PIGEON PEA PROTEIN CONTENT AND ITS POTENTIAL FOR DEVELOPING BINDER IN SAUSAGE MAKING

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EXTENDED ABSTRACT

The use of non-meat and natural binders like legumes to replace chemical binders in sausage making (stimulated by the increasing demand for healthy food) has become one of the common practices in the meat industry. The protein in pigeon pea can be exploited to improve sausage performance. This study was conducted to assess the potentiality of pigeon pea protein in developing beef sausage compared to industrial phosphate binder.

Pigeon pea sample varieties, local and improved, were collected and subjected to protein and anti-nutritional factors analyses. Beef sausages were formulated with eight levels that included control sausage (CB), phosphate sausage (PB) with 0.5% of phosphate, 2%, 4% and 6% of pigeon pea binder (PPB1, PPB2 and PPB3 respectively) and 2%, 4% and 6% pigeon pea flour (PPF1, PPF2 and PPF3 respectively). Sausage performance was assessed by determining the texture profile (TP) and water solubility index (WSI) of the sausages and sensory evaluation was performed.

The improved variety had higher protein content hence was used to develop pigeon pea binder. Anti-nutritional factors were present below the lethal dose hence were not analyzed in the binder developed. In texture profile, PPB sausages had the significantly ($p<0.05$) lowest hardness and the second highest value for springiness, cohesiveness and adhesiveness after PB sausage. For water solubility index (WSI), PPB3 sausages had significantly ($p<0.05$) higher WSI, PB sausage were second highest while other formulations had lower WSI.

For sensory evaluation, phosphate sausage (PB) had significantly ($p<0.05$) highest scores in sensory attributes similar to 6% pigeon pea binder (PPB3) sausage which were also most preferred.

Therefore, these findings revealed that 6% pigeon pea binder improved sausage performance with sensory profile similar to the phosphate chemical binder. It is recommended that, pigeon pea binder at 6% level can be used to replace phosphate in sausage making.

DECLARATION

I, Gomezulu, Alice David do hereby declare to neither the Senate of Sokoine University of Agriculture that this dissertation is the result of my own original work done within the registration period and has neither been submitted nor being concurrently submitted in any other institution.

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DEDICATION

This work is dedicated to my amazing parents, David Sifuel Gomezulu and Bertha Charles Msaki, who, for their constant love and faith have pushed me into becoming and discovering myself each single day.

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LIST OF ABBREVIATIONS

AOAC	Association of Official Analytical Chemists
BIB	Balanced Incomplete Block Design
CHNS/O	Carbon Hydrogen Nitrogen Sulphur and Oxygen Analyzer
CRD	Completely Randomized Design
DFTNCS	Department of Food Technology, Nutrition and Consumer Sciences
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
ISO	International Organization for Standardization
NM-AIST	Nelson Mandela African Institute of Science and Technology
PC	Principal Component
PCA	Principal Component Analysis
RCBD	Randomized Complete Block Design
SUA	Sokoine University of Agriculture
SUGECO	Sokoine University Graduate Entrepreneurship Cooperative
TP	Texture Profile
TPA	Texture Profile Analysis
UK	United Kingdom
USA	United States of America
USDA	United States Department of Agriculture
WSI	Water Solubility Index

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Legume seeds are important staple foods and are one of the richest and cheapest sources of proteins for majority of people living in developing countries (Maphosa and Jideani, 2017). The most commonly consumed are pigeon pea, kidney beans, black gram, chickpeas, green gram and lentils (Singhali *et al.,* 2014). Pigeon pea *(Cajanus cajan*), also known as dhal, red gram or tur, is an erect perennial legume shrub belonging to Family *Fabaceae* (Mathew *et al.,* 2015). It is heat-tolerant preferring hot moist conditions and is often grown as an annual crop, reaching 91 - 366 cm in height (Mathew *et al.,* 2015). It originated in the Indian subcontinent at least 3 500 years ago and is currently grown in subtropical and tropical regions of several countries (Odeny, 2017). Tanzania is the 4th world producer of pigeon pea with an annual production of 271 210 ton/year after India (4 870 000 tons), Burma (798 689 tons) and Malawi (470 630 tons) (FAOSTAT, 2017). The key production regions in Tanzania are Arusha, Dodoma, Manyara, Lindi and Mtwara (Mponda *et al*., 2014).

Pigeon pea is a good source of vitamins especially riboflavin, thiamine, choline and niacin which are water soluble (Olagunju *et al*., 2018). It is also a source of crude protein (22 - 27%), dietary fiber and antioxidants (Talari and Shakappa, 2018). These components impact human health in different ways such as regulation of blood pressure, growth and development, prevention of anemia as well as boosting the immune system (Olagunju *et al*., 2018). Bioactive compounds present in pigeon pea are important in modulating natural microbiota present in the gut hence reduce inflammation (Talari and Shakappa, 2018).

However despite its nutritional and health benefits, pigeon pea is still an underutilized crop as it is considered as poor peoples' food. Additionally, research and development have paid little attention to unlock its potential. Thus, diversification of pigeon pea utilization may be one of the strategies toward its increased consumption, marketability, farmer's income and wellbeing. Keshav (2015) elaborated that pigeon pea can be incorporated into food products like biscuits, noodles, pasta and restructured meats like sausages as a novel ingredient. This is due to its high fiber and protein content, gluten-free status, low glycemic index, antioxidant levels as well as functional properties like fat absorption and water binding capacity.

Restructured meat is meat which has been partially or completely disassembled then the meat pieces are bound together to form a cohesive mass (Bhaskar-Reddy *et al*., 2015). The cohesive mass resembles an intact muscle and sausage is an example of a restructured meat product (Bhaskar-Reddy *et al*., 2015). After slaughter, meat suffers loss of ability to hold water due to changes in the muscle as a result of loss of adenosine tri-phosphate. This condition is corrected by the addition of binders like phosphates, which improve the particle cohesion and water-binding capacity of the products (Teye and Teye, 2011). However, the use of phosphates in comminuted meat products is impeded by causing harmful residues (toxins) in the body (Teye and Teye, 2011) which may not be easily excreted due to poor lifestyle. Hence, prolonged consumption causes buildup of these toxins leading to health problems like digestive disorders, liver and kidney damage and also cancer (Inetianbor *et al*, 2015)

Addition of proteins in restructured meat facilitates water molecules to be bound by polar groups of proteins which is necessary for them to retain their spatial structure and remain intact (Pospiech and Montowska, 2011). Soy bean protein has been used as an alternative to chemical binder in processed meat products. This is for improving the water binding capacity and fat binding ability, enhancement of the emulsion stability and increasing yield (Badpa and Saghir, 2014). Despite its usage, soy is among the eight (8) most significant food allergens (Solomon *et al*., 2017). This study's focus is to investigate the protein and anti-nutritional quality of pigeon pea and its performance and sensory quality as a protein binder in beef sausage making.

1.2 Problem statement and study justification

Pigeon pea is an important crop for its nutritional value, medicinal properties and industrial application as water holding binder in meat based products (Abrams and Gerstner, 2015). It is considered as an economical source of nutrients that are crucial for human nutrition.

However, in spite of its potential, pigeon pea appears to be an underutilized legume (Adenekan *et al*., 2017) both for human consumption and industrial use. Furthermore, the situation has been worsened by recent close up of pigeon pea market in India (USDA, 2018). Consequently, its current cultivation and domestic utilization have significantly declined denying people both important nutrients and income (USDA, 2018). One of the approaches to increase pigeon pea production and utilization may be through exploitation of its protein component (Adenekan *et al*., 2017).

Currently, soybean bean protein is used as one of natural meat binder in meat industry as an alternative to chemical binders for improving water binding capacity of meat (Badpa and Saghir, 2014). This is because chemical binders pose a risk of leaving residues which can be harmful when such products are consumed over a long period of time (Teye and Teye, 2011). Unfortunately, although allergic reactions to the legume family are common

(Abrams and Gerstner, 2015), soybean protein has been listed among the 8 most significant food allergens (FAO and Taylor, 2018).

Based on those circumstances, there is a need for study to investigate an alternative protein binder in meat industry. Pigeon pea is cheaper than soy (Solomon *et al.*, 2017) and is not among the eight (8) most significant food allergens hence a probable choice for consumers. This study was therefore conducted to assess protein content of pigeon pea and its potential for developing natural binders for meat based products. Information obtained from this study will serve as basis for increased pigeon pea production, utilization and marketing in the country.

1.3 Objectives of the study

1.3.1 General objective

The general objective of the study was to determine the protein content of pigeon pea and its potential for developing binder for sausage making.

1.3.2 Specific objectives

The specific objectives were;

i. To evaluate protein content and anti-nutritional factors of two pigeon pea varieties (local and improved).
ii. To develop pigeon pea protein binder from the protein rich variety
iii. To assess performance i.e. water solubility index and texture profile of the developed pigeon pea protein binder against chemical binder in sausage.
iv. To determine the effect of the developed binder on sensory quality of sausages.

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CHAPTER TWO

MANUSCRIPT ONE

2.0 PROTEIN CONTENT, ANTI-NUTRITIONAL FACTORS OF PIGEON PEA VARIETIES AND ITS POTENTIAL FOR BINDER DEVELOPMENT IN SAUSAGE MAKING

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Abstract

The protein contents, four anti-nutritional factors of two pigeon pea varieties (local and improved) and the performance of binder in sausages in terms of water solubility index (WSI) and texture profile analysis (TPA) were investigated in this study. The binder was prepared by ultrafiltration method from protein rich variety (improved variety). Eight sausage samples were prepared at levels of 2, 4 and 6% of pigeon pea protein binder (PPB1, PPB2 and PPB3) and pigeon pea flour (PPF1, PPF2 and PPF3) respectively. Sausage without binder (CB) and phosphate chemical binder (PB) were also prepared and served as control samples. There was significant ($p<0.05$) variation in protein content between the two varieties with the improved variety having higher protein content than the local variety. There were no significant differences in anti-nutritional factors and the values obtained were lower than maximum allowed limits. Furthermore, the texture profile parameters differed significantly ($p<0.05$) between the formulations. PB formulation had the highest cohesiveness (0.54 ± 0.03 g) and adhesiveness (10.6 ± 0.57 mm) compared to other formulations. The PPB formulation had the lowest hardness with PPB3 having the lowest value of 278.0 ± 1.11 g. PPB3 also had the highest value for springiness (14.9 ± 0.10 mj). The PPF formulation had intermediate values for texture profile parameters. For water solubility index, CB and PPF formulations had the significantly ($p<0.05$) lowest WSI. PPB formulation had the highest WSI with PPB3 having the highest value (2.93 ± 0.03) compared to the rest of the formulations. PB was second highest with WSI of 2.75 ± 0.05. Therefore, these findings suggest that PPB3 can be used as an alternative replacement of the phosphate chemical binder in beef sausages. This is because it increases the WSI and TP, producing sausage products with comparable performance as that of phosphate chemical binder.

Keywords: Pigeon pea sausages; protein content; anti-nutritional factors; texture analysis; water solubility index.

2.1 Introduction

Legume seeds are important staple foods and one of the richest and cheapest sources of proteins for majority of people living in developing countries (Maphosa and Jideani, 2017). They are also an economic source of carbohydrate, minerals and B-complex vitamins (Singh *et al.*, 2018). They also contain anti-nutrients like tannin, cyanogenic glycosides, hemagglutinnin and alkaloids which inhibit the bio-availability of nutrients (Aruna and Devindra, 2016).

Pigeon pea (*Cajanus cajan*) is among the most commonly consumed legumes which belongs to the *Leguminosae* family (Talari and Shakappa, 2018). It can be incorporated into food products like biscuits, noodles, pasta and restructured meats like sausages as a novel ingredient (Keshav, 2015). This is due to its gluten free status, protein content as well as functional properties like fat absorption and water binding capacity (Keshav, 2015). Sausages, as processed and restructured meat products, are used in different and diverse cultures around the world (Hidayat *et al*., 2019). Non-meat protein ingredients are important in sausage formulation because they act as binding agents and contribute to a better final product (Muthia *et al*., 2012).

