ability and colour superior to one processed from post-rigour pork (Hasenhuettl & Hartel, 2019). Further, the chopping step involved in sausage production releases the soluble myofibrillar protein intensifying their contact and subsequent interaction with the fat particles during chopping. These solubilized proteins and water of the sausage concoction form a matrix that encapsulates the fat particles forming sausage batter (Lonergan et al., 2019).

The sarcomere is another protein in meat that has been found to significantly affect the final quality of meat and meat products (Ertbjerg & Puolanne, 2017). Sarcomere structure and length affect the meat quality, notably tenderness and WHC (Ertbjerg & Puolanne, 2017; Lonergan et al., 2019).

Salt is one of the essential non-meat ingredients used in food processing to improve the taste and flavour of the products (Lonergan et al., 2019). However, one of the key roles of salt is improving the texture of meat products by solubilizing myofibrillar protein, thus increasing the protein's water and fat retention ability (Chen et al., 2017; Hasenhuettl & Hartel, 2019; Hassan et al., 2019; Sun & Holley, 2011), reducing cooking losses and reducing the growth of microorganisms while in storage.

The functional properties of meat and plant products are highly variable and hence there is a challenge in matching the nutritional quality, functionality and sensory acceptability of these noble products with consumers' demand (Alcorta et al., 2021; Day et al., 2022; Igual and Martínez-Monzó, 2022; Sha and Xiong, 2020). As such, it is crucial to understand how plant-based material will fit into meat components and its impact on the products' nutritional, functional and sensory attributes (McClements, 2020). Loveday (2020) reported that animal possesses fibrous protein structure, whereas plant has globular form; these impact the texture, emulsion, fluid holding capacity and nutritional and sensory attributes differently. Yi-Shen et al. (2018) reported that mung green gram contains respectively 25 and 60%, albumin and global protein. Moreover, these properties vary from one plant product to the other and thus cannot be generalized (Day et al., 2022; Ma et al., 2022).

Plant protein can be substituted for animal-based protein for up to 80% or more (Hassan et al., 2019). However, the substitution alters the physicochemical properties of products and sensory quality which are the key criteria in consumer choice of meat and processed meat products (Danowska-Oziewicz, 2014). This is probably due to the difference between the protein of meat and plant-based protein sources. For instance, meat protein exhibits a fibrous structure, while plant protein exhibits a globular structure (Loveday, 2020). Hassan et al. (2019) revealed that partial or complete meat replacement with plant proteins in sausage formulation significantly reduced cooking loss and enhanced emulsion stability. The same study also reported that substituting chicken meat with plant proteins was significantly accepted in terms of texture, colour, odour and overall acceptability. Emulsion stability is one of the critical factors that influence the texture, colour and juiciness of sausages (Lee et al., 2010), which significantly influences the sensory acceptability of the product, consequently affecting the economic value of the product.

Danowska-Oziewicz (2014) found that partially substituting meat in meat with soy protein isolates and gluten during sausage production significantly improved emulsion stability and reduced cooking yield and frying loss. In addition, a study by Jeong and Han (2019) found that increasing Wanggasi-Chunyuncho fruit powder to sausage significantly reduced the cooking loss. Despite the challenges associated with the substitution, many studies have demonstrated that the substitution of plant protein for animal-based protein improves the products' nutritional quality and functional properties (Li et al., 2022). However, the effect of substitution using green gram on the physicochemical properties is largely unknown and thus needs to be explored.

2.3 Sensory quality of foods and influencing factors

The sensory properties of foods are critical determinants of the overall acceptability of foods (Chambers, 2019; Murray & Baxter, 2003; Wanjiru, 2018). These properties include; appearance, taste, odor, texture, and flavor. These individually or as a whole determines and influence consumers' choice of foods and products (Kong & Singh, 2011), they vary from product to product and are greatly influenced by the raw materials' micro and macrostructures (Cardona et al., 2023). Previous studies on sensory evaluation of different products have shown that consumers usually judge the product quality based on its colour, texture, aroma and taste before considering the nutritional qualities (Imtiyaz et al., 2021; Mccrickerd & Forde, 2016).

Determination of sensory attributes can be instrumental or non-instrumental (organoleptic) (Starowicz, Poznar, & Zieliński, 2022) and the latter is most preferred for product acceptability assessment. Colour is the first sensory characteristic that an average meat consumer considers; according to them, meat is supposed to have a bright red colour (Lonergan et al., 2019). The colour of minced meat products like sausages is affected by many factors such as processing methods, the status

of raw materials and addition of non-meat ingredients (Meng et al., 2022; Owus et al., 2022). Specifically for pork sausage, the major sensory quality parameters previously studied include flavour, colour, texture, juiciness and aroma (Salejda et al., 2017; Zhu, Zhang, and Peng 2021). It should however, be noted that the preference for food sensory attributes can vary based on consumer factors like age, gender, culture, religion, education, fashion, price, convenience, physical and psychological state (Ali & Ali, 2020; Cardona et al., 2023; Chen & Antonelli, 2020; Mielmann et al., 2022; Ojeda et al., 2021; Popoola et al., 2022; Wadołowska et al., 2008; Wanjiru, 2018). On the other hand, it is clearly emphasized in the literature that the introduction of foreign components into a food product may alter its sensory properties and hence its overall consumer acceptability (Liu et al., 2009; Meng et al., 2022; Owus et al., 2022). For instance, adding non-meat ingredients in sausage processing significantly alters the product's final colour (Owus et al., 2022; Velemir et al., 2020). Relatedly, the addition of sodium nitrite in sausage formulation resulted in cured meat colour as a result of a reduction of nitrite to nitrite gas (Lonergan et al., 2019). Lonergan et al. (2018) reported that water, as one of the key non-meat ingredients in sausage production, also affects the final product colour. Water is used to dissolve and disperse other non-meat ingredients in meat which consequently affect the colour through the distribution of the salt and spices amongst other ingredients such as flavours and preservatives (Lonergan et al., 2018).

Furthermore, previous studies on low-fat frankfurter meat products where meat was replaced with pea protein at 50% in the formulation led to substantial changes in the texture and colour properties (Revilla et al., 2022). Meanwhile, Argel et al. (2020) showed that the partial replacement of pork in the production of low-fat burgers with pulse flour did not affect the sensory properties. In both cases of meat substitution above, it should be noted whether or not the sensory properties were affected

negatively or positively depending on the level of substitution of the products. Thus, this study explored the effects of green gram substitution for pork ham on the sensory quality of pork sausages.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Raw materials and handling conditions

Two main raw materials were used in this study; green gram and pork ham. Green gram (variety NAROGRAM2) was bought from Gulu main market, sorted and washed to remove immature, rotten and weevilled seeds, and sundried for 3 days. The clean and sorted green gram was stored in triple bags at room temperature in the laboratory until further processed. Further processing involved roasting with mild heat. The roasted grain was then milled into flour using the certified commercial flour milling machine (Maize Grinding Mill, BLXF-330, China-made) in Gulu City. The procedure applied in the preparation of the green gram flour is consistent with the work of Karpagavalli and Amutha (2015). Fresh pork, ham (Biceps femoris) was bought from a certified butcher in Gulu main market and transported to the laboratory and kept at $\leq 7^{\circ}\text{C}$ until used. The pork was trimmed off subcutaneous and connective tissue, minced into 6-mm pieces and used as presented in subsection 3.2.

3.2 Development and production of green gram-pork sausages

Making use of a silent cutter (YC-12 meat grinder, model YC-12, made in China) 75, 50 and 25% minced pork ham (prepared as in subsection 3.1) was blended for five minutes with 25%, 50% or 75% green gram flour (prepared as in subsection 3.1) and other ingredients (fats, water, potato starch, soy protein isolate, salt, sugar and spices and seasoning) as presented in Table 2. The control sample was made from 100% pork and other ingredients but without green gram. Each formulation was separately filled in fibrous casings measuring 50 mm in diameter and cooked to a core

temperature of 72 °C. The cooked products were cooled to 4 °C and used for further analyses. The flow chart illustrating the production process is presented in Figure 1.

Table 2: Pork sausage formulations using various proportions of green gram

Ingredient (g)	Products formulated with various proportions (%) of green gram			
	0% (A)	25% (B)	50% (C)	75% (D)
Pork ham (low-fat)	52	39	26	13
Fat	15	15	15	15
Water	20	20	20	20
Potato starch	7.5	7.5	7.5	7.5
Soy protein isolate	3.0	3.0	3.0	3.0
Salt	1.3	1.3	1.3	1.3
Sugar	0.4	0.4	0.4	0.4
Spices and seasoning	0.8	0.8	0.8	0.8
Green gram flour	0.0	13	26	39
Total (g)	100	100	100	100

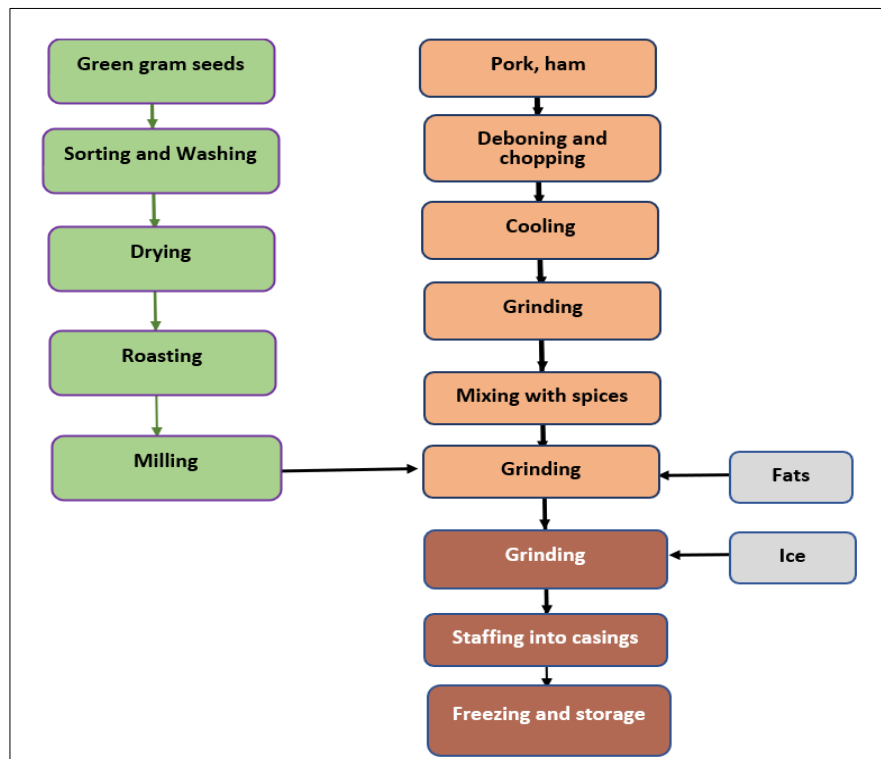


Figure 1: Flow chart for the production of green gram-pork sausages

3.3 Determination of nutritional quality

Nutritional quality was determined in terms of proximate composition, mineral content, protein digestibility, mineral bioavailability, fatty acid profile and antioxidant potential. Before analyses, freshly prepared samples were transferred to air- and water-tight glass containers to prevent water loss during storage and subsequent handling as recommended by the Association of Analytical Chemists (AOAC) (1990). Each sausage sample was removed from its case and passed rapidly 3 times through a food chopper with plate openings of 3 mm. The chopped materials were mixed thoroughly after each grinding and used immediately for analyses. In situations where analyses could not be done immediately, the prepared samples were stored at 4 °C to preserve the quality (Ayman et al., 2013).