Among the commonly used sausage binder in the industry is the chemical phosphate. Phosphates are of great importance in the meat industry for giving meat products superior properties through modifying the tertiary and/or secondary structures of related proteins (Teye and Teye, 2011). Furthermore, phosphates can increase the protein solubility as well as expose hydrophobic groups (Xue *et al.*, 2016). This leads to better water holding capacity, tenderness and other favorable properties of meat products (Xue *et al.*, 2016).

However, the incorporation of phosphates in comminuted meat products is hindered by causing harmful residues (toxins) in the body (Teye and Teye, 2011) which may not be easily excreted due to poor lifestyle. Hence, prolonged consumption causes buildup of these toxins leading to health problems like digestive disorders, liver and kidney damage and also cancer (Inetianbor *et al.*, 2015). Many researches have been conducted to explore the potentiality and feasibility of using non-chemical and plant-based ingredients. This aims at promoting a healthier meat sausage product while emphasizing on the physicochemical properties relating to the addition of new ingredients (Syuhairah *et al.*, 2016).

Texture profile (TP) and water solubility index (WSI) are among the most important properties of sausages. Texture profile depends on the matrix structure formed by proteins, water and non-meat ingredients (Wang *et al.*, 2018). To improve textural properties and decrease fat content, they are used as texture-improving ingredients (TIIs) in meat products as potential fat substitutes (Wang *et al.*, 2018). Water solubility index (WSI), also known as water holding capacity (WHC), determines the loss of water during processing of a sausage product (Abdolghafour and Saghir, 2014). These mentioned properties influence the consumers' willingness to purchase the product by determining the overall acceptability (Warner, 2017). This study was carried out to determine the protein quality of pigeon pea and its performance as a protein binder in beef sausage making.

2.2 Materials and Methods

2.2.1 Study area

The study was conducted at Sokoine University of Agriculture (SUA). Sausage preparation was done at Sokoine University Graduate Entrepreneurship Cooperative (SUGECO) SUA, Morogoro. Laboratory work was conducted at the Nelson Mandela African Institute of Science and Technology (NM-AIST) and at the Department of Food Technology, Nutrition and Consumer Sciences (DFTNCS), SUA.

2.2.1.1 Materials and their sources

Two varieties of pigeon pea (improved and local variety) were purchased from farmers in Lindi region. Ultrafiltration tubes for protein extraction were purchased from Dableen General Suppliers Company - Arusha, Tanzania. Fresh meat, sausage spices, phosphate binder and sausage lamb casing were purchased from a local market and butcher in Morogoro municipal.

2.2.1.2 Chemicals and reagents

Chemicals and reagents for protein profile and anti-nutritional factors analysis were obtained from NM-AIST and SUA laboratory. These were of analytical grade (Analar) and they included hydrochloric acid (HCl), potassium iodide (KI) solution, ethanol, sodium hydroxide (NaOH), distilled water (H_2O), concentrated sulphuric acid (H_2SO_4), acetic acid, sodium carbonate (Na_2CO_3) solution, tannic acid solution, Folin-Dennis reagent and concentrated ammonium hydroxide (NH_4OH).

2.2.2 Methods

2.2.2.1 Research design

Completely randomized design (CRD) was used in this study. The principal factors were sample varieties and binder type. The analyzed parameters were protein profile of sample varieties, anti-nutritional factors: texture profile and water holding capacity of sausage based on the binder used. The effect of these factors on analyzed parameters was determined. The designed mathematical model is depicted in Equation i.

$Yij = \mu + \alpha i + \varepsilon ij$ ………………...……………………….…………………….……... (i)

Where μ is the overall (grand) mean, αi is the effect due to the ith treatment effect (variety and binder type) and εij is the error term.

2.2.2.2 Pigeon pea flour and binder preparation

a) Flour preparation

Flour preparation was done based on the method described by Adenekan *et al*. (2017) with slight modifications. Improved variety of pigeon pea (4 kg) was sorted, washed and soaked in water (1 kg pigeon pea: 3 liters of water) for 24 hours at room temperature (22°C). It was then de-hulled and oven dried at 60°C for 24 hours followed by milling (Bunn G2 Black Model 875 miller, USA) into fine powder then stored in a desiccator (Desiccator; Stainless steel, Tempered Glass Windows, Series 100, USA).

b) Binder extraction

Binder extraction was done using the method described by Kett *et al*. (2004) and Pazmiño *et al.* (2018) with slight modifications. An amount of 750 g of pigeon pea flour was sieved through 90 micrometer sieve (GKL-Model KTL, Germany) then mixed with water at a ratio of 1:10 (flour: water, w/v). Its pH was adjusted to 8.5 with 1N NaOH. The mixture was left to stand for 30 minutes then agitated for 5 minutes until no foaming was observed in the mixture. It was then centrifuged (in an 800-1 Centrifuge, China) twice for 20 minutes at 4000 rotations per minute (rpm) then filtered through 0.45 micrometer ultrafiltration tubes (Merck Millipore Amicon™ - UK), freeze dried (BK-FD10S, China) for 48 hours at -44°C and 0.08106 bars to obtain pigeon pea protein binder. The binder was stored in a desiccator (Desiccator; Stainless steel, Tempered Glass Windows, Series 100, USA) prior to sausage preparation.

2.2.2.3 Beef sausage formulation

Sausage samples were formulated using methods described by Dzudie *et al*. (2002) and Teye and Teye, (2011) with slight modifications as shown in Table 2.1. The first two formulations served as control samples which consisted of basic ingredients only with no binder (CB) and the other one consisted of 0.5% per kg of meat of chemical phosphate binder. Another three formulations consisted of pigeon pea binder (PPB) at 2, 4 and 6% respectively. The other three formulations consisted of pigeon pea flours (PPF) at 2, 4 and 6% respectively (Dzudie *et al*., 2002; Teye and Teye, 2011) as depicted in Table 2.1.

Table 2. 1: Beef sausage formulations with different proportions of binders

	Proportions (%)/ kg of meat		
Sample	**Phosphate**	**Pigeon pea binder**	**Pigeon pea flour**
(Control, CB)	0	0	0
Phosphate binder (PB)	0.5	0	0
PPB1	0	2	0
PPB2	0	4	0
PPB3	0	6	0
PPF1	0	0	2
PPF2	0	0	4
PPF3	0	0	6

2.2.2.4 Sausage preparation

Sausage samples were prepared using methods as described by Dzudie *et al*. (2002) and Teye and Teye, (2011). Meat muscles were removed from the meat carcass after 24 hours chilling at 4ºC, trimmed of visible fat and connective tissues, and ground through a 3 mm plate using a meat grinder. The ground meat was sealed in 8 × 12 cm polyethylene zipper bags (500 g package) and stored at -18ºC for 24 hours. Prior to processing, the stored meat was thawed at 4ºC for 16 hours. To each formulation (presented in Table 2.1), a constant amount of 20 g salt, 300 g water, 1 g ground black pepper, 1 g ground white pepper and 4 g ground coriander (basic ingredients) were added. The sausage batters were processed by replacing beef with binders at levels of 2, 4 and 6% (Dzudie *et al*., 2002; Teye and Teye, 2011) of the weight of meat. The whole mixture (batter) and 1/3 of the total water (10°C) were chopped in a Stephan UMC 5 - 12 Electronic cutter (Marne-la-Vallee, UK) for 3 minutes. Binders and the remaining water (2/3) were added and the mix was chopped for 10 minutes and the final chopping temperature did not exceed 15°C. The sausage batters were stuffed into 22 mm lamb casings using a hand operated stuffer (VLA 13 - France) and formed into links of 15 cm in length.

The sausages were cooked at 85 - 90ºC in a water bath (PURA™ Series 30, UK) for about 45 minutes to an internal temperature of 72ºC. They were then rapidly chilled to 15 - 20ºC with cold water for 10 minutes and stored in polyethylene bags in a refrigerator at 4ºC for 48 hours before sensory analysis.

2.2.2.5 Determination of protein content

Protein content of the samples was determined by CHNS/O analyzer method as described in method 44.4.04 by AOAC (2005). The samples were combusted and the produced gases were carried by Helium flow to a second reactor filled with Copper. The gases were then swept through CO_2 and H_2O traps through a gas chromatography (GC) column (Series 4060, UK) and finally detected by a thermal conductivity detector (TCD Detector, Teledyne Series 100, Model 2020, USA). A complete report was automatically generated by software which automatically converts the nitrogen content into protein content. For this case, a specific protein factor of 6.25 was used.

2.2.2.6 Determination of anti-nutritional factors

a) Cyanogenic glycoside determination

Cyanogenic glycoside was determined by the alkaline titration method as described by Onwuka (2006). In this method, 20.0 g of each sample variety was placed in a quick fit flask. Distilled water (200 ml) was added and allowed to stand for 4 hours. It was then connected to a steam distiller and 150 ml distillate was collected in 20 ml of 25% NaOH solution. The distillate was diluted to 250 ml in a volumetric flask and 100 ml of it was measured out in a conical flask. To the 100 ml aliquot in the conical flask, 8.0 ml of 6 N NH_4OH solution was added, followed by 3.0 ml of 5.0% KI solution. The mixture was then titrated against 0.02 N $AgNO_3$ solution. The self-indicating titration had an end point marked by a faint but persistent turbidity which was easily visible against a black background provided by the use of carbon paper. The HCN content of the sample (mg/ kg or %) was calculated, thus:

$$1.0 \text{ ml of } 0.02 \text{ N } AgNO_3 = 1.08 \text{ mg HCN}$$

$$\text{HCN (mg/kg)} = (1.08 \times Vf/Va \times 1000/w)$$

Where; Vf = total distillate (volume = 250 ml),

Va = aliquot distillate (volume used = 100 ml),

W = weight of the sample analyzed and

λ = titer value.

$$HCN\ (mg/kg) = (1.08 \times 250/100 \times 1000/20)\ \lambda = 135\ \lambda$$

b) Tannin determination

The Folin–Denis spectrophotometric method was used to determine tannins in pigeon pea varieties as described by Onwuka (2006). A measured weight of each sample variety (1.0 g) was dispersed in 10 ml distilled water and agitated. This was left to stand for 30 minutes at room temperature, shaking after every 5 minutes. At the end of the 30 minutes, it was centrifuged (in an 800-1 Centrifuge, China) and the extract was recovered. The supernatant (extract) (2.5 ml) was dispersed into a 50 ml volumetric flask. Similarly, 2.5 ml of standard tannic acid solution was dispersed into a separate 50 ml flask. Folin–Denis reagent (1.0 ml) was measured into each flask, followed by 2.5 ml of saturated Na_2CO_3 solution. The mixture was diluted to 50 ml and incubated for 90 minutes at room temperature. The absorbance was measured at 250 nm in a 6000 electronic spectrophotometer (Genway model, Japan). Readings were taken with the reagent blank at zero. The tannin content was given as follows:

$$\%\ Tannin = An/As \times C \times 100/w \times Vf/Va$$

Where; An = absorbance of the test sample,

As = absorbance of the standard solution,

C = concentration of the standard solution,

w = weight of the sample used,

Vf = total volume of the extract and

Va = volume of the extract analyzed.

c) Alkaloid determination

The gravimetric method as described by Onwuka (2006) was used in Alkaloid determination. A measured weight (5 g) of each sample variety was dispersed in 50 ml of 10% acetic acid solution in ethanol. The mixture was shaken and allowed to stand for 4 hours before it was filtered. The filtrate was evaporated to one quarter (1/4) of its original volume. Concentrated NH_4OH was added drop wise to precipitate the alkaloids. The precipitate was filtered off and washed with 1% NH_4OH solution. The filtering was done with a weighed filter paper. The precipitate in filter paper was dried in the oven at 60°C for 30 minutes and reweighed. By weight difference, the weight of alkaloid was determined and expressed as a percentage of the sample weight analyzed as described below;

$$\% \text{ Alkaloids} = (w2 - w1)/ w1 \times 100/1$$

Where; w = weight of the sample

w1 = weight of the empty filter paper

w2 = weight of the paper plus precipitate.

d) Hemagglutinin determination

The method of hemagglutinin determination as described by Onwuka (2006) was used. A measured weight of each processed sample (0.5 g) was dispersed in 10 ml of normal saline solution buffered at pH 6.4 with a 0.01 M phosphate buffer solution and allowed to stand at room temperature for 30 minutes. This was centrifuged (in an 800-1 Centrifuge, China) and the extract was obtained. One milliliter of the extract was used for the test. The diluent (0.1ml) was added to the test tube with 1 ml of trypsinated rabbit blood added to it while the other tube contained only the blood cells. Both tubes were allowed to stand for 4 hours at room temperature. One milliliter of normal saline was added to all the test tubes and allowed to stand for 10 minutes after which the absorbance was read at 620 nm in electronic spectrophotometer (Genway model, Japan). The test tube that contained only the blood cells and normal saline served as the blank. The result was expressed as hemagglutinin units per milligram of the sample.

Therefore;

$$\text{Hemagglutinin unit/ mg} = (b - a) \times F$$

$$F = (1/w \times Vf/Va)\, D$$

Where; b = absorbance of the test sample solution

a = is the absorbance of the blank control

F = experimental factor

w = weight of the sample

Vf = total volume of the extract,

Va = volume of the extract used in the assay

D = dilution factor.

2.2.2.7 Texture profile measurements

The textural properties (hardness, cohesiveness, adhesiveness and springiness) were determined using a texture analyzer (Genway Universal Testing Machine, Japan). After peeling off the casing, texture profile was performed using the central cores from three slices of each cooked sausages (Jung *et al*., 2012). All measurements were performed in triplicate.

2.2.2.8 Water solubility index (WSI) measurements

The WSI of sausages was measured as expressible moisture (EM %) by centrifugation, according to the modified method of Menegassi *et al.* (2011). Approximately 1.5 g of each cooked sausage was wrapped with dried filter paper (Whatman no.3) and weighed. After centrifugation (in an 800-1 Centrifuge, China) at 3000 rotations per minute (rpm) for 15 minutes, the expressible moisture (EM %) was calculated as weight difference between the sample weight before centrifugation and sample weight after centrifugation.

2.2.2.9 Statistical Data Analysis

Data was analyzed by using R statistical package (R development Core Team, Version 3.0.0 Vienna, Austria) for analysis of variance (ANOVA). Mean were separated using Tukey's honest significant difference test (HSD) at $p<0.05$. Also, principal component analysis (PCA) was used to determine the systematic variations between texture characteristics and sausage formulations. Results were presented as arithmetic mean and standard deviation in tables and PCA bi plot.

2.3 Results

2.3.1 Protein composition of pigeon pea flour and binder

The protein composition of the two pigeon pea varieties (local and improved) and protein binder of improved variety are shown in Table 2.2. There was a significant ($p<0.05$) difference ($p<0.05$) in protein content for local and improved varieties. Furthermore, the binder contained a higher protein content compared to both varieties as depicted in Table 2.2.

Table 2. 2: Protein contents (g/100 g dm) of pigeon pea varieties and binder

	Protein content (g/100 g dm)	
Variety	**Flour**	**Binder**
Local	22.10 ± 0.05^a	-
Improved	25.0 ± 0.03^b	32.0 ± 0.01

Mean values are expressed as mean ± s.d. (n = 3). Mean values with different superscript letters are significantly different at p<0.05

2.3.2 Anti-nutritional factors in pigeon pea

The results of the anti-nutritional properties of the local and improved variety of pigeon pea samples are shown in Table 2.3. There were no significant ($p>0.05$) differences in the anti-nutritional factors between the local and improved varieties.

Table 2. 3: Anti-nutritional factors (mg/100 g dm) of pigeon pea varieties

Anti-nutritional Factor	Variety	Concentration (mg/ 100 g)	Reference Lethal dose (mg/100 g)
Alkaloid	Local	0.41 ± 0.02^{a}	≥20 (Nimenibo-Uadia *et al.*, 2017)
	Improved	0.57 ± 0.01^{a}	
Hemagglutinin	Local	29.3 ± 0.06^{a}	≥50 (Solomon *et al.*, 2017)
	Improved	32.3 ± 0.07^{a}	
Tannin	Local	0.21 ± 0.04^{a}	≥1.05 (Balogun, 2013; Talari and Shakappa, 2018)
	Improved	0.20 ± 0.01^{a}	
Cyanonegic Glycoside	Local	9.6 ± 0.02^{a}	≥12.42 (Aja *et al.*, 2013; Talari and Shakappa, 2018)
	Improved	7.5 ± 0.06^{a}	

Mean values are expressed as mean ± s.d. (n = 2). Mean values with different superscript letters are significantly different at p<0.05

2.3.3 Texture profile of the sausage samples

Table 2.4 shows the results of texture profile parameters of sausage formulations which where hardness, cohesiveness, adhesiveness and springiness. The CB formulation had the highest value of hardness and lowest value of cohesiveness, adhesiveness and springiness.

For PB, high cohesiveness and adhesiveness was observed compared to other formulations. The PPB formulation had the lowest value for hardness with PPB3 being the lowest and had the highest value for springiness. PPB3 was second highest in cohesiveness and adhesiveness parameters. The PPF formulation had intermediate texture values (Table 2.4).

Table 2. 4: Texture profile parameters of sausage samples

Formulation	Hardness (g)	Cohesiveness (g)	Adhesiveness (mm)	Springiness (mj)
CB	424.0 ± 1.53^{a}	0.29 ± 0.04^{d}	2.5 ± 0.10^{e}	14.0 ± 0.35^{b}
PB	361.3 ± 4.35^{ab}	0.54 ± 0.03^{a}	10.6 ± 0.57^{a}	14.3 ± 0.11^{ab}
PPF1	359.0 ± 8.19^{ab}	0.33 ± 0.01^{cd}	4.1 ± 0.56^{d}	14.5 ± 0.17^{ab}
PPF2	348.0 ± 1.67^{b}	0.37 ± 0.07^{bcd}	5.9 ± 0.31^{c}	14.5 ± 0.29^{ab}
PPF3	344.7 ± 2.88^{bc}	0.42 ± 0.04^{bc}	6.0 ± 0.66^{c}	14.6 ± 0.17^{ab}
PPB1	342.7 ± 1.50^{bc}	0.43 ± 0.02^{bc}	6.3 ± 0.25^{c}	14.6 ± 0.17^{ab}
PPB2	298.0 ± 1.09^{bc}	0.44 ± 0.03^{ab}	7.0 ± 0.10^{c}	14.8 ± 0.11^{a}
PPB3	278.0 ± 1.11^{c}	0.47 ± 0.04^{ab}	9.2 ± 0.25^{b}	14.9 ± 0.10^{a}

Values are expressed as mean ± s.d. (n = 8). Mean values with different superscript letters are significantly different at $p<0.05$

Key: CB - Control sausage, PB - Phosphate binder, PPB1 - Pigeon pea binder (2%), PPB2 - Pigeon pea binder (4%), PPB3 - Pigeon pea binder (6%), PPF1 - Pigeon pea flour (2%), PPF2 - Pigeon pea flour (4%), PPF3 - Pigeon pea flour (6%).

2.3.3.1 Multivariate approach

Figure 2.1 shows the relationship between texture attributes and sausage formulations. The hardness characteristic was closely related with CB formulation and had the highest value of hardness. Cohesiveness and adhesiveness were strongly related with PB formulation; while springiness was strongly associated with PPB3 and PPB2 formulations. The formulations PPF1, PPF2, PPF3 and PPB1 were not strongly correlated with any specific texture characteristic.

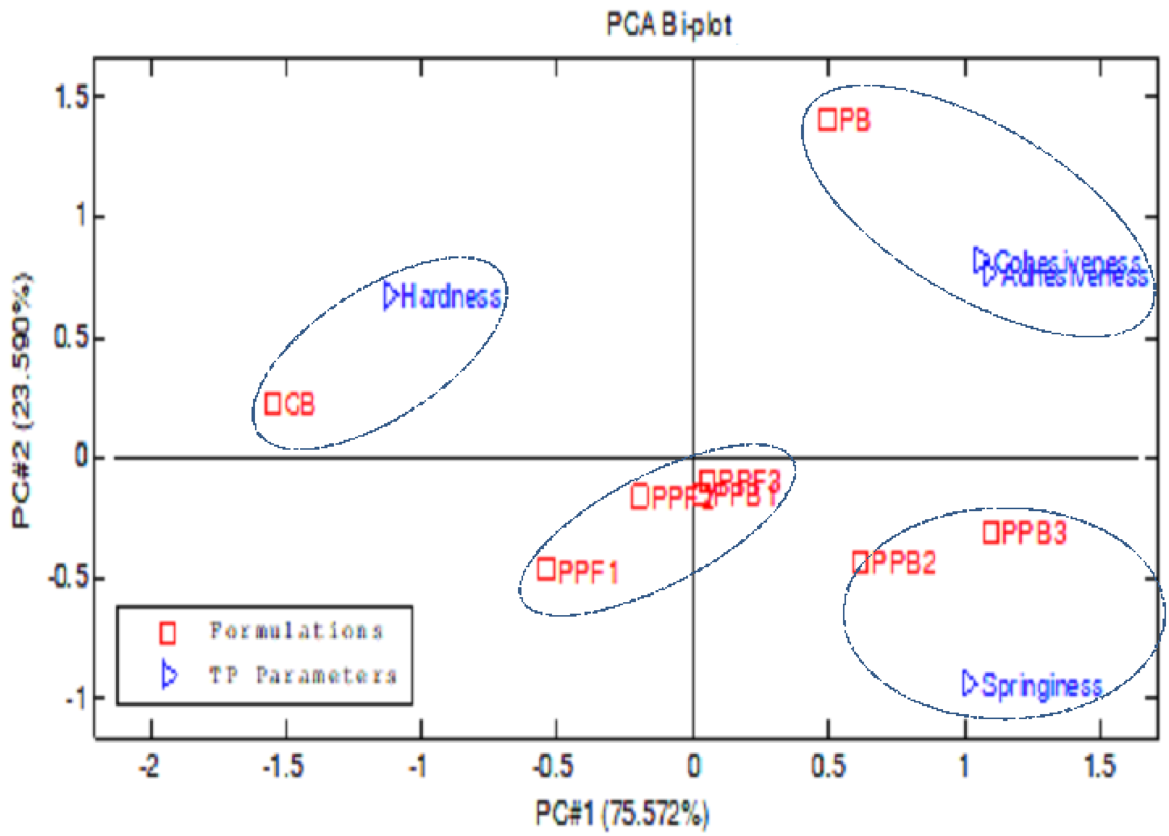

Figure 2. 1: PCA bi-plot showing relationship between texture characteristics and sausage formulations

2.3.4 Water solubility index (WSI)

Table 2.5 shows the value of WSI of sausages with PB, PPB, PPF and with no binder (CB) with means and standard deviation. The CB and PPF3 formulations had the significantly lowest WSI. PPB formulation had the highest WSI with PPB3 having the highest value compared to the rest of the formulations. PB was second highest in WSI and the PPF formulations were second lowest.

Table 2. 5: Water solubility index of sausages developed by pigeon pea flour and binder

Sample	Water solubility index (WSI)
CB	1.04 ± 0.21^{c}
PB	2.75 ± 0.05^{ab}
PPB1	2.69 ± 0.05^{ab}
PPB2	2.92 ± 0.11^{a}
PPB3	2.93 ± 0.03^{a}
PPF1	2.46 ± 0.11^{ab}
PPF2	2.36 ± 0.21^{b}
PPF3	1.04 ± 0.21^{c}

Values are expressed as mean ± s.d. (n = 8). Bars with mean values bearing different superscript letters are significantly different at p<0.05
Key: CB - Control sausage, PB - Phosphate binder, PPB1 - Pigeon pea binder (2%), PPB2 - Pigeon pea binder (4%), PPB3 - Pigeon pea binder (6%), PPF1 - Pigeon pea flour (2%), PPF2 - Pigeon pea flour (4%), PPF3 - Pigeon pea flour (6%).