3.3.1 Determination of proximate composition

Proximate constituents analyzed included moisture, crude fibre, crude protein, crude fats and ash contents. The moisture content was determined according to AOAC (1990). Briefly, 2 g (W1) of each sample was weighed into a dry crucible. The crucible and sample (W2) were then placed in a moisture extraction hot oven with a fan at 100°C for 5 hours. The dried samples were then put in desiccators to cool and weighed. The weight of the dried sample and crucible was denoted as W3. The difference in weight was calculated and presented as a percentage of moisture content.

$$\text{Percentage moisture} = \frac{W2-W3}{W1} \times 100 \dots\dots\dots \text{Equation 1}$$

The incineration method was used for the determination of ash content (Harris and Marshall 2017). Briefly, 5g of the sample was placed in a 50 mL petri-dish and heated at 100°C until the water was expelled. A few drops of pure olive oil was added and heated slowly over a flame according to

Suzanne Nielsen, (2017). The dish was placed in a furnace at 525°C and left until white ash was obtained. The ash formed was moistened with water and re-ashed at 525°C till a constant weight was attained. The ash content was then calculated on a dry weight basis as the percentage of the ratio of ash weight to the weight of the sample as in equation 2.

$$\text{Percentage Ash content} = \frac{\text{Ash weight}}{\text{Sample weight}} \times 100 \dots \text{Equation 2}$$

Crude fat content was determined using the Soxhlet method as applied by Fogarasi et al. (2018). Thus, 3g of the samples were transferred to the extraction thimbles followed by the addition of 1g of sand. The sample and the sand were mixed with the aid of a glass rod and spread on the side of the thimble added into the extraction thimble. The thimble with the rod was placed in a 50 ml beaker and dried in the oven at 125°C for 1.5 hours. Thereafter, the thimble was placed in the extraction apparatus and the fat was extracted for 4 hours. Following fat extraction, the solvent was distilled off and the resultant fat was dried in a hot oven at 100°C for 30 minutes to remove the remaining solvent. The solvent-free fat was cooled in a desiccator. Fat content was calculated according to equation 3.

$$\text{Percentage fat on a dry weight basis} = \frac{\text{Weight of fat}}{\text{Weight of dried sample}} \times 100 \dots \text{Equation 3}$$

Crude protein was determined using the Kjeldahl method as described by Suzanne Nielsen, (2017). Briefly, 2g of the sample was mixed with 10 ml of concentrated Sulphuric acid (H₂SO₄) in a heating tube. One tablet of selenium catalyst was added to the tube and the mixture was heated inside a fume hood until a colourless solution appeared. The resulting solution was then transferred into distilled water, after which a 10-milliliter portion of it was mixed with an equal volume of 45% sodium hydroxide (NaOH) solution. Subsequently, this mixture was poured into a Kjeldahl distillation apparatus. The mixture was distilled and the distillate collected into a 4% boric acid

solution containing 3 drops of methyl red indicator. A total of 50 ml distillate was collected and titrated against 0.02 N HCl. Determination was conducted in triplicate and the average value was taken. The Nitrogen content was calculated and multiplied by 6.25 (used as a conversion factor) to obtain the crude protein content according to Equation 4.

$$\text{Percentage Crude protein} = \frac{100 \times N \times 14 \times VF}{VA} \times 100 \dots \text{Equation 4}$$

Where; N = Normality of the titrate (0.1 N); VF = Total volume of the digest = 100 m; T = Titre Value; and VA = Aliquot Volume distilled

The fatty acid profile was determined using Gas chromatographic (GC) quantification of fatty acid methyl ethers using the internal addition method (Christie, 2003). The internal standard used was methyl pentadecanoate (C15:0). The standard (20mg/mL) was added to the fat extract to determine the quantity of each fatty acid in the extract. Fatty acid was trans-esterified following the method described by Leite et al. (2015) with some modifications. Briefly, 4 mL of sodium methoxide solution was added to the fraction, vortexed every 5 min for 20 min at room temperature followed by addition of 4 mL of Sulphuric acid solution (in methanol at 50%) to form fatty acid methyl esters (FAMES). The resultant mixture was vortexed for 30 seconds followed by addition of 2 mL of distilled water. Finally, 1µL of FAMES was injected into the GC for quantification. The flame ionization detector was used.

3.3.2 Determination of mineral content and bioavailability

The following minerals were determined; iron, zinc and calcium. These minerals were selected because their bioavailability is affected by processing methods and ingredients used (Castro-Alba

et al., 2019; Singh and Prasad, 2023). The atomic absorption spectrophotometer (model 2300 FOSS, Shelton, USA) method was used for analyses (AOAC, 1990).

Briefly, 2g of each sample was burned for 3 hours into ash at 550°C in a Vulcan furnace. The ash was dissolved in 65% HNO₃ solution (3ml) and filtered in a 100ml volumetric flask using Whatman filter paper No. 41. Following that, the extract was diluted with distilled water up to the mark. The standard solutions for each mineral element were prepared according to AOAC (2005), with concentrations ranging from 0.1 to 1.0 mg/L for Zn, 0.5 to 4.0 mg/L for Fe, and 0.3 to 3.0 mg/L for Ca. The absorbance of calcium (Ca), iron (Fe), and zinc (Zn) was measured at 422.7, 248.3, and 213.9 nm for both the samples and the standard solutions, respectively, using an air acetylene flame. Each diluted standard solution of Zn and Fe received 5ml of-Lanthanum nitrate solution (10% w/v) and HNO₃ (0.2%), and Ca received 5ml of 0.2% HNO₃ (3mol/L). To minimize interference, the Fe and Zn samples were run at lamp currents of 12 and 10mA, with band passes of 6.2 and 0.5nm, respectively. A standard curve was plotted by graphing the absorbance of individual mineral element ions against their respective concentrations in parts per million (ppm). This standard curve was then employed to ascertain the mineral element ion content within each ash sample by extrapolating the sample's absorbance values. The mineral quantities were subsequently quantified in milligrams per 100 grams of the sample.

3.3.3 Determination of protein digestibility

This was carried out using in-vitro enzyme digestion method with pepsin, trypsin and chymotrypsin as described by Plank (2017). Each of the samples was weighed using an analytical balance to 1±0.005g into 50ml centrifuge tubes. A mixture (10mL) of Pepsin and HCl (1Pepsin:19HCl) (1mol/L) was added to each sample. The pH was adjusted to 2.0 and the mixture

was incubated at 37°C under continuous shaking at 150 rpm for 2 hours. Thereafter, 2.5mL of intestinal enzyme concentrate, Trypsin was added to each sample followed by 5.0mL of Tyrode buffer. The pH in each sample was adjusted to 7.5 by dropwise additions of 1M NaOH and 1M HCl solutions. The final volume was made to 25mL with distilled water. Incubation was further done at 37°C with continued shaking at 150 rpm for 2 hours. At the end of the trypsin/chymotrypsin digestion, the samples were placed in a boiling water bath for 10min. After the incubation period, the samples were extracted from the water bath and subjected to vortexing. Following this step, the samples were allowed to cool completely to ambient room temperature for a duration of 20 minutes. Subsequently, 1 mL of a 40% Trichloroacetic Acid (TCA) solution was introduced into the mixture. The samples were then subjected to another incubation, this time at a temperature of 4°C, for an overnight duration. Following the overnight incubation, the samples underwent centrifugation for a period of 10 minutes at a speed of 6,000 revolutions per minute, all conducted at room temperature. After the centrifugation, 1mL of supernatant was removed and mixed with 0.5mL of 2% Ninhydrin followed by 0.5mL of Phosphate buffer saline (PH 8.0), mixed by vortexing and incubated at 99°C for 15mins. The absorbance of the resultant solution was measured at 570 nm, after being diluted to 25mL. Free amino acids content was calculated against a standard calibration curve made with glutamate standard as a reference.

3.3.4 Determination of anti-oxidant potential

Weighed (0.25g) sample was added to a clean test tube. Then, 5.0ml of acidified methanol was added and the mixture was homogenized and centrifuged at 6000rpm at 4°C for 10 minutes. Approximately 0.5mL of the supernatant was transferred into a clean test tube and 3.0mL of solution contain 0.6M Sulphuric acid, 28mM sodium phosphate and 1% ammonium molybdate.

The resultant mixture was incubated at 95°C for 10 minutes to complete the reaction. Spectrophotometric reading was taken at 695nm against the reagent blank.

3.4 Determination of functional properties

The functional properties determined are cooking loss, water holding capacity, and emulsion stability. These were selected because they greatly impact on the final quality of sausages. The cooking loss was measured as the difference in weight of the sausages before and after cooking (water temperature 80 °C, the internal temperature of sausages 72 °C), divided by the sample weight before heating, expressed as a percentage as applied by Hassan et al. (2019). The tests were carried out in triplicate.

$$\text{Cooking loss (\%)} = \frac{\text{Cooked weight}}{\text{Raw Weight}} \times 100 \dots\dots\dots \text{Equation 5}$$

Water holding capacity was measured by centrifugation method as previously described by Santana et al. (2013). Briefly, 5g of the sausage were added into a 50ml centrifuge tube, accompanied by the addition of 10ml of distilled water. Following thorough homogenization, the resulting suspension underwent centrifugation at 5000rpm for 10 minutes, at a temperature of 15 °C. Subsequently, the supernatant was carefully decanted, and the ultimate weight of the pellet was determined. Water holding capacity was then calculated as the weight difference between raw sausage before and after centrifugation according to equation 6.

$$WHC = \left(1 - \frac{\text{Weight}_{\text{Start}} - \text{Weight}_{\text{End}}}{\text{Weight}_{\text{Start}}} \right) \times 100 \dots\dots\dots \text{Equation 6}$$

Emulsion stability was determined in terms of total fluids released, total water and total fat released. In the case of total fluids released, 15g of raw sausages in a 50ml centrifugal tube was centrifuged at 2500rpm for 5 minutes. Subsequently, the tube was heated in a water bath at 80 °C

for 30 minutes. The tube was left to stand upside-down for 50 minutes to allow the exudation of fluids. The supernatant was then decanted and the total fluid released was determined as a percentage of the sample weight as in equations 7 and 8.

$$\text{Total fluid released (TFR)} = (\text{weight of centrifuge tube} + \text{sample}) - (\text{weight of tube} + \text{pellet}) \dots \text{Equation 7}$$

$$\text{Percentage TFR} = \frac{\text{Total fluid released}}{\text{Sample weight}} \times 100 \dots \text{Equation 8}$$

The total water released was determined as the percentage of weight loss after evaporating the total fluid released in an oven at 100 °C for 16h (Cofrades et al., 2008).

$$\text{Water released} = (\text{Weight of TFR} - \text{Dried weight of TFR}) \dots \text{Equation 9}$$

Total fat released (%) was calculated as the difference between TFR and released water as applied by Hassan et al. (2019).

$$\text{Fat released} = \text{Weight of Dried TFR} \dots \text{Equation 10}$$

3.5 Sensory evaluation

Sensory evaluation was carried out on cooked sausages by 60 untrained panelists comprising students and staff of the Faculty of Agriculture and Environment (FAE) as previously applied by Santana et al. (2013). Sharif et al. (2017) stated that between 50 to 150 should be used for untrained sensory panelists. The panelists included students (50) and staff (10) of the FAE of Gulu University. The samples were evaluated based on color, texture, juiciness, flavor, and overall acceptability, utilizing a 5-point hedonic scale, as prescribed by Mousavi et al. (2019). This scale ranged from 1 (indicating strongly dislike) to 5 (indicating strongly like).

Before the evaluation exercise, the panelists were informed of the definition of each sensory characteristic being tested. Each sample was separately served on a clean disposable plate. Samples were coded as 111 (sample A, 0% green gram), 504 (sample B, 25% green gram), 999 (sample C, 50% green gram), and 747 (sample D, 75% green gram). Two inches of each sample were served to each participant. Samples were randomly given to the panelists in a nonsequential order and mineral water (Rwenzori Mineral Water®) was provided to rinse the mouth in between sample tests to minimize carryover effects.

3.6 Statistical analysis

Data were summarized as mean $\pm$ SD (standard deviation). A normality test was performed to check for the normality of the dataset and One-way analysis of variance (ANOVA) was used to check if the means are significantly different from each other and the means were separated using Duncan's multiple range test at 5% level of significance ($p < 0.05$). The analyses were performed using SPSS version 26 (IBM Analytics, USA).

3.7 Ethical consideration

Ethical clearance for the study was duly acquired from the Gulu University Research Ethics Committee (GUREC)-(GUREC-2022-451), and informed consent was sought from sensory evaluation participants in this study.

CHAPTER FOUR: RESULTS

4.1 Effect of green gram substitution on nutritional quality

Nutritional quality was evaluated in terms of proximate composition, protein digestibility, total antioxidant capacity, contents of selected minerals and bioavailability. Results on proximate composition of pork sausages formulated with various levels of green gram are shown in Table 3.

Table 3: Proximate composition of pork sausages incorporated with various levels of green gram

Sample ID	Proximate content (%)				
	Moisture content (Mean $\pm$ SD)	Crude Fiber (Mean $\pm$ SD)	Ash Content (Mean $\pm$ SD)	Crude Protein (Mean $\pm$ SD)	Crude Fat (Mean $\pm$ SD)
A	49.20 $\pm$ 6.57 ^a	0.78 $\pm$ 0.35 ^a	1.61 $\pm$ 0.03 ^a	13.55 $\pm$ 0.19 ^a	8.59 $\pm$ 0.29 ^a
B	44.45 $\pm$ 4.17 ^b	1.49 $\pm$ 0.19 ^b	1.66 $\pm$ 0.05 ^a	14.15 $\pm$ 0.61 ^a	7.77 $\pm$ 0.14 ^b
C	39.17 $\pm$ 2.73 ^c	1.72 $\pm$ 0.06 ^b	1.98 $\pm$ 0.01 ^b	15.75 $\pm$ 1.00 ^b	7.24 $\pm$ 0.11 ^c
D	34.57 $\pm$ 0.18 ^d	2.41 $\pm$ 0.44 ^c	2.06 $\pm$ 0.03 ^c	18.26 $\pm$ 1.01 ^c	7.04 $\pm$ 0.12 ^c

The values are Means $\pm$ SD; n =3. Values in the same column with different superscripts are significantly different at 5% level of significance (Duncan's multiple range test). A: (100:0 % Pork: GG); B: (75:25% Pork: GG); C: (50:50 % Pork: GG) and D: (25:75% Pork: GG).

Significant differences were observed ($p < 0.05$) in the values of various proximate parameters as a function of the level of green gram addition. The moisture content significantly ($p < 0.05$) reduced with increase in the levels of green gram addition. The Crude fibre was significantly higher ($p < 0.05$) in samples containing green gram (B, C and D) compared to the control (A). Pork sausages containing 50 and 75% green gram had significantly higher ($p < 0.05$) ash content above the control. Generally, the crude protein content increased with increase in green gram substitution. However, a significant ($p < 0.05$) effect started to be registered at substitution level of 50%. With respect to crude fat, generally, a significant reduction in the content of proximate parameters with increase in the proportion of green gram in the product formulation. In specific terms, whereas the

control sample (A) had significantly higher fat content than all the other formulations that had green gram substitutions, the formulation with 25% green gram (B) was also significantly richer ($p<0.05$) in fat compared to those with higher green gram substitution level.

Figure 2 presents results on the protein digestibility of pork sausages formulated with various levels of green gram. In general, protein digestibility levels of samples containing green gram were significantly lower than that of the control and the effect increased with the level of green gram substitution ($p<0.05$).

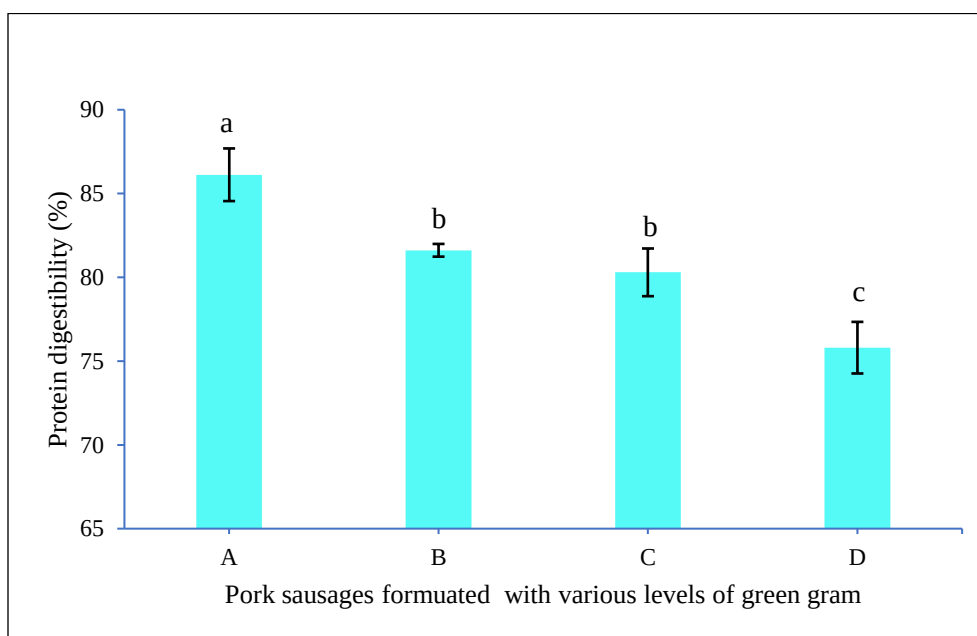


Figure 2: Protein digestibility of pork sausages formulated with various levels of green gram. A: (100:0 % Pork: GG); B: (75:25% Pork: GG); C: (50:50 % Pork: GG) and D: (25:75% Pork: GG). The values are Means $\pm$ SD; $n = 3$. Bars with different letters are significantly different at 5% level of significance (Duncan's multiple range test) and error bars show standard deviation ($n=3$).

Figure 3 presents results on total antioxidant capacity measured in terms of Trolox equivalent. All the formulations containing green gram had significantly ($p<0.05$) higher total antioxidant capacity compared to control. However, the effect increased with increased in the level of green gram incorporated into the product.

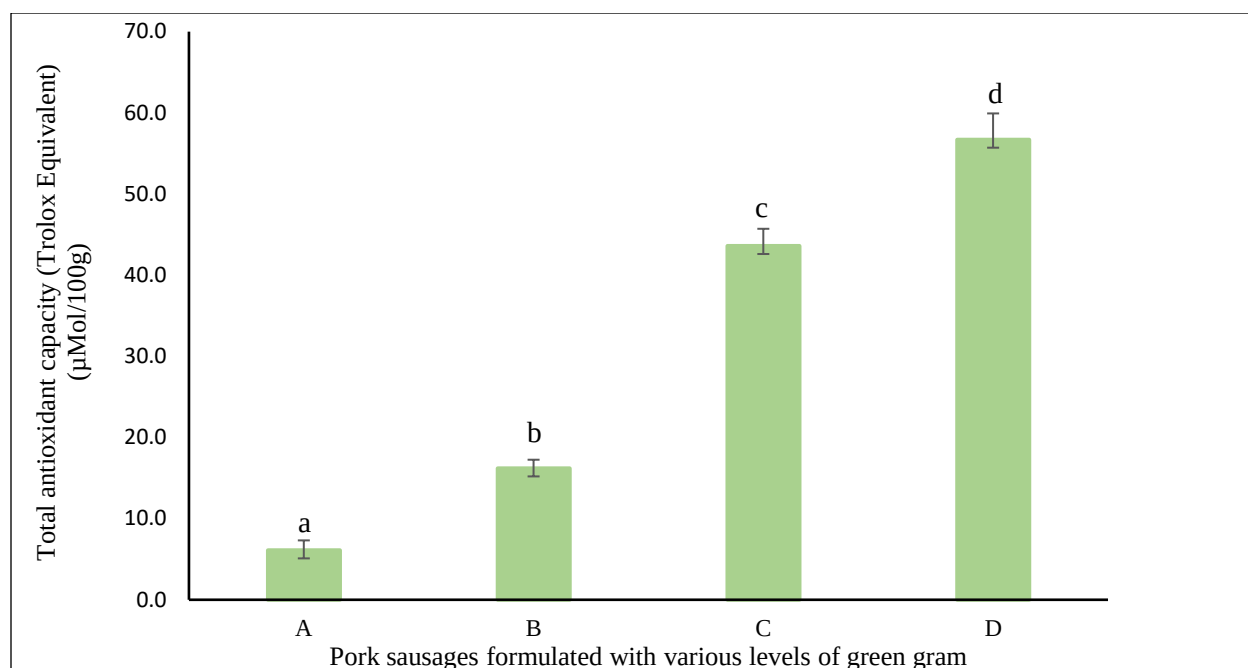


Figure 3: Antioxidant potential of pork sausages formulated with various levels of green grams. A: (100:0 % Pork: GG); B: (75:25% Pork: GG); C: (50:50 % Pork: GG) and D: (25:75% Pork: GG). The values are Means $\pm$ SD; n =3. Bars with different letters are significantly different at 5% level of significance (Duncan's multiple range test) and error bars show standard deviation (n=3).