2.4 Discussion

2.4.1 Protein content

The observed significant difference in protein content (Table 2.2) between the two varieties of pigeon pea may be due to the cultivar type where the improved variety may be of superior hybrid than the local variety (Cheboi *et al*., 2019; Aruna and Devindra, 2016). Also seasonal variations may have affected the protein content. Aruna and Devindra (2016) also documented that the protein content of commonly cultivated varieties (local and improved) of pigeon pea range between 21.1% and 28.1%, thus complying with the observed results. The relatively high protein content of the improved pigeon pea binder (Pazmiño *et al*., 2018) showed that it could be incorporated into sausages to improve water solubility index (WSI) and the texture of the sausage.

2.4.2 Anti-nutritional factors

The lethal dose for alkaloid is ≥20 mg/100 g (Nimenibo-Uadia *et al.*, 2017), for hemagglutinin is ≥50 mg/100 g (Solomon *et al.*, 2017), for tannin is ≥1.05 mg/100 g (Balogun, 2013) and for cyanide is ≥12.42 mg/100 g (Aja *et al.*, 2013). These limits were much higher than the results from this study (Table 2.3). Hence, the sample varieties were within a safe range. This may be associated with cultivar type of the samples and seasonal variations (rainfall and temperature) that tend to affect the presence of anti-nutrients (Aruna and Devindra, 2016).

2.4.3 Texture profile (TP)

Hardness is a maximum force required to compress a sample. The result of hardness presented in Table 2.4 shows a decrease in sausage hardness with increasing levels of PPB and PPF. Sausages developed with PPB especially PPB3, had the lowest hardness values than PPF. This may be due to an increase in protein which tends to decrease hardness in sausage (Abdolghafour and Saghir, 2014). The CB sample was strongly associated with hardness compared to other sausage samples probably due to higher meat concentration and the absence of a binding agent. This results to water separation from the protein matrix caused by destabilization of meat structure (Hidayat *et al.*, 2018). A Similar result of high hardness value in control sample was also observed by Syuhairah *et al*. (2016). PB formulation was second highest in hardness, this may be associated with the fact that phosphate tends to decrease hardness in meat (Wang *et al.*, 2009). That is why CB sausage were observed to be harder than PB sausages. Also, the effect of PB and PPB on sausage hardness was antagonistic (Table 2.4). PB sausage were harder while PPB3 decreased sausage hardness. This observation may be associated with the difference in interaction between phosphate (PB) and protein (PPB) with meat. The presence of phosphate ions in phosphate binder tend to increase sausage hardness in non-fat meat (Hemung and Chin, 2015). On the other hand, protein tends to decrease sausage hardness (Abdolghafour and Saghir, 2014).

Cohesiveness is the degree of difficulty in breaking down the internal structure of the sausage. There was an observed directly relationship between increase in levels of PPB in formulations with cohesiveness characteristic. Increasing levels of concentration resulted to an increase in cohesiveness for PPB. This may be associated with the use of protein in sausage which tends to increase cohesiveness (Abdolghafour and Saghir, 2014) due to increase in protein interactions in meat. A similar observation was reported by Syuhairah *et al.* (2016) where an increase in non-meat ingredient resulted to a slightly higher degree of cohesiveness. CB formulation had the lowest cohesiveness probably due to the absence of binder. A similar observation was reported by Shand (2000) where potato starch, waxy barley and wheat flour meal treatments had more cohesiveness than the control. PB had the highest cohesiveness value but was not significantly different from that of PPB3 and PPB2 samples. This implies that the effect of PBB3 and PPB2 is similar to that of PB on sausage cohesiveness.

Adhesiveness is the necessary work required to overcome the forces of attraction between the food surface and the surface of other materials in contact with the food (Wambui *et al.*, 2017). There was also an observed directly proportional relationship between increase in levels of PPB and PPF formulations with adhesiveness characteristic. Increasing levels of formulation resulted to an increase in adhesiveness with PPB values being higher than PPF. CB formulation had the lowest, significantly different adhesiveness probably due to the absence of binder (Syuhairah *et al.*, 2016). PB had the highest significantly different adhesiveness value of all formulations.

Springiness is the sample ability to recover its original form after the force of deformation is removed (Wambui *et al.*, 2017). As shown in Table 2.4, there was a relationship between increase in levels of PPB and PPF concentration with springiness characteristic. Increasing levels of their concentration resulted to an increase in springiness with PPB values being higher than PPF values. Thus, the addition of protein (pigeon pea protein binder) was probably associated with an increase in springiness of beef sausage. The effect was noted to be significant when 6% of the protein binder (PPB3) was used compared to the complete absence of binder in sausage (CB). Similar results of increase in springiness with increasing protein concentration were reported by Wambui *et al*. (2017). CB formulation had the lowest springiness which was significantly different from PPB3 formulation. These results contradicts with those of Syuhairah *et al.* (2016) who reported that the control sample had the highest score in springiness compared to formulations with binders. The observed difference is probably due to use of beef instead of chicken meat in the sausage preparation (Syuhairah *et al.*, 2016). PB was the second lowest with a springiness value of 14.3 but was not significantly different compared to PPB3 formulation.

Principal component analysis bi-plot (Figure 2.1) also supports the above results. Cohesiveness and adhesiveness were strongly related with PB formulation. This may be due to the fact that phosphate tends to increase cohesiveness and adhesiveness because it improves binding ability of meat proteins (Xue *et al.*, 2016).

2.4.4 Water solubility index (WSI)

Water solubility index (WSI) is the ability of meat to retain and hold moisture including any fluids added during processing of the meat and moisture initially present in the meat muscle (Abdolghafour and Saghir, 2014). It indicates the ability of the sample (sausage) to bind and hold water (Hidayat *et al.*, 2018). From Table 2.5, there was an increase in water holding capacity of sausages extended with pigeon pea binder (PPB) as the amount of binder increased from 2% (PPB1) to 6% (PPB3). PPB3 and PPB2 had the highest water holding capacity compared to all other treatments including the industrial phosphate binder (PB). This may be due to the presence of salt soluble protein present in plant-based protein (PPB) which influences water holding capacity (Reddy *et al.*, 2015). Thus, the water is both entrapped in the open myofibrillar structure of meat as well as bound to the negative charges of the protein.

Also, an increase in the concentration of pigeon pea protein binder at 4% to 6% was observed to increase WSI compared to the rest of the treatments. The observed results in this research may be similar to several types of research done where the addition of other substitution material rich in protein like bean flour and gelatin increased WSI in sausages (Lee and Chin, 2016; Souissi *et al*., 2016; Dzudie *et al.*, 2002). Also sausages extended with PPF1 and PPF2 were observed to be significantly different in WSI compared to the control sausage (CB). But PPF3 had the same WSI like the control sample (CB) and there was no significant difference between them. This indicated that the increase in levels of PPF in sausage resulted to a decreased WSI. This observed result agrees with Kenawi *et al*. (2009). They reported a similar observation where mung bean powder was used as a meat binder and extender but lead to the reduction of sausage moisture content. Hence, the use of pigeon pea flour (PPF) in binding water for sausage may not be efficient compared to its protein binder (PPB).

2.5 Conclusion

In a view of the results, the improved variety of pigeon pea had a higher protein content compared to the local variety. Also, the level of anti-nutrients in both varieties were not above maximum allowable limits. Therefore, the improved variety can be used to develop pigeon pea protein binder in sausage making with safe levels of anti-nutrients. Also, the six percent level (6%) of PPB formulation (PPB3 sausage) increases the ability of sausage to hold moisture (water solubility index). It also increases the texture (cohesiveness, adhesiveness and springiness; and lowered hardness of sausages) compared to the rest of the formulations. This performance is similar to that of PB formulation and therefore PPB3 can be recommended to be used in sausage making as a replacement of phosphate binder.

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CHAPTER THREE

MANUSCRIPT TWO

3.0 SENSORY PROFILE, CONSUMER ACCEPTABILITY AND PREFERENCE MAPPING OF BEEF SAUSAGES PREPARED BY USING PIGEON PEA FLOUR AND BINDER

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Abstract

The quantitative descriptive profile, consume acceptability as well as the relationship between descriptive data and acceptability data to ascertain the drivers for consumer liking of sausage samples prepared from pigeon pea protein binder were investigated in this study. Eight sausage samples were prepared from 2, 4 and 6% of pigeon pea protein binder (PPB) and pigeon pea flour (PPF). Sausage without binder (CB) and commercial chemical phosphate binder (PB) served as control samples. The quantitative descriptive analysis (QDA) was performed by a trained panel of 9 assessors. Consumer tests; hedonic and preference ranking were performed by 59 consumers using a 9- point hedonic and 5-point ranking scales respectively employing balance incomplete block (BIB) design. Preference mapping by partial least squares regression analysis was used to identify the drivers of acceptance of the sausage samples by consumers. The results showed that phosphate sausage binder (PB) had the highest significant ($p<0.05$) saltiness (5.8 ± 1.56), mouth feel (6.9 ± 1.20), moistness (7.6 ± 1.23) and compactness (6.4 ± 1.81) mean intensity scores followed by PPB3 which was second highest in the same attributes. The lowest liking was observed for PPF1 and CB for these attributes. Furthermore, sample PB had the highest hedonic scores in appearance (6.7 ± 2.0), taste (6.6 ± 1.8), softness (6.3 ± 2.1) and moistness (6.4 ± 1.8) followed by PPB3 which was second highest in the same attributes. PPF1 had the lowest liking in these attributes. PPB3 and PPB2 were the most preferred samples with rank sum of 159. Moistness, mouth feel, saltiness and compactness of sausage were major drivers for consumer liking of samples by consumers. The findings suggest that PPB3 can be used as an alternative replacement of phosphate binder in meat industry to produce sausages with comparable sensory profile and consumer acceptability to phosphate chemical binder.

Keywords: Quantitative descriptive analysis; consumer test; sausages; pigeon pea binder; pigeon pea four.

3.1 Introduction

Pigeon pea (*Cajanus cajan*) is among the most commonly consumed legume in the semi-arid tropics (Maphosa and Jideani, 2017). It is a source of crude protein (22 - 27%) and water soluble vitamins like thiamine, riboflavin and niacin (Olagunju *et al.*, 2018). These impact human health by regulating blood pressure, growth and development, prevent anemia as well as boosting the immune system (Talari and Shakappa, 2018).

Despite of its potential, pigeon pea is an underutilized legume crop (Adenekan *et al.*, 2017) compared to other legumes like common beans and cowpeas. Diversification of pigeon pea utilization may be one of the strategies toward its increased consumption. Pigeon pea can be incorporated into restructured meats like sausages, as a novel ingredient due to its functional properties like water binding and fat absorption (Keshav, 2015). According to Omojola *et al.* (2013), the inclusion of non-meat ingredients in the meat processing industry is an important strategy in maintaining sensory and nutritional qualities of products.

After slaughtering of an animal, changes in the meat muscle occur as a result of loss of energy (adenosine tri-phosphate, ATP) which leads to inability of meat to hold water. This condition is corrected by addition of chemical binders like phosphate binder that improves the water-binding capacity and particle cohesion of the products (Teye and Teye, 2011). However, the use of phosphates in comminuted meat products is impeded by causing harmful residues (toxins) in the body (Teye and Teye, 2011) which may not be easily excreted due to poor lifestyle. Prolonged consumption causes buildup of these toxins leading to health problems like liver and kidney damage and also cancer (Inetianbor *et al.*, 2015). This suggest for development and application of non-harmful natural foods like pigeon pea while maintaining nutritional and sensory qualities acceptable to consumers.