Table 4 shows the content of selected minerals as a function of green gram substitution level.

Table 4: Contents of selected minerals in pork sausages formulated with various levels of green gram

Sample ID	Mineral content (mg/100g)			
	Fe	Ca	Mg	Zn
A	2.22 $\pm$ 0.27 ^a	4.85 $\pm$ 1.00 ^a	1.34 $\pm$ 0.25 ^a	1.63 $\pm$ 0.03 ^a
B	2.62 $\pm$ 0.12 ^a	7.84 $\pm$ 0.26 ^b	1.69 $\pm$ 0.16 ^{ab}	1.44 $\pm$ 0.07 ^b
C	3.44 $\pm$ 0.12 ^b	9.67 $\pm$ 0.72 ^c	2.19 $\pm$ 0.12 ^b	1.37 $\pm$ 0.06 ^{bc}
D	4.72 $\pm$ 0.54 ^c	13.00 $\pm$ 0.22 ^d	2.89 $\pm$ 0.52 ^c	1.31 $\pm$ 0.08 ^c

The values are Means $\pm$ SD; n =3. Values in the same column with different superscripts are significantly different at 5% level of significance (Duncan's multiple range test). A: (100:0 % Pork: GG); B: (75:25% Pork: GG); C: (50:50 % Pork: GG) and D: (25:75% Pork: GG).

Generally, with exception of zinc, there was a significant increase in the iron, calcium and magnesium content of the pork sausages with increase in the level of green gram substitution.

However, there were peculiarities with respect to the level of green gram substitution at which the

effect started to be registered. In the case of iron and magnesium, the effect started to be registered at 50% while for calcium, the effect started from 25% green gram substitution level. Conversely, zinc content decreased significantly with green gram substitution and was apparent right from the low level of green gram substitution.

Table 5 presents results on bioavailability of calcium, iron and zinc from pork sausages formulated with various levels of green gram. Except for sample containing 75% green gram, there was no significant ($p>0.05$) difference in the calcium and Iron bioavailability. With respect to zinc bioavailability, only the formulation containing 25% green gram had a significantly ($p<0.05$) higher zinc bioavailability compared to the control and other formulations.

Table 5: Mineral bioavailability of pork sausages formulated with various levels of green gram

Sample ID	Mineral Bioavailability (%)		
	Ca	Zn	Fe
A	10.45±0.46 ^a	3.02±0.09 ^a	11.47±0.96 ^a
B	10.12±0.23 ^a	3.62±0.36 ^b	11.10±0.12 ^a
C	10.11±0.31 ^a	3.22±0.23 ^a	10.75±0.95 ^a
D	8.93±0.25 ^b	3.22±0.22 ^a	7.69±0.15 ^b

The values are Means ± SD; n =3. Values in the same column with different superscripts are significantly different at 5% level of significance (Duncan's multiple range test). A: (100:0 % Pork: GG); B: (75:25% Pork: GG); C: (50:50 % Pork: GG) and D: (25:75% Pork: GG).

Table 6 presents results on the fatty acid profile of pork sausages formulated with various levels of green gram. Generally, there was a significant ($p<0.05$) reduction in the total saturated fatty acids with increase in green gram substitution level. Specifically, there was a significant ($p<0.05$) reduction in C18:0 (Stearic acid) and C16:0 (Palmitic acid) content with an increase in green gram substitution level. The content of MUFA reduced with green gram substitution levels. However, the actual reduction was registered from 50% substitution level onwards.

Table 6: Fatty Acids composition of pork sausages formulated with various levels of green gram

Fatty acids (%)	Sample ID			
	A	B	C	D
C14:0	0.13±0.02 ^a	0.10±0.01 ^a	0.10±0.01 ^b	0.08±0.03 ^b
C16:0	24.38±0.25 ^a	20.27±0.29 ^b	18.56±0.12 ^c	17.56±0.29 ^d
C18:0	13.08±0.10 ^a	8.55±0.29 ^b	7.98±0.07 ^c	5.47±0.10 ^d
C20:0	0.76±0.02 ^a	0.31±0.04 ^b	0.28±0.04 ^c	0.21±0.06 ^c
ΣSFA	38.36±0.19^a	29.21±0.31^b	26.96±0.10^c	23.38±0.35^d
C14:1	0.19±0.01 ^a	0.15±0.19 ^b	0.13±0.02 ^a	0.10±0.04 ^a
C16:1	0.15±0.08 ^a	0.12±0.03 ^a	0.16±0.01 ^a	0.09±0.01 ^a
C18:1	36.52±0.11 ^a	34.56±0.40 ^b	31.55±0.54 ^c	28.53±0.21 ^d
C20:1	0.11±0.03 ^a	0.19±0.02 ^a	0.29±0.02 ^b	0.32±0.04 ^c
ΣMUFA	39.42±0.44^a	38.19±0.18^a	36.39±0.52^b	33.81±0.19^c
C18:2	5.70±0.41 ^a	7.06±0.47 ^b	9.45±1.04 ^c	10.42±0.44 ^c
C18:3	7.92±0.04 ^a	8.36±0.47 ^a	15.92±0.42 ^b	21.03±0.24 ^c
ΣPUFA	13.62±0.43^a	15.42±0.75^b	25.38±1.31^c	31.45±0.39^d
<u>UFA</u>				
<u>SFA</u>	1.38±0.01^a	1.84±0.02^b	2.29±0.00^c	2.79±0.03^d

The values are Means ± SD; n =3. Values in the same row with different superscripts are significantly different at 5% level of significance (Duncan's multiple range test). A: (100:0 % Pork: GG); B: (75:25% Pork: GG); C: (50:50 % Pork: GG) and D: (25:75% Pork: GG). SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, and PUFA: Polyunsaturated fatty acid. C14:0 (Myristic acid), C16:0 (Palmitic acid), C18:0 (Stearic acid), C20:0 (Arachidic acid), C14:1 (Myristoleic acid), C16:1 (Palmitoleic), C18:1 (Oleic acid), C20:1 (Eicosenoic acid), C18:2 (Linoleic acid) and C18:3 (Linolenic acid).

With respect to specific fatty acids, peculiarities were observed. This is because whereas the content of C18:1 reduced significantly with the addition of green gram in the formulation, there was a significant ($p<0.05$) increase in C22:1 content instead. There was also a significant ($p<0.05$) increase in the content of PUFA with an increase in the proportion of pork ham substituted with green gram. Particularly, the contents of C18:2 and C18:3 significantly ($p<0.05$) increased with the addition of green gram. Finally, there was a significant ($p<0.05$) increase in the ratio of unsaturated fatty acids to saturated fatty acids with the increase in green gram content.

4.2 Effect of green gram substitution on functional properties of pork sausages

Functional properties of pork sausages formulated with various levels of green gram are presented in Table 7. Addition of green gram significantly reduced cooking loss by about 37% ($p < 0.05$) but was independent of the level of addition. Water holding capacity significantly ($p < 0.05$) increased with the level of green gram substitution. In the context of emulsion stability, total fluid released, total water released and total fat released were all reduced by green gram substitution. The effect was more pronounced at green gram substitution levels of 50% and 75% where total emulsion stability was achieved.

Table 7: Functional properties of pork sausages formulated with various levels of green gram

Sample	Cooking Loss (%) (Mean $\pm$ SD)	Water Holding Capacity (%) (WHC) (Mean $\pm$ SD)	Emulsion Stability (Mean $\pm$ SD)		
			% Total fluid Released	% Total water Released	% Total Fat Released
A	21.30 $\pm$ 3.59 ^a	5.36 $\pm$ 0.96 ^a	4.63 $\pm$ 0.54 ^a	4.49 $\pm$ 0.96 ^a	0.14 $\pm$ 0.00 ^a
B	15.35 $\pm$ 2.21 ^b	17.40 $\pm$ 0.75 ^b	0.28 $\pm$ 0.09 ^b	0.24 $\pm$ 0.54 ^b	0.04 $\pm$ 0.00 ^b
C	13.56 $\pm$ 1.36 ^b	22.21 $\pm$ 1.77 ^c	0.00 ^b	0.00 ^b	0.00 ^c
D	11.06 $\pm$ 4.01 ^b	26.78 $\pm$ 1.37 ^d	0.00 ^b	0.00 ^b	0.00 ^c

The values are Means $\pm$ SD; n =3. Values in the same column with different superscripts are significantly different 5% level of significance (Duncan's multiple range test). A: (100:0 % Pork: GG); B: (75:25% Pork: GG); C: (50:50 % Pork: GG) and D: (25:75% Pork: GG).

4.3 Effect of green gram substitution on sensory acceptability of pork sausages

Figure 4 presents the results of the sensory acceptability of sausages with varied levels of green gram flour substituted for pork ham.

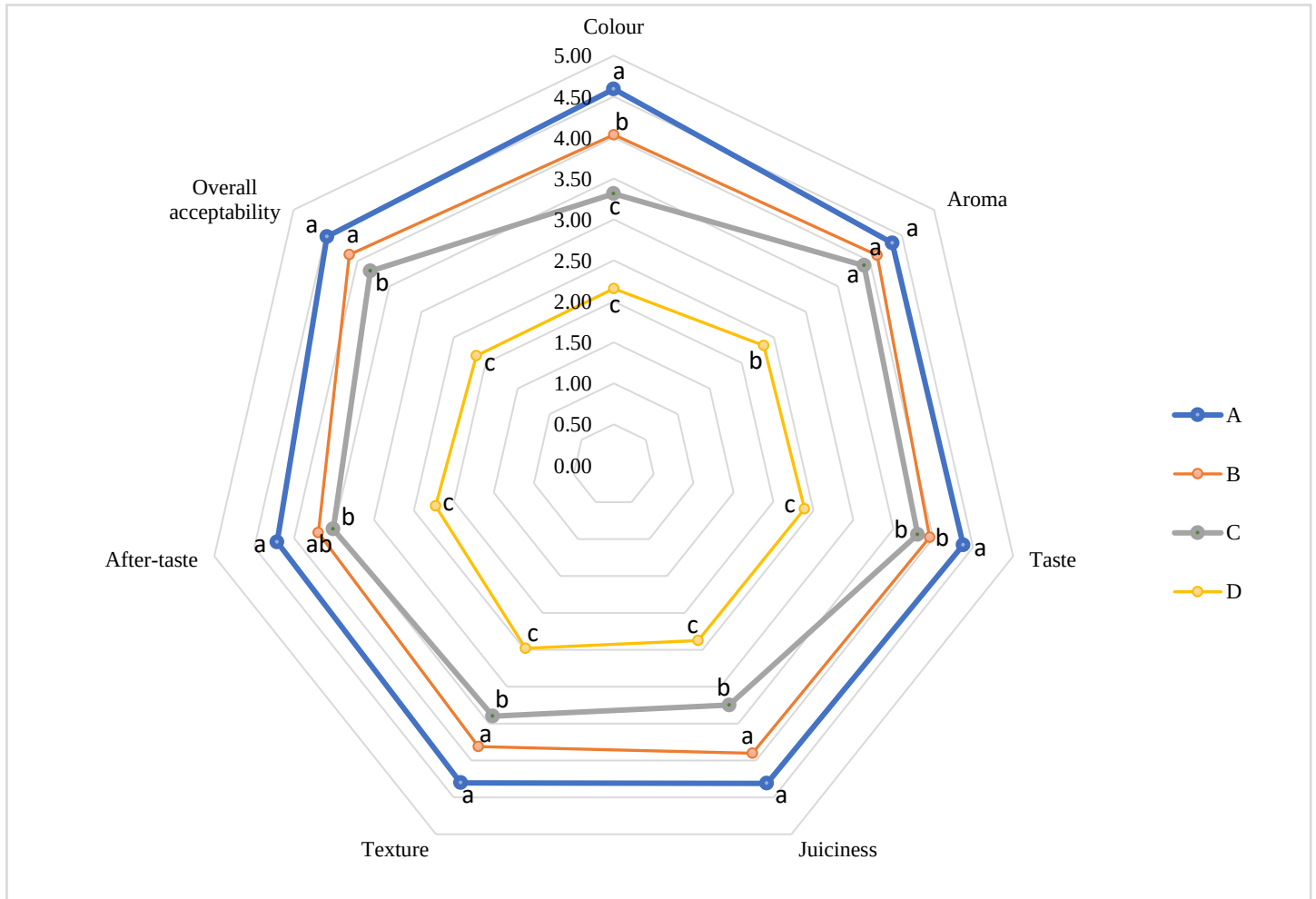


Figure 4: Sensory rating of pork sausages formulated with various levels of green gram. Sensory parameters with different letters are significantly different at 5% level of significance (Duncan's multiple range test). A: (100:0 % Pork: GG); B: (75:25% Pork: GG); C: (50:50 % Pork: GG) and D: (25:75% Pork: GG).

In terms of colour, addition of green gram impacted negatively on consumer preference for the colour of the sausages. The rating for colour decreased significantly with the level of green gram addition. For aroma, green gram addition affected negatively the rating of the sausages. However, the effect was dependent on the concentration and was significant only at 75% level of green gram

inclusion. The taste rating of the sausage was significantly affected negatively by green gram addition and the effect increased with increase in green gram substitution level. The rating for juiciness was also significantly affected negatively by green gram addition and depended on the level of addition. A significant reduction in the rating of juiciness was registered at green gram substitution level of 50% and a further significant reduction was observed with increase in substitution level of 75%. In the case of texture and after-taste, the rating of the parameters followed the same trend as in the case of juiciness. The overall acceptability significantly reduced with green gram addition and the extent of reduction increased with the level of green gram substitution.

CHAPTER FIVE: DISCUSSIONS

5.1 Effect of green gram substitution on nutritional quality of pork sausages

Nutritional quality of food is an indicator of the overall value it offers to the consumers (Fiore et al., 2019; Lingiardi et al., 2022). Essentially, the healthiness of food is measured by its ability to supply the required proportion and quality of nutrients and contribute positively to the body's normal functioning (Lartey, Meerman & Wijesinha-Bettoni, 2018; Shin, Lee & Park, 2012). This is largely derived from the individual profiles of the food ingredients used in the formulation and the interactions between the ingredients (Gunes et al., 2022; Moradi et al., 2022; Nasirpour et al., 2006). This study investigated how substitution of pork ham with green grams affected the nutritional quality of the resulting pork sausage. Specifically, proximate composition (crude protein, crude fats, ash content, fibre, moisture content and carbohydrates content), protein digestibility, micronutrient content, bioavailability, fatty acid profile and antioxidant capacity were evaluated.

The results (Table 3) showed a significant increase in protein content with increasing levels of substitution with green gram. This concurs with the findings of Bohrer (2019), who reported that pork-plant-based analogue had higher protein content than conventional ground pork products. Previous studies indicated that green gram may contain up to 20.9 to 31% proteins (Sharanagat et al., 2019; Venkidasamy et al., 2019; Yi-Shen, Shuai & FitzGerald, 2018), which is higher than the 18.8 to 21% previously reported in pork ham (Halagarda et al., 2017; Longvah et al., 2017). Therefore, the observed increase in protein content of the formulations containing green gram in the case of the current study can be attributed to the high protein content of green gram. Further, as expected, the higher the level of substitution, the richer the protein level of the formation. This

result corroborates the findings of Kenawi et al. (2009) who found that substituting 10% of green gram flour into Buffalo meat patties resulted in a significant protein enrichment from 14.86 to 16.52g. However, these results contradicted a study reported by Reddy et al. (2022b) who found that the addition of 5 to 15% green gram paste to chicken nuggets significantly decreased the protein content of the product. The highlighted disparity is probably due to the high protein content of chicken meat compared to green gram and the dissimilarity with the current study is due to the high protein content of green gram compared to pork ham which resulted in high protein content when pork ham is substituted. Protein is a fundamental nutrient required for human health and as such the increase observed provides a possibility to supply a higher portion of plant-based protein per unit of sausage consumed. With the associated advantages of increased plant protein intake, the new sausage formulation developed in this study could therefore contribute to health benefits, including decreased risks of coronary diseases and other chronic diseases such as cancers, type 2 diabetes and lowering low-density cholesterol that have been reported to be associated with plant protein source foods (Craig 2010; Lamberg-Allardt et al., 2023; Narayan et al., 2014).

Both the crude fibre and ash content of the sausages increased with increasing substitution levels of pork ham with green gram. It is well known that legumes inherently have a high content of dietary fibre relative to meat (Singh, 2017). Yahya and Ting reported that chicken sausages had a crude fibre content was less than 0.8%. In fact for green gram, its crude fibre content ranges from 4.6 to 17.0g/100g on a dry weight basis (Ganesan & Xu, 2018; Sudhakaran & Bukkan, 2021) which is higher than most legumes (Longvah et al., 2017). The observed results indicate potential health benefits of the pork-green gram sausage. For instance, Dhingra et al. (2012) and Soliman (2019) reported that consumption of diets rich in dietary fibre confers positive health benefits that help prevent the development and progression of many metabolic diseases and digestion disorders.

Moreover, dietary fibres may also improve the functional properties of plant-based meat substitutes (Asgar et al., 2010; Talukder 2015a; Twarogowska, Van Droogenbroeck, and Fraeyet, 2022), and are associated with increased satiety index of foods (Munekata et al., 2021; Reverri et al., 2017). In a similar study using green gram paste in chicken Nuggets, Reddy et al. (2022) reported increased crude fibre content with increasing levels of the paste added. By implication, therefore, using pork harm as a carriage means for dietary fibre, may increase the overall intake of beneficial dietary fibres. This assertion conforms with the findings of Päivärinta et al. (2020) from a 12-week clinical trial that replacing animal-based foods with plant-based foods significantly increased fibre intake. In addition, high fibre content has been shown to contribute to weight loss, thus lowering the risk of developing numerous NCDs (Maphosa & Jideani, 2017). By way of inference, these results imply that substituting pork with green gram can be beneficial in increasing the uptake of dietary fibre and thus contribute to fighting NCDs.

In terms of ash content, a similar trend of increase with increasing green gram inclusion was observed. The results concur with observation in a previous study where, the use of green gram and black beans (*V. mungo*) paste as an extender in the formulation of Kababs resulted in a significant increase in the ash content of chicken Kababs (Bhat & Pathak 2009, 2011). The increase could be an indirect indication of mineral enrichment in the formulations where pork was substituted with green grams. This is because the ash content of food is usually directly proportional to the mineral content of the food (Harris & Marshall, 2017; Marshall, 2010).

The inclusion of green gram portions as a replacement for pork harm was associated with a significant reduction in moisture and crude fat contents of the sausages. Specifically, both moisture and crude fat content were found to decrease significantly with an increase in the proportion of green gram. This result agrees with a study by Pietrasik and Soladoye (2021), which showed that

adding pulses to sausages decreased the moisture content, although Bhat (2009) and Pathak (2011) reported that the addition of green gram and black beans (*V. mungo*) paste to Kababs did not significantly affect the moisture content of the chicken kababs. The difference could be because only 15% of green gram flour was used in the study of Bhat and Pathak (2009) rather than 25% as the case in the present study.

Moisture content is an important factor in determining the product's quality characteristics such as colour, texture, taste, and most importantly, the shelf life (Steele, 2004). Similarly, Barberá et al. (2021) also reported a significant decrease in moisture content when black quinoa flour was added to beef patties. Because pulses such as green gram are relatively rich in dietary fibre compared to meat, an increase in the proportion of pulses increased dietary fibre content, which in turn increases the water absorbability of final products due to increase in myofibrillar water entrapment capacity (Kurt & Kilincceker, 2012). This can lead to a reduction in product moisture content as observed with green gram flour in this study. Although it was not the subject of this study, the reduction in moisture content could also confer additional advantage of prolonged shelf life on the green gram-pork-based sausages. This is because the lower moisture content is known to minimize food spoilage and thus enhance product shelf life (Bozoglu & Erkmen, 2016; Kong & Singh, 2016).

Crude fat content of the sausages was significantly reduced with increment in the proportion of green gram substituted for pork ham. Pork ham has a higher fat content than green gram and as such, decreased portions of pork due to substitution is expected to reduce the crude fat content. In another meat product, Bhat and Pathak (2011) found that the addition of green gram at 20% to chicken seekh kababs significantly lowered the crude fat content. Fat content is an important parameter that affects not only the nutritional quality of comminuted meat products but also the functional properties such as oil absorption, water holding capacity, emulsion stability and others

(Abzhanova et al., 2022; Badar et al., 2021; Barbut, 2011). For instance, Crehan et al. (2000) reported that fat content was indirectly related to cooking loss and directly related to the emulsion stability of frankfurters. Similarly, Lorenzo et al. (2011) also reported a marked increase in the shear force and firmness of dry-cured duck sausages with decreasing fat contents. Fonseca et al. (2015) also report that fat levels significantly affected the physicochemical properties of dry-ripened sausages.