Sensory quality analysis is the evaluation of perceptible attributes by the five sense organs such as odor, color, touch, texture and taste (Lawless and Heyman, 2010). It determines the quality of food by using basic techniques such as consumer test, preference mapping and descriptive sensory analysis (Mongi *et al*., 2013). Descriptive sensory analysis is based on perceptions of a qualified group of assessors who provide quantitative descriptions of all the sensory attributes of food products. On the other hand, consumer test assesses whether the consumers like, accept or prefer the product over another product (Lawless and Heyman, 2010). Preference mapping describes which attributes contributed to consumer liking by using a perceptual map that shows the relationship between descriptive sensory data and consumers' hedonic judgments (Tenenhaus *et al*., 2005).

Currently, there is a challenge in the food industry to formulate healthy sausage that can help to lower health risks due to chemical binders (Hidayat *et al*., 2017). Research attention has thus been directed toward increasing utilization of plant protein sources for food use which includes the use of pigeon pea (Omojola *et al.*, 2013). Despite the efforts, information on the sensory profile, consumer acceptability and preference and drivers of consumer liking of sausage samples prepared by using pigeon pea binder is inadequate. Therefore, this study aimed at evaluating the effects of pigeon pea binder developed as an alternative replacement of chemical phosphates binder on sensory profile, consumer acceptability and preference mapping of sausage samples.

3.2 Materials and methods

3.2.1 Study area

The study was conducted at Sokoine University of Agriculture (SUA), Morogoro and Nelson Mandela African Institute of Science and Technology (NM-AIST), Arusha in

Tanzania. Sausage preparation and sensory evaluation were respectively conducted at Sokoine University Graduate Entrepreneurship Cooperative (SUGECO) and the Department of Food Technology, Nutrition and Consumer Sciences laboratory (DFTNCS), SUA. Binder development was carried out at NM-AIST laboratory.

3.2.1.1 Materials and their sources

Two varieties of pigeon pea (improved and local variety) were purchased from farmers in Lindi region. Ultrafiltration tubes (Merck Millipore Amicon™ - UK) for protein extraction were purchased from Dableen General Suppliers Company - Arusha, Tanzania. Fresh meat, sausage spices, phosphate binder and lamb casing were purchased from a local market and butcher in Morogoro municipal. Materials for sensory evaluation were obtained from the market and supermarkets in Morogoro region.

3.2.1.2 Chemicals and reagents

Chemicals and reagents for pigeon pea binder preparation were obtained from NM-AIST laboratory. These were of analytical grade (Analar) and they included hydrochloric acid (HCl), sodium hydroxide (NaOH) and distilled water (H_2O).

3.2.2 Methods

3.2.2.1 Research designs

Balanced incomplete block design (BIB) was used in this study. The BIB design (ISO 29842, 2011) is applied to sensory tests in which the total number of samples is greater than the number that can be evaluated, before sensory and psychological fatigue set in. In BIB designs, each assessor evaluates only a subset of the total number of samples in a single session randomly. The principal factors were assessors and sausage samples prepared from different binders. The effects of these factors on sausages sensory attributes and consumer acceptability and preferences were determined and compared. The mathematical expression is depicted in Equation i.

$Yij = \mu + \tau i + \beta j + \varepsilon ij$ ….……….……………………………………..…………….. (i)

Where μ is the overall mean, τi is the i^{th} treatment effect (binder type), βj is the j^{th} block effect (assessors) and εij is the random effect.

3.2.2.2 Pigeon pea binder preparation

a) Flour preparation

Flour preparation was done based on the method described by Adenekan *et al*. (2017) with slight modifications. Improved variety of pigeon pea (4 kg) was sorted, washed and soaked in water (1 kg pigeon pea: 3 liters of water) for 24 hours at room temperature (22ºC). It was then de-hulled and oven dried at 60ºC for 24 hours followed by milling (Bunn G2 Black Model 875 miller, USA) into fine powder then stored in a desiccator (Desiccator; Stainless steel, Tempered Glass Windows, Series 100, USA).

b) Binder extraction

Binder extraction was done using the method described by Kett *et al*. (2004) and Pazmiño *et al.* (2018) with slight modifications. About 750 g of pigeon pea flour was sieved through 90 micrometer sieve (GKL-Model KTL, Germany) then mixed with water at a ratio of 1:10 (flour: water, w/v). Its pH was adjusted to 8.5 with 1N NaOH. The mixture was left to stand for 30 minutes then agitated for 5 minutes until no foaming was observed. It was then centrifuged (in an 800-1 Centrifuge, China) twice for 20 minutes at 4000 rotations per minute (rpm) then filtered through 0.45 micrometer ultrafiltration tubes (Merck Millipore Amicon™ - UK), freeze dried (BK-FD10S, China) for 48 hours at -44ºC and 0.08106 bars to obtain pigeon pea protein binder. The binder was stored in a desiccator (Desiccator; Stainless steel, Tempered Glass Windows, Series 100, USA) prior to sausage preparation.

3.2.2.3 Beef sausage formulation

Sausage samples were formulated using methods described by Dzudie *et al*. (2002) and Teye and Teye (2011) with slight modifications as shown in Table 3.1. The first two formulations served as control samples which consisted of basic ingredients only with no binder (CB) and the other ones consisted of 0.5% per kg of meat of chemical phosphate binder. Another three formulations consisted of pigeon pea binder (PPB) at 2, 4 and 6% respectively. The other three formulations consisted of pigeon pea flours (PPF) at 2, 4 and 6% respectively (Dzudie *et al*., 2002; Teye and Teye, 2011) as depicted in Table 3.1.

Table 3. 1: Beef sausage formulations with different proportions of binders

	Proportions (%)/ kg of meat		
Sample	**Phosphate**	**Pigeon pea binder**	**Pigeon pea flour**
(Control, CB)	0	0	0
Phosphate binder (PB)	0.5	0	0
PPB1	0	2	0
PPB2	0	4	0
PPB3	0	6	0
PPF1	0	0	2
PPF2	0	0	4
PPF3	0	0	6

3.2.2.4 Sausage preparation

Sausage samples were prepared using methods as described by Dzudie *et al*. (2002) and Teye and Teye (2011). Meat muscles were removed from the meat carcass after 24 hours chilling at 4°C, trimmed of visible fat and connective tissues, and ground through a 3 mm plate using a meat grinder. The ground meat was sealed in 8 × 12 cm polyethylene zipper bags (500 g package) and stored at -18°C. Prior to processing, the stored meat was thawed at 4°C for 16 hours. To each formulation (presented in Table 3.1), a constant amount of 20 g salt, 300 g water, 1 g ground black pepper, 1 g ground white pepper and 4 g ground coriander (basic ingredients) were added. The sausage batters were processed by replacing beef with binders at levels of 2, 4 and 6% (Dzudie *et al.*, 2002; Teye and Teye, 2011) of the weight of meat. The whole mixture (batter) and 1/3 of the total water (10°C) were chopped in a Stephan UMC 5 - 12 Electronic cutter (Marne-la-Vallee, UK) for 3 minutes. Binders and the remaining water (2/3) were added and the mix was chopped for 10 minutes and the final chopping temperature did not exceed 15°C. The sausage batters were stuffed into 22 mm lamb casings using a hand operated stuffer (VLA 13 - France) and formed into links of 15 cm in length.

The sausages were cooked at 85 - 90ºC in a water bath (PURA™ Series 30, UK) for about 45 minutes to an internal temperature of 72ºC. They were then rapidly chilled to 15 - 20°C with cold water for 10 minutes and stored in polyethylene bags in a refrigerator at 4°C for 48 hours before sensory analysis.

3.2.2.5 Sensory evaluation

i) Quantitative descriptive analysis (QDA)

Quantitative descriptive analysis test was conducted at the Department of Food Technology, Nutrition and Consumer (DFTNCS) laboratory at SUA involving a trained panel of 9 assessors comprising of 7 male and 2 females with age ranging from 22 to 28 years according to the method described by Lawless and Heyman (2010). The assessors were selected and trained for three (3) days according to ISO 8586 (2012). During training panelists developed descriptors describing differences between samples and they agreed on the following attributes; color, saltiness, mouth feel, moistness, compactness and hardness (Table 3.2). They also developed and agreed an unstructured 9- line scale for rating the intensity of an attribute. The left side of the scale corresponded to the lowest intensity of each attribute (value 1) and the right side corresponded to the highest intensity (value 9). The samples were coded with 3- digit random numbers and were served to each panelist in a randomized order using BIB design. The obtained average responses were used in the univariate and multivariate analyses. Both pre-trial test and panel performance assessment were done to ascertain agreement of panelist in discriminating samples and their reproducibility.

Table 3. 2: Definitions of sensory attributes used in descriptive sensory analyses

Attribute	Description	Reference	Scale ranges(1-9)
Color	Characteristic of visual perception described through color categories	Himalaya	1- Pale Himalaya 9- Himalaya
Saltiness	The quality of being salty	Table salt (NaCl)	1- Less salty 2- Very salty
Mouth feel	The spread of particles while chewing	Beef Vienna Sausage	1- Loose particles 2- Dense particles
Moistness	Moisture experienced by the finger feel	Beef Vienna Sausage	1- Not moist 2- Very moist
Compactness	The denseness of meat particles in sausage as perceived by the eye	Beef Vienna Sausage	1- Not compact 2- Very compact
Hardness	Characteristic of the product as perceived for the first teeth bite	Beef Vienna Sausage	1- Not hard 2- Very hard

Source: Study QDA Panel (2020)

ii) Consumer Test

a) Hedonic test

The hedonic test was conducted at the Department of Food Technology Nutrition and Consumer Sciences (DFTNCS) by 59 untrained consumers of both sexes aged between 20 - 45 years using a 9- point hedonic scale as described by Lawless and Heyman (2010). The sausages were thawed and warmed in an oven (Turbofan 3000, Blue seal, UK), sliced into uniform sizes (about 2 cm in length) then served on white disposable plates which were randomly coded with 3- digit numbers. Then the plates were served to the panelists in a randomized order on the day of evaluation using BIB design. They were then asked to evaluate and express their degree of liking for sausage product attributes on appearance, color, aroma, taste, softness, moistness and finally expressing judgment on overall acceptability using a 9- point hedonic scale (where 1 = dislike extremely and 9 = like extremely). Good sensory practices such as blind labelling and mouth rinsing between tastes were observed.

b) Preference test

The preference test was conducted at the Department of Food Technology, Nutrition and Consumer Sciences (DFTNCS) by 59 untrained consumers of both sexes between 20 - 45 years using a 5- point ranking scale described by Lawless and Heyman (2010). The sausages were thawed and warmed in an oven (Turbofan, Blue seal, UK), sliced into uniform sizes (about 2 cm in length) then served on white disposable plates which were randomly coded with 3- digit numbers. The samples were then served to the panelists in a randomized order on the day of evaluation using a BIB design and panelists were asked to test and rank the sample according to their preference using a scale provided (where 1 = most preferred and 5 = least preferred).

.iii) Relationship between descriptive and acceptability data by PLS (Preference mapping)

Correlation between QDA and hedonic data for the overall impressions of the consumers was determined. The purpose was to allow the identification of descriptive terms that positively and negatively affected the acceptance of different sausage samples at a confidence interval of 95%. This correlation was determined using the partial least squares regression analysis (PLS) where QDA data was regressed into acceptability data (Tenenhaus *et al*., 2005).

3.2.2.6 Statistical data analysis

QDA and hedonic data were analyzed by using the R statistical package (R Development Core Team, Version 3.0.0 Vienna, Austria) for analysis of variance (ANOVA) using two factors (panelist and sample formulations). Means were separated by Tukey's honest significant difference (HSD) test. At $p<0.05$, non-parametric Friedman rank sum test was performed for preference data. Furthermore, principal component analysis (PCA) was used to determine the systematic variations in sensory data using Latentix software (Latentix Aps Team, version 2.12, Frederiksberg Denmark) (Martens and Martens, 2001). Partial least square regression (PLSR) was computed to determine relationship between QDA and hedonic data to ascertain drivers for consumer liking. Results were presented as arithmetic mean and standard deviation in tables and graphs as well as in PCA bi plots.

3.3 Results

3.3.1 Quantitative descriptive analysis of sausage

Mean intensity ratings of descriptive attributes between sausages samples are as shown in Table 3.3. There were significant differences ($p<0.05$) in mean intensity scores of attributes between different sausage samples. Increasing levels of PPB formulation resulted to an increase in color, saltiness, aroma, mouth feel, moistness and compactness attribute while decreasing intensity of hardness attribute. For PPF formulation, the increase in levels resulted to an increase in aroma, mouth feel and moistness attribute while decreasing intensity of color, saltiness and hardness attributes (Table 3.3).

Table 3. 3: Mean intensity scores of sausage samples with different binders

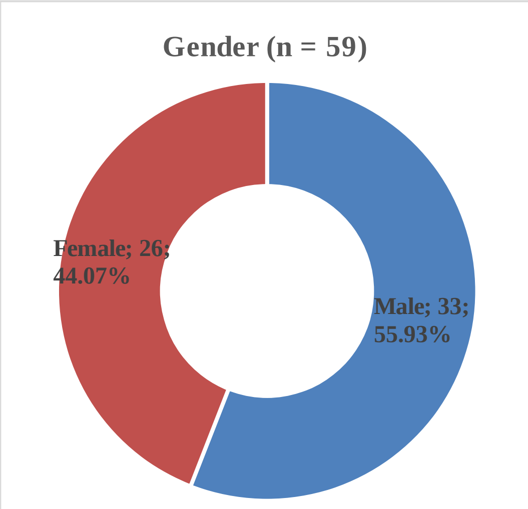
Sample	Color	Saltiness	Aroma	Mouth feel	Moistness	Compactness	Hardness
CB	8.2 ± 1.09^{a}	5.0 ± 1.87^{c}	7.7 ± 1.11^{ab}	4.7 ± 1.40^{e}	5.1 ± 1.96^{e}	4.3 ± 2.40^{c}	6.8 ± 2.71^{a}
PB	8.2 ± 1.30^{a}	5.8 ± 1.56^{a}	7.8 ± 1.09^{ab}	6.9 ± 1.20^{a}	7.6 ± 1.23^{a}	6.4 ± 1.81^{a}	6.0 ± 2.82^{c}
PPF1	7.1 ± 1.56^{d}	5.6 ± 1.67^{a}	7.4 ± 1.59^{c}	5.4 ± 1.54^{d}	6.0 ± 2.34^{d}	4.2 ± 2.11^{c}	6.4 ± 2.51^{b}
PPF2	7.1 ± 2.15^{d}	5.0 ± 1.73^{c}	7.9 ± 1.54^{a}	5.9 ± 2.14^{c}	6.3 ± 1.92^{c}	4.2 ± 2.22^{c}	6.1 ± 3.00^{c}
PPF3	7.0 ± 2.40^{d}	5.1 ± 1.83^{bc}	7.8 ± 1.30^{ab}	5.7 ± 2.22^{c}	6.9 ± 3.21^{b}	4.2 ± 1.88^{c}	5.8 ± 2.54^{c}
PPB1	7.4 ± 1.51^{c}	4.9 ± 1.62^{c}	7.6 ± 1.42^{bc}	5.9 ± 2.09^{c}	6.9 ± 1.11^{b}	4.2 ± 2.33^{c}	6.7 ± 2.06^{a}
PPB2	7.8 ± 1.79^{b}	5.1 ± 1.83^{bc}	7.7 ± 1.41^{ab}	5.9 ± 2.09^{c}	7.0 ± 2.00^{b}	4.2 ± 2.28^{c}	6.0 ± 2.83^{c}
PPB3	8.3 ± 1.00^{a}	5.3 ± 1.66^{b}	7.6 ± 1.88^{bc}	6.1 ± 1.94^{b}	7.4 ± 3.00^{a}	4.8 ± 2.22^{b}	5.8 ± 2.54^{c}

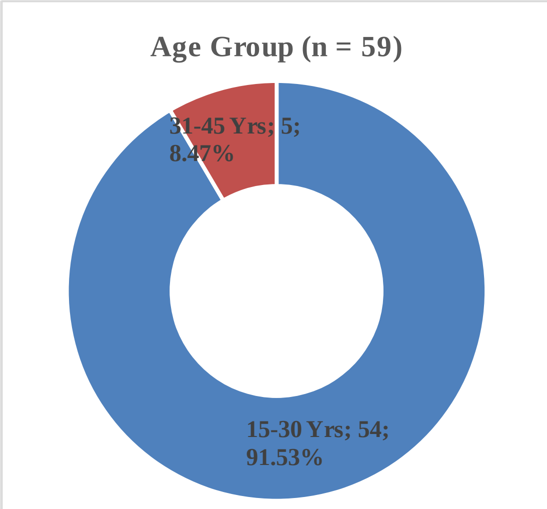
Values are expressed as mean ± s.d. (n = 8). Mean values with different superscript letters along the columns are significantly different at p<0.05.
Key: CB - Control sausage, PB - Phosphate binder, PPB1 - Pigeon pea binder (2%), PPB2 - Pigeon pea binder (4%), PPB3 - Pigeon pea binder (6%), PPF1 - Pigeon pea flour (2%), PPF2 - Pigeon pea flour (4%), PPF3 - Pigeon pea flour (6%).

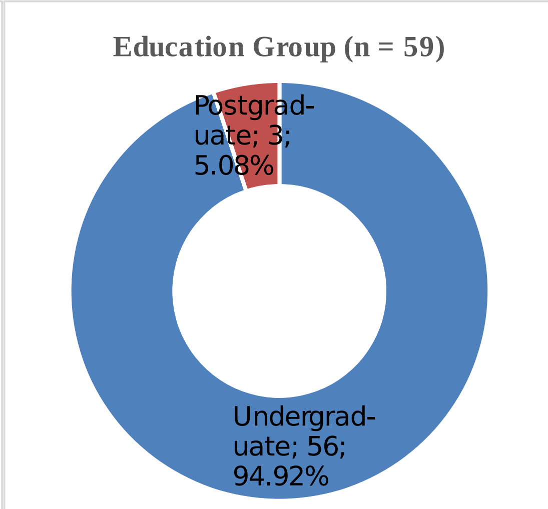
3.3.2 Consumer test

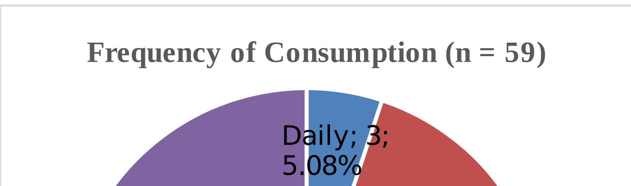
3.3.2.1 Consumer panel characteristics

The consumer panel was comprised of 59 panelists whereby 56% were male and 44% were female. Undergraduate students were 95% and postgraduate students were 5%. Also 92% of the panelists were aged between 15 - 30 years while 8% were between 31 - 45 years. Five percent (5%) of the panelists were frequent users of sausage on daily basis, 39% once a week, 29% once per month and 27% were seldom users of sausage. The finding shows that 80% of the consumer panelists were willing to purchase the sausage products once introduced in the market while 20% were not willing to purchase the products (Figure 3.1).


Gender (n = 59)
Female; 26; 44.07%
Male; 33; 55.93%


Age Group (n = 59)
31-45 Yrs; 5; 8.47%
15-30 Yrs; 54; 91.53%


Education Group (n = 59)
Postgrad-uate; 3; 5.08%
Undergrad-uate; 56; 94.92%


Frequency of Consumption (n = 59)
Daily; 3; 5.08%

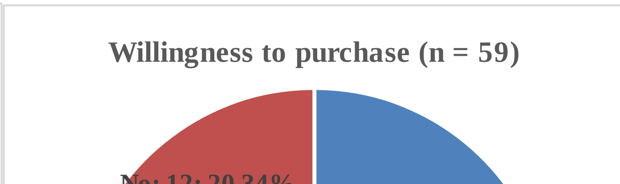

Willingness to purchase (n = 59)

Figure 3. 1: Characteristics of the consumer acceptability panel (n = 59)

3.3.2.2 Acceptability test

Acceptability of different formulated sausage samples prepared by different binders is shown in Table 3.4. There were significant ($p<0.05$) differences in consumer hedonic scores for taste, softness and moistness attributes. The increase in levels of PPB and PPF formulation resulted to an increase in liking for these respective attributes. Consequently, the overall acceptability also increased with the PPB formulation being the most acceptable after PB formulation while CB and PPF formulations were least acceptable.

Table 3. 4: Hedonic scores of sausage samples

Sample	Appearance	Color	Aroma	Taste	Softness	Moistness	Overall acceptability
CB	6.0 ± 2.1^{a}	5.9 ± 2.1^{a}	5.8 ± 2.4^{a}	5.9 ± 2.0^{b}	5.6 ± 1.9^{ab}	5.7 ± 2.0^{ab}	5.7 ± 2.12^{ef}
PB	6.7 ± 2.0^{a}	6.0 ± 2.4^{a}	5.8 ± 2.0^{a}	6.6 ± 1.8^{a}	6.3 ± 2.1^{a}	6.4 ± 1.8^{a}	7.3 ± 1.88^{a}
PPB1	6.2 ± 2.4^{a}	6.1 ± 2.0^{a}	6.2 ± 1.8^{a}	6.1 ± 2.4^{a}	6.0 ± 2.4^{a}	6.0 ± 2.4^{ab}	6.3 ± 2.11^{d}
PPB2	6.4 ± 1.9^{a}	6.2 ± 1.9^{a}	6.3 ± 1.9^{a}	6.3 ± 1.8^{a}	6.1 ± 1.9^{a}	6.0 ± 1.7^{ab}	6.7 ± 1.84^{c}
PPB3	6.3 ± 1.8^{a}	6.1 ± 1.9^{a}	6.4 ± 1.6^{a}	6.6 ± 1.6^{a}	6.3 ± 1.6^{a}	6.2 ± 1.4^{a}	7.0 ± 1.67^{b}
PPF1	6.0 ± 2.0^{a}	5.7 ± 2.0^{a}	5.6 ± 1.7^{a}	4.9 ± 2.2^{c}	5.1 ± 2.0^{b}	5.1 ± 1.9^{b}	5.1 ± 2.03^{g}
PPF2	6.0 ± 1.8^{a}	6.0 ± 1.6^{a}	5.6 ± 1.8^{a}	5.3 ± 2.0^{bc}	5.4 ± 2.1^{ab}	5.4 ± 1.9^{ab}	5.5 ± 3.11^{f}
PPF3	6.1 ± 2.0^{a}	5.8 ± 2.0^{a}	5.8 ± 1.8^{a}	5.7 ± 1.9^{b}	6.1 ± 1.9^{a}	5.9 ± 1.9^{ab}	5.9 ± 1.11^{e}

Values are expressed as mean ± s.d. (n = 8) Mean values with different superscript letters along the columns are significantly different at p<0.05.
Key: CB - Control sausage, PB - Phosphate binder, PPB1 - Pigeon pea binder (2%), PPB2 - Pigeon pea binder (4%), PPB3 - Pigeon pea binder (6%), PPF1 - Pigeon pea flour (2%), PPF2 - Pigeon pea flour (4%), PPF3 - Pigeon pea flour (6%).

3.3.2.3 Preference test

Preference results are as shown in Table 3.5. There were significant ($p<0.05$) variations in consumer preference among samples. Samples CB, PB, PPFs and PPB1 had higher rank sums and medians while samples PPB2 and PPB3 had lower rank sums and medians. This suggests that, PPB2 and PPB3 were the most preferred samples by consumers. Samples CB, PB, PPFs and PPB1 were the least preferred (Table 3.5).