Except for Zn, the contents of Fe, Ca and Mg increased in green gram-substituted pork sausages. Generally, the contents of Fe, Ca and Mg in pork ham have been reported as 1.5mg/100g, 14mg/100g and 25mg/100g respectively, while that of green gram is 6.7mg/100g, 132 mg/100g and 189mg/100g, respectively (Longvah, et al., 2017; Sudhakaran & Bukkan, 2021). Therefore, it is expected that the level of these minerals would increase when higher portions of green gram are used in sausage formulation. A study by Venkatachalam and Nagarajan (2017) reported a significant increase in Fe content with an increase in green gram flour as a substitute for wheat flour in crackers. The results also correlate with a study by Harnack et al. (2021) that reported a significant increase in Fe content in plant-based ground beef products. Equally, the significant reduction in Zn content with increase in green gram substitution for pork ham is not peculiar to this study. Swing et al. (2021) reported a significant reduction in Zn content of plant-based beef products compared to ground beef products. This could be because of the replacement of pork ham which is relatively high (3.46mg/100g) in Zn content, with green gram, which is relatively low (1.2-2.67mg/100g) in the mineral (Nagrle et al., 2018; Sudhakaran & Bukkan, 2021). Consequently, this study, therefore, demonstrated the possible avenue for increasing the mineral content of pork sausages, and thus contributing to enhancement of dietary intake of mineral micronutrients such as Fe, Mg and Ca.

Bioavailability and digestibility of nutrients are critical factors that demonstrates the efficiency of utilization of nutrients obtained from diets (Melse-Boonstra, 2020; Rein et al., 2013). Although food formulation may increase nutrient density in foods, the ultimate proportions of the nutrients that are available from the diets become the most important outcome indicator of the nutritional enhancement objective (Affonfere et al., 2021; Fernández-García et al., 2009). In previous studies, it has been widely reported that nutrient bioavailability and digestibility may be influenced by the functional nature of different food ingredients used (Bagchi & Ohia, 2022). Essentially, some nutrients may either act as enhancers or prohibitors of bioavailability of others due to interactions in the digestive pathways or due to chemical changes occasioned by their co-existence (Caballero, 2023; Kan et al., 2022; Kumar et al., 2020; Watzke, 1998; Zhang et al., 2014).

This study investigated the bioavailability of Fe, Zn and Ca and the digestibility of proteins in the formulated products. Substitution of green gram for pork did not significantly alter the Zn, Fe and Ca bioavailability. In fact, the bioavailability of Fe did not significantly change at 50% and 75% substitution levels respectively while the Fe bioavailability was significantly higher with 16% increase at 75% substitution level. Essentially, the results indicate that with substitution levels at 25% and 50%, the net nutritive value of the sausage as estimated by invitro bioavailability determination would be maintained or even improved as in the case of pork sausages formulated with 50 and 75% green gram.

The results for Fe and Ca bioavailability from the current study are similar to the one reported in a study on the effect of thermal processing on the mineral bioavailability of pork (Cilla et al., 2019), where the Fe and Ca bioavailability was detected at 8-12% (Menezes et al., 2018). The observed reduction in Zn bioavailability in this study could be due to the high concentration of iron in the formulation. Singh et al. (2016) reported that high Fe levels reduced Zn bioavailability

and is due to the divalent nature of Fe which interferes with Zn absorption. Another probable reason for the reduced Zn bioavailability observed in green gram substituted pork products could be due to the high level of dietary fibre observed with an increase in green gram substitution for pork. This assertion draws credence from the work of Hemalatha et al. (2007) that showed that both insoluble and soluble fractions of dietary fibre interfere with bioavailability of zinc.

The utilization of protein obtained from foods depends on how much of the protein is broken down into free amino acids and small peptides available for physiological functions (Qin, Wang, and Luo 2022c). The digestibility of proteins is dependent on the hydrolysis of peptide bonds that are characteristic of proteins and is, therefore, a principal factor for absorbing the amino acids by the human body (Hertzler et al., 2020; Joye 2019; Raju, 2019). The results showed a significant decrease in protein digestibility with increase in green gram substitution levels. The highest digestibility (86.12%) was recorded in formulation with 0% green gram, and the values decreased with the substitution of pork with 25%, 50% and 75% green gram. The lowest digestibility (75.8%) was exhibited by formulation with the highest (75%) proportion of green gram. This result is not exceptional to this study. Zhou et al. (2021) reported a significant reduction in protein digestibility in plant-based beef products. Quantitatively, protein digestibility recorded was 85.2% and 69.8% for beef and soybean-beef product, respectively.

However, both values for protein digestibility were lower than found in this study. The probable reason could be that soybean contains a significantly higher amount of anti-nutritional factors than green gram (Emire, Jha, & Mekam, 2013; Guillamón et al., 2008). Roasting of green gram as in this study could have contributed to the high protein digestibility observed, as it could have led to a reduction in antinutrient factors. Veena and Bhattacharya (2012) reported that roasting green gram significantly increased the protein digestibility of green gram paste. Previously Yadav and

Bhatnagar (2017) reported that roasting legumes significantly reduced the content of antinutrients in the product and improved the bioavailability of nutrients. Furthermore, Sá et al. (2020) documented that protein from animal-based foods exhibits better digestibility than plant-based protein due to the high antinutritional factors in plant foods. Therefore, even though there is a significant decrease in the digestibility of protein in formulations containing green gram, the reduction is counterbalanced by a significant increase in the protein content of the product due to an increase in the proportion of green gram substituted for pork. Therefore, substituting green gram for pork has minimal effect on protein digestibility, and this lends credence to the recommendation to apply it as an alternative to meat in producing mixed meat and plant-based food products.

Fats and fatty acid composition of foods is an integral aspect that directly relates to the risk and prevalence of NCDs and other diseases (Billingsley et al., 2018; Mirmiran et al., 2020; Schwingshackl et al., 2022). Many studies have provided evidence of how different fatty acids influence NCDs differently (Billingsley et al., 2018; Hayes, 2002). For instance, a high proportion of saturated and trans-fatty acids are associated with increased cases of NCDs such as hypertension, obesity and certain types of cancers (Beulen et al., 2018; Billingsley et al., 2018). Therefore, determining the fatty acid composition, as in this study, was crucial. From the results (Table 6), the content of saturated fatty acids decreased significantly with an increased proportion of green gram substituted for pork ham in the formulation. This finding is similar to that reported by Curtain and Grafenauer (2019), Katidi et al. (2023) and Costa-Catala et al. (2023) where a reduction in the saturated fats content in plant-based meat products compared to meat products such as sausages, burgers and meatballs. The probable reason for the decreased proportion of saturated fats in sausages containing green gram observed in the current study could be due to the

decrease in the proportion of pork known to be relatively high in SFA compared to the added green gram, which is relatively low in SFA.

It was apparent that the content of total MUFA decreased with increase in green gram proportion in sausages. Of significance, C18:1 (Oleic acid) content significantly decreased as the proportion of pork ham substituted with green gram increased. This is in line with a study by Marchi et al. (2021) that reported a higher content of C18:1 (Oleic acid) in meat-based burgers than in plant-meat burgers. This could probably be due to the low content of C18:1 (Oleic acid) in green gram (Sudhakaran and Bukkan 2021) than in pork (González-Domínguez et al., 2020; Longvah et al., 2017). Thus, it is clear that reducing pork ham proportion has a negative effect on C18:1 (Oleic acid) content. The ratio of unsaturated fatty acids to saturated fatty acids detected in green gram substituted sausages (1.37-2.82) fell within the favourable ratio (1.0-4.0) for CVD risk reduction (Kang et al., 2005). It is largely known that replacing saturated fats with unsaturated fats improves cardiovascular health (Siri-Tarino et al., 2010). From these analyses, therefore, it can be concluded that the modification of fatty acids profile and content of pork sausages through green gram incorporation provides pork sausages that may support better NCD outcome.

Besides providing nutrients, nutraceutical functions of foods are crucial for achieving better health outcomes (Alali et al., 2021; Díaz, Ruiz & Cámara, 2020; Ruchi et al., 2017). In the course of intensive metabolic activity, reactive free radicals are released, which can cause oxidative damage to the cells and nerves with detrimental health consequences (Lobo et al., 2010; Phaniendra, Jestadi & Periyasamy 2015; Pizzino et al., 2017; Sachdev, Ansari & Ansari 2023). The protective roles of food in preventing oxidative damages have long been documented (Kumar & Pandey 2013; Kurutas 2016). Numerous compounds in plant-based foods have been reported to be associated with free radical scavenging properties (Abeyrathne et al., 2022; Lobo et al., 2010; Sachdev et al.,

2023). These include phenolic and non-phenolic compounds such as phytates, tocopherols, vitamin C, saponins and carotenoids (Ganesan & Xu, 2018; Guo et al., 2012). In the present study, substitution of green gram flour for pork ham resulted in a significant increase in the anti-oxidant potential of pork sausages. This result is not peculiar to this study, Venkatachalam and Nagarajan (2017) evidenced that substituting green gram flour for wheat flour significantly improved the antioxidant activity of wheat crackers.

The increase in antioxidant potential observed in this study could be due to the high level of phenolic compounds and flavonoids reported in green gram (Mehta et al., 2021; Sudhakaran and Bukkan, 2021). In fact, Sharma and Giri (2022) noted that phenolic and flavonoids in legumes are the major contributors to their anti-oxidant properties and that green grams have higher antioxidant activity compared to other legumes. On the other hand, previous studies have shown that green grams are rich in waters-soluble polysaccharides that have free radical scavenging activities, consequently contributing to the increased antioxidant potential of products containing them (Song et al., 2019a, 2019b). Indeed, the protective nature of green gram against cancer-causing free radical species has been explored by Ganesan and Xu (2018) and Sudhakaran and Bukkan (2021) in a review of the health benefits of green gram. This observation further stretches relevance to the objective of increasing availability and access to more healthy meat and protective products to consumers. Therefore, it can be inferred that incorporating green gram in sausages as illustrated in the current study is a potential strategy for offering consumers with pork sausages that confer nutritional and nutraceutical benefits.

5.2 Effect of green gram substitution on functional properties of pork sausages

Alongside the nutritional quality, functional properties of food are pivotal in determining the sensory appeal and overall acceptability. The key functional properties that affect the quality of sausages are cooking loss, water holding capacity and emulsion stability (Igual & Martínez-Monzó, 2022). Generally, substituting pork with green gram flour in pork sausage recipes significantly improved the functional properties of the sausages. These results tend to support the findings of Boye et al. (2010) who noted that incorporating pulses in products like sausages offered not only nutritional benefits but also conferred desirable functional properties that improve consumers' sensory acceptability.

Specifically, adding green gram flour significantly increased the WHC of sausages in all three formulations investigated in this study. The WHC of food measures the ability to retain water, as Pearce et al. (2011) defined. The result positively relates to the finding of Saikia et al. (2019), who observed an increase in WHC of duck patties with the addition of black gram flour. The improvement in WHC observed could be attributed to the increase in protein and fibre content of the sausages with the increasing addition of green gram flour. The presence of- and the increase in protein and fibre content makes it bind better to the water, hence increasing water holding capacity (Tiwari et al., 2020). This could also have additional advantages in improving the product texture. Indeed, the potential of legume fibres to increase water and fat retention capacity has been utilized to improve the texture of foods such as sausages (Tosh & Yada, 2010).