Table 3. 5: Friedman rank sum test of the sausage samples

Sample	Median	Rank Sum
CB	3	181^{ab}
PB	3	177^{ab}
PPF1	5	228^{b}
PPF2	4	212^{b}
PPF3	3	194^{ab}
PPB1	3	164^{a}
PPB2	2	159^{a}
PPB3	2	159^{a}

Friedman chi-squared = 29.683, p-value = 0.0001085 and least significant rank difference (LSRD) is 43.3. Mean values with different superscript letters along the column are significant different at $p<0.05$. Scale (1 = most preferred and 5 = least preferred).
Key: CB - Control sausage, PB - Phosphate binder, PPB1 - Pigeon pea binder (2%), PPB2 - Pigeon pea binder (4%), PPB3 - Pigeon pea binder (6%), PPF1 - Pigeon pea flour (2%), PPF2 - Pigeon pea flour (4%), PPF3 - Pigeon pea flour (6%).

3.3.2.4 Correlation between descriptive attributes and acceptance by the PLSR

i. Principal component analysis of quantitative descriptive analysis data

Figure 3.2 shows bi-plot with the two first significant principal components from principal component analysis (PCA) on average sensory attributes of sausages. Principal component 1 (PC 1) accounted for 64.85% of the total variations while PC 2 accounted for 23.73% of total the variations. PC 1 is a contrast between PB, PPB3 and PPB2 correlated with aroma, color, compactness, moistness, mouth feel and saltiness; and CB and PPFs samples associated with hardness attribute (mainly between binder and flour). PC 2 is a contrast between CB and PB correlated with color, compactness, saltiness and hardness; and PPFs and PPBs samples associated with mouth feel, moistness, and aroma (mainly between control and formulated samples).

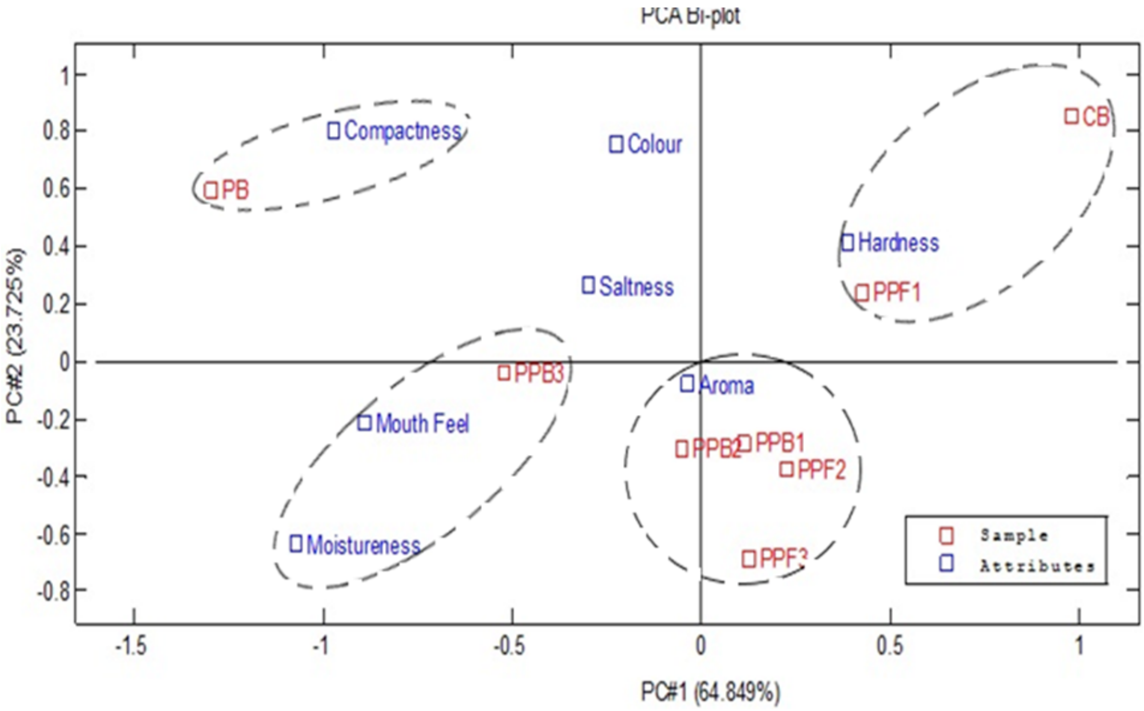


Figure 3. 2: PCA bi-plot showing relationship between sausage samples and descriptive attributes

ii. Correlation between descriptive attributes and acceptance by the partial least square regression (PLSR)

Results from a partial least square regression (PLSR) using descriptive data as X-variables and liking rated by the consumers as Y-variables are given in Figure 3.3. The first two significant components indicate 33% of the variations in X and 25% in Y. It was observed that the consumers fall to the right of the vertical Y-axis, outside the 50% explained circle. This means that, the acceptance values of these consumers go in the direction of liking of sausages made from PB and PPB3 due to their high association with color, saltiness, moistness, mouth feel and compactness attributes.

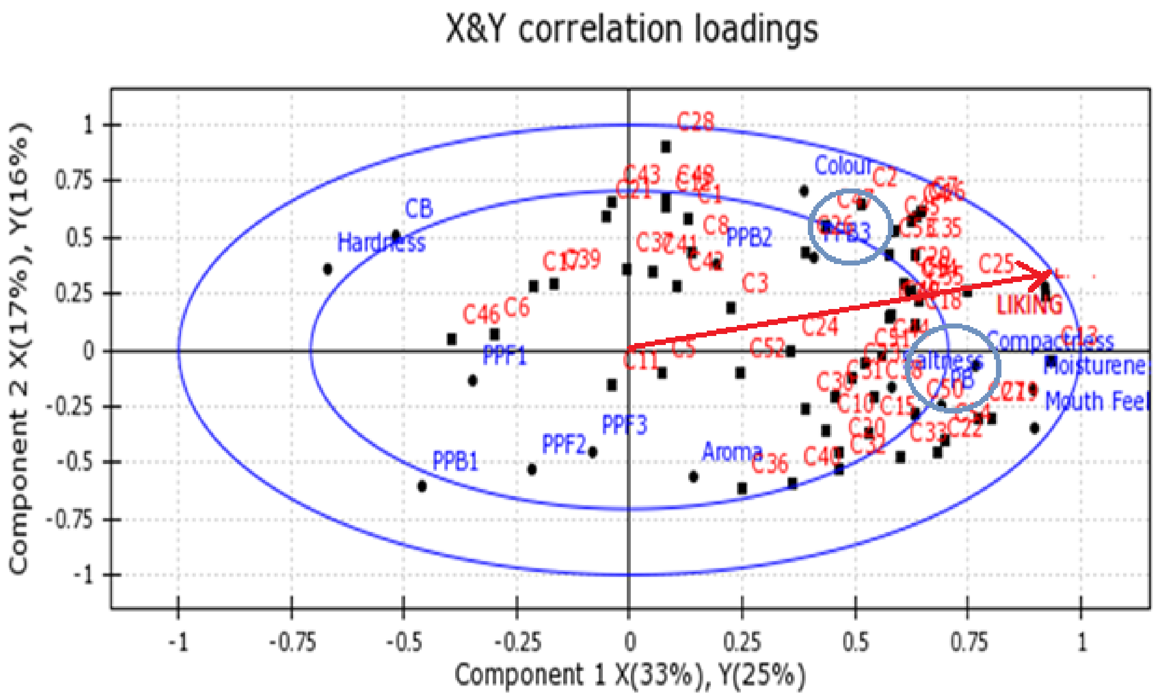


Figure 3. 3: **Correlation loadings from a partial least squares regression of sausage samples made from different binders with descriptive data as X variables and hedonic rating as Y variables**

This is further supported by Figure 3.4 which shows the contribution of each attribute on acceptability of sausage through correlation loading. Mouth feel, moistness and compactness contributed strongly and positively towards sample acceptability compared to color, saltiness and aroma that showed a weak positive contribution. Contrarily, hardness contributed weakly and negatively to acceptance of sausages samples. These results support the acceptability and preference of consumers towards moist and compact sausages with good mouth feel from the correlation loading plot in Figure 3.3.

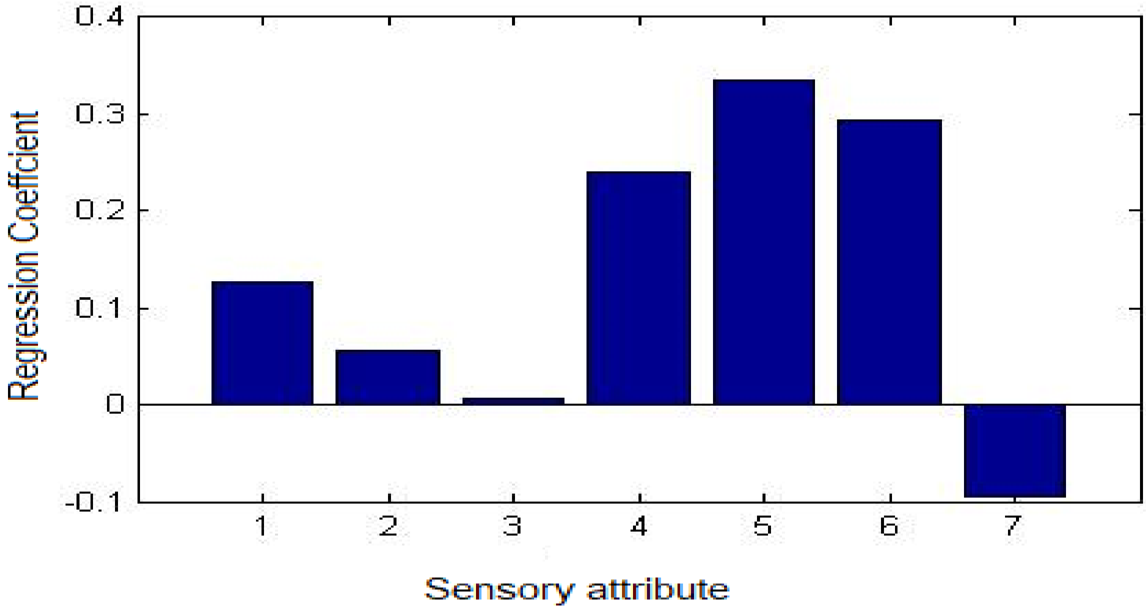


Figure 3. 4: Drivers of preference for sausage consumers according to the partial least squares regression analysis. Key: 1- Color, 2- Saltiness, 3- Aroma, 4- Mouth feel, 5- Moistness, 6- Compactness, 7- Hardness

3.4 Discussion

3.4.1 Quantitative descriptive analysis

The PB formulation had high intensity scores for saltiness, mouth feel, compactness and color (Table 3.3). The high saltiness in PB formulation is because addition of phosphate increases the salt content of sausage because phosphates are salts by nature hence a high salt content (Glorieux *et al.*, 2017). The observed high color intensity score in sample PB could be due to added phosphate which has an impact on the color of sausages by increasing the buffering capacity of meat which helps retain and maintain the fresh stable color of meat by changing the meats pH (Long *et al.*, 2011). For sausages, a stable color is among the main physical characteristics that determine the acceptability of a product to consumers and is a parameter that can easily be altered by the proportion of non-meat ingredients in the formulation (Syuhairah *et al.*, 2016). Furthermore, the addition of phosphate improves the compactness and mouth feel of the sausage by holding the water molecules together (Long *et al.*, 2017; Peng *et al*., 2009).

The high protein content in PPB is responsible for moistness in sausage since proteins tend to interact with water and myofibrillar protein in meat. This forms stable hydrophobic interactions and greater moisture holding capacity (Wi *et al*., 2020) resulting to a compact and moist sausage. Also, the high color intensity in PPB3 is probably due to yellow tone color of pigeon pea protein which was favorable to panelist especially at 6% formulation (PPB3). It can thus be assumed that the continuous replacement of beef with PPB increases the intensity of sausage color. Similar results were reported by Hidayat *et al.* (2018) and Babatunde *et al.* (2013) were the use of legume proteins increased the sausage color performance. The lower intensities for color, mouth feel, moistness, compactness and high hardness in PPF samples than PPB3 and other formulations, may be due to inadequate amount of protein present in the flours.

CB formulation was associated with high hardness, low mouth feels and moistness score. Control formulations have poor performance in sensory attributes due to absence of binders that enhance their properties. Similar results were reported by Babatunde *et al.* (2013) and Teye and Teye (2011) where control sausages didn't perform well. The high hardness in the control sausage (CB) may be due to high meat concentration as the CB formulation was not extended with any binder. Also, CB had high similar color intensity score as PB; this could be due to absence of binder in CB formulation.

3.4.2 Consumer test

From the results, there was high acceptability of PB and PPB3 sausage formulations than other formulations due to contribution of individual attributes like appearance, taste, moistness and softness (Table 3.4). All these attributes had influence on the overall acceptability of the sausage and similar results were reported by Oluwaseun (2019) and Syuhairah *et al.* (2016). In product development, consumer testing is considered to be one of the most important tests and its primary purpose is to assess the personal response by current and potential customers of a product or specific product characteristics (Soma, 2013).

3.4.3 Relationship between descriptive attributes and acceptance by the partial least square regression (PLSR)

Figure 3.2 shows the relationship between sausage samples and descriptive attributes through principal component analysis (PCA). PC 1 accounted for most of the variations observed (64.849%) and PB and PPB3 were grouped on one side and the rest of the formulations were grouped on the other side showing that PB and PPB3 have similar sensory profile. PB formulation was strongly related to compactness attribute and PPB3 was strongly related with moistness and mouth feel attributes. The high value in these attributes is because for PB, the use of phosphate is associated with improved texture and compactness due to meat stabilization (Long *et al.*, 2017)**.** As for PPB, presence of high protein content increases hydrophobic interactions and meat texture (Hidayat *et al.*, 2017). Therefore, PPB3 may replace PB in sausage making due to similar effect on sausage attributes and has an added advantage of being natural hence free from harmful chemicals in the body. CB and PPF formulation were on the right side of variations explained by PC 1. Hence, they had similar sensory profile associated with hardness and aroma attributes. This is because, as noted previously, the absence of binder in CB and low protein concentration in PPF formulation results to low scores of attributes. Thus, for sausage to be acceptable to consumers it should contain a binder. Pigeon pea flour (PPF) is not recommended to be used as a binder.

Sample PPB3 correlated positively with mouth feel attribute while sample PB correlated positively with compactness attribute. Aroma attribute was positively correlated to samples PPB2, PPB1, PPF2 and PPF3 while hardness correlated with PPF1 and CB samples. Saltiness and color attribute had no high correlation with any sample at varying levels of binders used.

PPB3 was highly correlated with moistness attribute and these results agree with Omojola *et al.* (2013) who reported that sausages extended with legume proteins had higher moisture content. Thus, the higher the protein proportion in the sausage the more the moisture. The observed high hardness for CB may be associated with water loss from the sausage during refrigerated storage and inability of the sausage to effectively hold water. Because the sausage had no any binder, which results to negative effect on sensory quality (Wang *et al.,* 2009). PPF1 was also highly related to hardness indicating that the concentration of proteins in the sample was not effective at holding water.

The observed high consumer acceptability and preferences for PB and PPB3 samples than CB and PPFs samples (Figure 3.3) was associated with the high higher moistness, compactness and mouth feel attributes. This is supported by Figure 3.4, which shows the contribution of each attribute towards the acceptance of sausage formulations. It provides insight into the sensory attributes that are important to individual's consumer preference of sausage while moistness, compactness and mouth feel attributes are strong contributors; color, saltines and aroma are weak contributors.

Also from Table 3.5, Friedman rank sum test shows that PPB3 and PPB2 sausage formulations were the most preferred samples with a rank sum of 159. This was less than PB which had a rank sum of 177 while the least preferred sample was for PPF formulation. These result shows that sausage formulation made from pigeon pea protein binder of 6% (PPB3) and 4% (PPB2) levels may be used to replace phosphate binder (PB) due to higher preference of PPB3 and PPB2 over PB, CB and PPFs.

Despite the preference for PPB3 and PPB2 sausage formulations being the same, the acceptance of PPB3 was higher than PPB2 (Table 3.4), thus PPB3 was superior to PPB2. Also the acceptance of PPB3 and PB was associated with the same attributes and since PPB3 is natural with low cost and easily accessible compared to PB; it implies that the protein binder can replace phosphate in sausage making.

3.4.4 Cost benefit analysis

The costs of acquiring binders to prepare 1 dozen of sausage are presented in Table 3.6. For one (1) sausage with phosphate binder, 0.25 g of phosphate is required (Teye and Teye, 2011). This was obtained by dividing 5 g of phosphate to 20 sausages produced from 1 kg of meat. Also, for one (1) sausage with pigeon binder, 3 g of pigeon pea protein binder is required (based on sensory quality findings for PPB3). This was obtained by dividing 60 g (6%) of pigeon pea binder to 20 sausages produced from 1 kg of meat. The total cost for a dozen of phosphate sausages is Tshs. 7 644.22 and for a dozen of pigeon pea binder sausages is Tshs. 10 230.00 (Table 3.6). Based on these, manufactures opt to use phosphate because it is much cheaper than pigeon pea binder. Therefore, more research is required in this area so as to optimize/ minimize production cost.

Table 3. 6: Cost of acquiring and processing the binders used in the experiments

Activity	Recipe	Quantity	Unit Cost	Amount (TZS)	Number	Amount per dozen (TZS)
1. Sausage development using PB	Phosphate binder	0.25 g	8.07	2.02	12	24.22
	Meat	50.00 g	8.00	400.00	12	4 800.00
	Spices	0.25 g	40.00	10.00	12	120.00
	Casing	15.00 cm	1.33	20.00	12	240.00
	Electricity	0.50 kwh	400.00	200.00	12	2 400.00
	Water	15.00 g	0.33	5.00	12	60.00
	Sub total			**637.02**	**12**	**7 644.22**
2. Sausage development using PPB						
2.1. Binder development (I kg)						
	Raw pigeon pea	1.00 kg	2 500.00	2 500.00	1	2 500.00
	Electricity	60.00 kwh	333.33	20 000.00	1	20 000.00
	Reagents	2.00	25 000.00	50 000.00	1	50 000.00
	Sub total		**72 500.00**	**72 500.00**	**1**	**72 500.00**
						1 g = 72.50
2.2. Sausage development						
	PP binder	3.00 g	72.50	217.50	12	2 610.00
	Meat	50.00 g	8.00	400.00	12	4 800.00
	Spices	0.25 g	40.00	10.00	12	120.00
	Casing	15.00 cm	1.33	20.00	12	240.00
	Electricity	0.50 kwh	400.00	200.00	12	2 400.00
	Water	15.00 g	0.33	5.00	12	60.00
	Sub total			**852.50**	**12**	**10 230.00**

3.5 Conclusion

The findings of the present study indicate that sausage prepared from chemical phosphate binder (PB) had similar sensory profile as sausages prepared by 6% pigeon pea binder (PPB3) to consumers. The major attributes for the acceptance of PPB3 sausage samples were mouth feel, moistness and compactness. Therefore, the use of pigeon pea binder at 6% level as a binding agent in beef sausage making may be recommended. Because apart from having a similar sensory acceptability as the phosphate, it also presents an excellent alternative as binder in sausage making. This is due to its accessibility, availability and is natural hence doesn't pose health risks to consumers compared to phosphate which is not easily accessible. Despite high costs, pigeon pea binder production should be optimized to reduce the production costs so as to encourage its utilization in the sausage industry. The study may be useful for sausage producers, since it provides feedback of acceptance of this new product (pigeon pea sausage binder) by consumers. Also, there is a description of consumer mapping preferences which will facilitate meeting of market needs.

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CHAPTER FOUR

4.0 OVERALL CONCLUSIONS AND RECOMMENDATIONS

4.1 Conclusions

The findings have revealed that, the improved variety of pigeon pea had a higher protein content compared to the local variety and the level of anti-nutrients in both varieties were within safe limits. Therefore, the improved variety can be used to develop pigeon pea protein binder in sausage making which is free from anti-nutrients. Also, pigeon pea binder of six percent (6%) level (PPB3) can be used in sausage making due to improved texture and water solubility of sausages. Apart from that, the PPB3 sausages had similar sensory profile as that of sausages developed by chemical binder (phosphate) commonly used in the sausage industry. Also, the use of pigeon pea flour as a sausage binder resulted to poor performance and poor sensory quality of sausage.

4.2 Recommendations

Recommendations are made based on the study findings as follows:

i. For sausage to be of good quality and acceptable to consumers it should contain a binder. Pigeon pea flour is not recommended to be used as a binder in sausage making.

ii. The use of pigeon pea binder in sausage making to replace chemical binders is recommended. Also initiation of production of pigeon pea binder within the country at a large scale for sausage industry for reducing importation of binders from outside the country which are costly and hard to access is recommended too.

iii. Provision of technical and financial support to pigeon pea farmers so as to encourage them to increase pigeon pea cultivation with high protein content for the inside market

iv. Further study to evaluate the effect of storage time on the physicochemical quality and microbiological safety of sausage developed by pigeon pea binder is recommended. Also assessment of the effect of the binder on other types of sausages like chicken and pork sausage in the presence of additional ingredients like fat is recommended.

v. Optimization studies on the use of pigeon pea binder (PPB) as a cheaper alternative source of binder compared to phosphates.

APPENDICES

Appendix 1: Quantitative descriptive analysis

Quantitative descriptive sensory evaluation form

Sensory Evaluation Form

Quantitative Descriptive Analysis (QDA) of Sausage Samples

Sex………………..………………………… Age………………………… Time……….

Please evaluate each coded ample in the order they are listed. Choose appropriate number in a scale from 1 to 9, where 1 is low intensity and 9 is high intensity. How do you find the following characteristics for sausage? Put the appropriate number against each characteristic.

Sample number ________

Color

Pale Himalaya 1 2 3 4 5 6 7 8 9 Himalaya

Saltiness

Less salty 1 2 3 4 5 6 7 8 9 Salty

Mouth feel

Loose particles 1 2 3 4 5 6 7 8 9 Dense particles

Moistness

Not moist 1 2 3 4 5 6 7 8 9 Very moist

Compactness

Not compact 1 2 3 4 5 6 7 8 9 Very compact

Hardness

Not hard	1	2	3	4	5	6	7	8	9	Very hard

Thank you for your cooperation!

Appendix 2: Consumer study of sausage samples

i. Hedonic test

P. No..

Time..

Please evaluate each of the eight (8) coded sausage samples from left to right. Indicate how much you like or dislike each sample by checking the appropriate sample attribute and indicate your degree of liking (9 - 1) in the column against each attribute. Put the appropriate number against each attribute (Table 1.1).

Key: 9- Like extremely, 8- Like very much, 7- Like moderately, 6- Like slightly, 5- Neither like nor dislike, 4- Dislike slightly, 3 -Dislike moderately, 2- Dislike very much, 1- Dislike extremely.

Table 1.1. Hedonic test

	Sample code							
Attribute	563	734	449	897	251	106	953	315
Appearance								
Color								
Aroma								
Taste								
Softness								
Moistness								
Overall acceptability								

Comments...

....

ii. Preference test

You are provided with eight sausage samples. Please assess each sample and determine which one you prefer based on the scale (5 - 1), 1 being most preferred and 5 being least preferred 9 (Table 1.1). Put the appropriate number against each sample code in the rating space.

Table 1.2: Preference test

Sample code	Preference rating
563	
734	
449	
897	
251	
106	
953	
315	

Comments...

Lastly, we would be happy if you will answer some additional questions. We need some information about our consumers, and would appreciate it if you could answer the following questions:

1. Gender

□ Female

□ Male

2. Age

□ 15-30

□ 31-45

□ 46-60

3. Which group do you fit?

□ Undergraduate

□ Postgraduate

□ Staff

4. How often do you consumer sausage?

□ Daily

□ Once in a week

□ Once a month

□ Seldom

5. Willingness to purchase

□ Yes

□ No

Many Thanks for Your Cooperation!