Cooking loss is an important parameter that affects sensory appeal to consumers (Bertola et al., 2019), as well as the stability of the product under storage (Abdolmaleki et al., 2016). From the current study, adding green gram flour significantly lowered cooking loss of sausages although the

reductions were independent of the level of substitution. The lowering of cooking losses is an important result because it provides avenue for maintaining the sales weight of the sausages after cooking. Similar to this finding, a study conducted by Pietrasik and Soladoye (2021) reported that adding pea flour and chickpea flour to sausages significantly lowered the cooking loss. Hassan et al. (2019) also reported that partial or total meat replacement with pulses reduced cooking loss in sausages. Similarly, Tahmasebi et al. (2016) reported that adding pigeon pea flour to sausage resulted in products with reduced cooking loss. The reduction in cooking loss could be attributed to the decrease in moisture content of the product following addition of green gram flour. This is evidenced by the perceived juiciness of the products expressed by pannelists during sensory evaluation.

Emulsion stability measures how well the meat emulsion retains juices, that is, water and fats (Faridah et al., 2023; Ugalde-Benítezt, 2012; Youssef & Barbut, 2010). The emulsion stability of commuted meat products has been shown to influence the texture, colour and juiciness (Lee et al., 2010; Nieto & Lorenzo, 2021). Generally, substitution of pork ham with green gram resulted in products with improved ability to retain water and fats. In particular, there was significantly high total fluid released, total water released, and total fat released from pure pork sausages compared to other formulations with green gram. In fact, no fluid was released in samples with 50% and 75% green gram implying that the potential of green gram to improve emulsion stability of pork sausages increases with the level of substitution. The observed increase in emulsion stability is probably due to the high dietary fibres from green gram bound moisture and fats in the emulsion. This is because addition of dietary fiber to emulsified meat products was reported to reduce water loss and fat loss (Mehta et al., 2015; Mehta et al., 2019; Talukder, 2015b). Song et al. (2019) showed in the analysis of functional properties of water-soluble polysaccharide isolate of green

gram that the isolate confers good oil absorption properties. This could also explain the observed low total fat released from products containing green gram compared to the control observed in the present study. Overall, it is clear that using green gram flour to substitute for pork in pork sausage improved important functional properties of the final products.

5.3 Effect of green gram substitution on sensory acceptability of pork sausages

Sensory attributes of food are critical determinants of consumers' acceptability (Kim et al., 2010; Melovic et al., 2020). Therefore, it is essential that in developing new food formulations, sensory properties are maintained or improved to ensure product uptake (Saldaña et al., 2021). In this study, the preference levels of key sensory attributes (colour, taste, flavour, texture, juiciness, after-taste) and overall acceptability as rated by the pork consumers were examined. In general, these sensory attributes are well-known primordial factors that affect consumer appeal (Bushong et al., 2010; Hutchings 1994; Shepherd 1990), their effects largely depend on the food ingredients and processing method applied (Chigwedere et al., 2022; Lai & Lin, 2007).

The results (Figure 4), showed that samples of pure pork sausages and that formulated with 25% green gram were the most preferred of all four formulations. This is well illustrated by the fact that substitution of green gram flour for pork above 25% negatively affected all the sensory parameters of the products. Considering colour as one of the essential sensory parameters, adding green gram flour significantly reduced the colour scores of the products. Similarly, Reddy et al. (2022) reported a reduction in colour ratings when green gram paste was added to chicken nuggets. The same study also reported a significant ($p < 0.05$) reduction in the preference rating of flavour and overall acceptability of nuggets. Further, a study using black bean paste in chicken seekh-kababs by Bhat and Pathak (2011) reported similar results where there was a reduction in the sensory

scores for juiciness, texture and overall acceptability when the levels of black beans were increased. Saikia et al. (2019) also reported the same trend in the results when black gram flour was used in duck meat patties. With respect to the current study, the reduction in the colour scores for sausages following green gram addition might be due to dilution of pork pigment and the overshadowing of the light red colour of meat with the green colour of green gram. On the other hand, the observed reduction in aroma scores of sausages with increased levels of green gram could partially be due to the dilution of flavour compounds in meat as more meat is replaced with plant-based ingredients (Malav et al., 2013; Saikia et al., 2019; Talukder, 2012). This is because several factors including the protein-flavour binding process, the chemical structure of the flavour compounds and proteins, moisture content and temperature of the food matrix; and the processing conditions of the food matrix have been reported to affect the flavour of plant-based meat products (Coggins, 2012; Zhang et al., 2021).

With regards to juiciness, pork sausages formulated with 25% received the same ratings as the control. This could be due to the high moisture content of pork sausages formulated with 25% green gram. This result is in line with that reported by Kenawi et al. (2009) where 10% green gram did not significantly affect the juiciness of buffalo meat products. Consumer rating for taste significantly reduced with addition of green gram. Kenawi et al. (2009) reported a significant reduction in taste rating of buffalo meat products extended with green gram. Notably for after-taste, formulations with 0 and 25% green gram did not significantly vary.

Interestingly, the overall acceptability of green gram-pork sausages followed the order of 0%, 25%, 50% and 75% green gram substitution. Generally, findings from this study confirm that formulations with more green gram had less favourable sensory ratings and overall acceptability. Previous studies have observed a similar relationship (Fiorentini, Kinchla & Nolden, 2020). For

the specific case of pork sausages examined in this study, the product colour, taste and aroma seem to be the most important sensory attributes that drive acceptability. This is because formulations with significantly higher scores for the three attributes also had the highest overall consumer acceptability. Considering the fact that sensory acceptability is a key factor that drives consumer food choice, future studies need to design strategies to enhance sensory attributes of the green gram substituted pork sausages. This is necessary in order to take advantage of the enhanced functional characteristics and nutritional quality that have been demonstrated to accrue when green gram is used in pork sausage formulation.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study has demonstrated the potential application of green gram in pork sausage formulation. Substitution of pork with green gram portions in sausage formulation particularly at 25% level produced a highly acceptable product comparable to the non-substituted pork sausage. It can further be concluded that adding green gram as a substitute for pork in pork sausages improves: i) the micronutrient (Fe, Ca, and Mg) profile; ii) the fatty acid profile with respect to increased UFA; iii) the antioxidant capacity; and iv) important functional properties such as water holding capacity, emulsion stability and cooking loss. On the other hand, it reduced the crude fat content and protein digestibility. Further, the mineral bioavailability is maintained at the substitution rates studied. Therefore, this product can contribute to promoting plant protein intake with its attended benefits. With the ever-increasing cases of NCDs, this study presents a product that can be used to promote healthy food consumption thus contributing to reducing the occurrence and progression of different NCDs, such as obesity, type 2 diabetes, cancer and CVDs. Specifically, reduced content of SFA, increased crude fibre, UFA content and antioxidant capacity observed make these products a healthy alternative available to consumers.

6.2 Recommendations

On the basis of the results and considering food choice, the recommended green gram substitution level is 25%. This is based on the fact that the acceptability of the product containing 25% green gram was identical to that of pure pork sausages while other formulations containing more green gram had significantly lower acceptability. Considering that products containing more than 25%

green gram had superior nutritional and functional properties despite negative sensorial appeal, there is need to improve the sensorial appeal of those product formulations to enable consumers derive more nutritional and health benefits of green gram substituted pork sausages.

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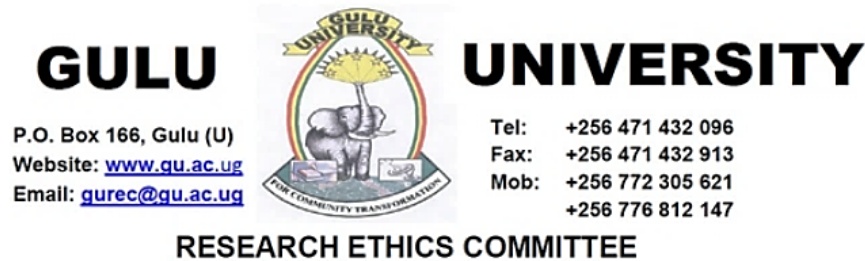
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APPENDICES

Appendix A: GUREC Approval



03/03/2023

To: Agako Alod

0785940056

Type: Initial Review

Re: GUREC-2022-451: Effect of green gram substitution rate on the quality of pork sausage , ,

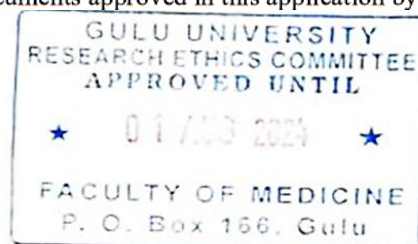
I am pleased to inform you that at the **94th** convened meeting on **17/11/2022**, the Gulu University REC, committee meeting, etc voted to approve the above referenced application.

Approval of the research is for the period of **03/03/2023** to **03/03/2024**.

As Principal Investigator of the research, you are responsible for fulfilling the following requirements of approval:

1. All co-investigators must be kept informed of the status of the research.
2. Changes, amendments, and addenda to the protocol or the consent form must be submitted to the REC for re-review and approval **prior** to the activation of the changes.
3. Reports of unanticipated problems involving risks to participants or any new information which could change the risk benefit: ratio must be submitted to the REC.
4. Only approved consent forms are to be used in the enrollment of participants. All consent forms signed by participants and/or witnesses should be retained on file. The REC may conduct audits of all study records, and consent documentation may be part of such audits.
5. Continuing review application must be submitted to the REC **eight weeks** prior to the expiration date of **03/03/2024** in order to continue the study beyond the approved period. Failure to submit a continuing review application in a timely fashion may result in suspension or termination of the study.
6. The REC application number assigned to the research should be cited in any correspondence with the REC of record.
7. You are required to register the research protocol with the Uganda National Council for Science and Technology (UNCST) for final clearance to undertake the study in Uganda.

The following is the list of all documents approved in this application by Gulu University REC:



No.	Document Title	Language	Version Number	Version Date
1	Research proposal	English	3.0	2023-02-06
2	Translated consent if applicable to your study	Acholi	3.0	2023-02-06
3	Data collection tools	English	2.0	2023-01-26
4	Informed Consent forms	English	2.0	2023-01-26
5	COVID-19 & EBOLA risk management plan for the study	English	2.0	2023-01-26

Yours Sincerely

Julaina A. Obika

Julaina A. Obika (PhD)

For: Gulu University REC



Appendix B: Informed Consent form in English language

Title of the study: Effect of green gram substitution rate on the quality of pork sausage

Investigator(s): AGAKO Alod

Institution(s): Gulu University

Introduction

Good morning/afternoon/evening. My name is Agako Alod, a student of Gulu university pursuing a master of science in food security and community nutrition in the study on '*Effect of green gram substitution rate on the quality of pork sausage*'. The study seeks to investigate the effect of green gram substitution rate on the nutritional, physico-chemical and sensory properties of pork sausages. This informed consent explains the study to you. After the study has been explained, any questions you may have are answered, and you have decided to participate in the study, you will be asked to sign a consent, which you will be given a copy to keep.

Purpose:

The study seeks to develop pork sausage mixed with green gram by substituting some component of pork ham. This study will provide information and make available, pork sausages with reduced proportion of animal protein thus improving the healthiness of the conventional pork sausages sold in the markets. This will be a one study that will contribute to the global efforts of reducing animal protein consumption while increasing plant protein consumption for a better NCDs outcome.

Procedures:

Your participation in this study will involve participating in the interview by means of testing of the product samples and you will respond appropriately by filling the questionnaire with your level of liking of the products.

Who will participate in the study?

You have been chosen to participate in this sensory evaluation study because you are a consumer of pork and pork products like pork sausage.

Risks/discomforts:

There is no foreseeable risk of harm or discomfort that will arise from your participation in this study. The only risk or discomfort will be the inconvenience in terms of time spent during the interview.

Benefits:

Furthermore, we assure you that research participants will get feedback on findings and progress of the study, and that any new information that affects the study participants (including incidental findings) will be made available to research participants. You will also be given 5,000/= Uganda shillings for your time.

Confidentiality:

Your identity will not be revealed to any one as we shall only use codes to identify participants. Information obtained will only be accessible by the research team. Soft copies of the data will be protected by password. Confidential information will only be accessed by the principal investigator.

Alternatives:

You do not have to participate in this study if you are not interested. You will not lose any benefit in case of no participation.

Cost:

There will be no any additional cost incurred as a result of participating in this study.

Questions:

If you have any questions related to the study, or your rights as a research participant, you can contact the principal investigator Agako Alod on mobile number 0785940056 or via email on: alodagako@gmail.com.

Statement of voluntariness:

Participation in the research study is entirely voluntary and you may join on your own free will. You have a right to withdraw from the study at any time without penalty.

If you have any issues pertaining to your rights and participation in the study, please contact the Chairperson, Gulu University Research Ethics Committee, Dr. Gerald Obai Tel: No., 0772305621; email: lekobai@yahoo.com / lekobai@gmail.com; or the Uganda National Council for Science and Technology, on plot 6 Kimera road, Ntinda, Kampala on Tel 0414705500.

Statement of consent

..... has described to me what is going to be done, the risks, the benefits involved and my rights as a participant in this study. I understand that my decision to participate in this study will not affect me in any way. In the use of this information, my identity will be concealed. I am aware that I may withdraw at any time. I understand that by signing this form, I do not waive any of my legal rights but merely indicate that I have been informed about the research study in which I am voluntarily agreeing to participate. A copy of this form will be provided to me.

Name Signature/thumbprint of participant.....Date
Name.....Signature of witness.....Date.....
Name.....Signature of interviewer.....Date.....

Appendix C: Informed Consent form Translated in Acholi

Title of the study: Effect of green gram substitution rate on the quality of pork sausage

Investigator(s): AGAKO Alod

Institution(s): Gulu University

Ngute

Itye nining, an nyinga obedo Agako Alod, latin kwan me Gulu Univeristy atye akwano degree ma malo ame olwongo ni food security and community nutrition dok atye akwedo lok ikom alokaloka me medo choroko ikom ringo punu loko kede mita ringo punu ame orego ot yupu kwede sausages. Waraga ma tye me miyi niang ilok kom kwed man. Ka dong atito boti lok kom kwan man ci iniang iye, ibino dong yero me bedo ikwed man onyo pe. Ka iye bedo iye, ibino keto cingi ni iye and obino miyo yiri waraga man.

Tyen lok me kwed mans

Kwed man tye me konyo yuzu ringo punu ame omedo choroko iye. Kwed man bi konyo weko oyubu ringo punu bedo ame camo kelo yot kom. Kwed man nang bi konyo wilobo iyore me dwoko piny rwom me camo ringo me wek dwok woko two mogo ma dono nwongo a calo adwogi me cam ringo ame moo dwong iye macalo two acuny.

Kit ma kwed obi cito kwede

Bedo ni ikwed man be bedo me bilo ringo punu man ma kimedo kwede choroko mi ite Waco kit ma inwongo kwede kun icoyo lagam ma meggi I papara me kibi mini.

Anga ma romo bedo ikwen man?

Yin ki yeri me bedo ikwed man pien yin I bedo dano ma camo ring opego.

Gin marac ma romo time ikwed man.

Gin maromo time ki adwogi ne ma luke ki gamo lapeny magi, Pe tye gin mo ma rac ma obe time ma luke ki gamo lapeny magi, amito ngec ma i tye kwede. Abe gwoko mung ma tut ma i be miya, dok pe abe weko ngat mo ngeyo ni in i miya ngec magi, pea be coyo nyingi i buk man, dok a tiyo ki nukta me ka-ka nying

Gin bai bi nwongo.

Mukene doki, wa mini gen ni adwogi pa kwed man bino dwogo cen bot yin and ngec mo keken ma konyo yin ma aa ikon kwed man obi dwogo cen bot yin

Ngec ma kwako imug me komi

Ngec mo keke ma kwako kumi pe kibi miyo bot ngat mo keken pien wa bi tiyo ki nukta to ngeyo yin ma nwongo ngat mukene romo niang kuu. Ngec ikumi bi bedo bot lakwed lok man keken and lok ma pire tek bi bedo bot lakwed keken.

Iromo kwero me bedo ikwed man ka miti ni peke iye. Kwero ni pe bino weki ikeng gin mo.

Pe ibi rwenyo gi cingi mo keken ma calo adwogi me yin ibedo ikwed man.

Lapeny

An nyinga obedo Agako Alod, latin kwan me Gulu Univeristy atye akwano degree ma malo ame olwongo ni food security and community nutrition pi lapeny mo keken ma lube ki lok kom kwed man, go cim boti iyi numba cimi 0785940056 onyo cwala email alodagako@gmail.com.

Lok me ye piri keni

Ye me bedi ikwed man tye itwero ni, iromo donyo ikwed man ka imito and dok twero ni tye me yaa weko kwed man icawa moo keken and pwod mo peke ma kibi keto ikumi.

Kube me niang i kum twero ma i tye kwede, ka itye ki kit lapeny moo i kum kwed man i twero meri i romo goyo cim bot Lapwony madit me kwed (Dr) Gerald Obai i cim namba 0772305621.

Ladit man aye loyo lok kum woro me kwed i Gulu University

Lok ma moko yee.

..... owaco yira gin ma obi time, gin mo marac me twero time, ber pa an me bedo ikwed man ki twero pa an ma lube ki bedo pa an ikwed man. Aniang dok ni ye me bedo pa an ikwed man pe bino tima kit gin mo keken ikuma I yo mo keken. Do aniang ni itic ki lok kumi ikwed pe bi ngutu anga ma an abedo. Dok atye kiniag ni aromo aa weko bedo ikwed ma icawa moo keken. Dok aniang ni ka aketo cinga I papara ma, no dong aye ma pira kena me bedo ikwed man labongo dic mo keken. Papara man ma an aketo cinga iye ni bene ki bi mina.

Nying lagam peny cinga ninodwe.....

Nying caden.....cing caden ninodwe.....

Nying lapeny.....cing lapeny ninodwe.....

Appendix D: Data collection tool

Laboratory analysis data entry form

Pork sausages will be produced using varying proportions of green gram flour.

Different samples will be produced and each will be produced in triplicates and analysis shall be done in duplicate.

Samples	Production	Analysis results	
		1 st	2 nd
Sample A	1	1 (Parameters)	2 (Parameters)
	2	1 (Parameters)	2 (Parameters)
	3	1 (Parameters)	2 (Parameters)
Sample B	1	1	2
	2	1	2
	3	1	2
Sample C	1	1	2
	2	1	2
	3	1	2
Sample D	1	1	2
	2	1	2
	3	1	2

Total number of samples = 4

Number of samples (duplicates per production) to be analyzed= 24

Appendix E: Sensory evaluation data entry form

SENSORY EVALUATION OF GREEN GRAM PORK SAUSAGE

SEX: MALE FEMALE AGE DATE / / 2023

INTRODUCTION

You are given diverse sausages samples that you should assess. Wisely start from left to right, observe the sample and assess them for; (i) **Colour** (ii) **Aroma** (iii) **Texture** (iv) **Juiciness** (v) **Taste** (vi) **After-taste** (vii) **Overall acceptability**. Please indicate the number which best describes your feeling. **1.** Dislike strongly **2.** Dislike **3.** Neither like nor dislike **4.** like **5.** like strongly. Please take a sip of water and rinse your mouth before and after testing any sample.

1. Dislike strongly **2.** Dislike **3.** Neither like nor dislike **4.** like **5.** like strongly

Sample code	Colour	Aroma	Taste	Juiciness	Texture	After-taste	Overall acceptability
111							
504							
999							
747							

Appendix F: Plagiarism check certificate

GULU
P.O. Box 166
Gulu, Uganda



UNIVERSITY

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Fax: +256 471343095
Emails: ul@gu.ac.ug
URL: www.gu.ac.ug

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PLAGIARISM CHECK

Declaration for Originality Form

This form must be completed and signed for all scholarly work produced.

Name: ALOD AGAKO
Personal File No.(staff) Reg. (student). 21/U/2401/MFN/PS
Faculty/Institute: AGRICULTURE & ENVIRONMENT
Department: FOOD SCIENCE & POST HARVEST HANDLING TECHNOLOGY
Title and bibliographic description of the work
EFFECT OF GREEN GRAM SUBSTITUTION ON THE QUALITY OF PORK SAUSAGE

Declaration.

1. I understand what plagiarism is and I am aware of the University policy on this regard,
2. I declare that this ...**Thesis**... (thesis, dissertation, essay, assignment, paper, report etc.) is my original work and has not been submitted elsewhere for examination, award of a degree or publication. Where other people's work or my work has been used, this has been properly acknowledged and referenced in accordance with Gulu University requirements,
3. I have not sought or used the services of any professional agency to produce this work,
4. I have not allowed, and shall not allow anyone to copy my work with the intention of passing it off as his/her own,
5. I understand that any false claim of this work shall result in disciplinary action in accordance with University Policy on Plagiarism.

Signature

Date

08th/09/2023

Originality Report

The article/thesis/document titled above have been subjected to plagiarism check and the level was found to be.....**20%** (percent) this is classified as:

- ☒ **Minor**
☐ **Moderate**
☐ **Serious**

Yours sincerely,

Dr. Ongaya Kizito (PhD)
Senior Librarian