PROTEIN, ANTI-NUTRIENT AND SENSORY QUALITIES OF COMPLEMENTARY FOOD MADE FROM MALTED FINGER MILLET *(Eleusine coracana* L.) AND COWPEA *(Vigna unguiculata* L.) COMPOSITE FLOUR

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DECLARATION AND RECOMMENDATION

Declaration

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

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Recommendation

This thesis has been submitted for examination with our approval as the official University supervisors.

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DEDICATION

The work is dedicated to my parents; Mr and Mrs Syeunda, they have set a good example of love, sheer hard work, discipline and dedication to education that have brought me this far. I am fortunate to have been born to the two of you.

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ABSTRACT

Protein-energy malnutrition remains a huge problem for children in sub-Saharan Africa. Kenyan children are no exception. These children rely on cereal porridge such as finger millet (*Eleusine coracana*) for nutrient supply. Cowpea (*Vigna unguiculata)*, a locally available nutritious legume, could be an excellent complement to lysine-deficient millet diets. Therefore, the present study aimed at innovatively improving the protein quality and sensory properties of the complementary food, by evaluating the effect of malting on improved finger millet genotypes (U15, P224, KNE741, KNE629 and Snapping green) and compositing with cowpea to enable selection of the best varieties with superior nutritional credentials post process. Prepared selected finger millet and cowpea flours were composited in a ratio recommended by the World Health Organisation to deliver minimum protein content for complementary formula with, 0% cowpea as the control. Impact of malting and compositing on protein and anti-nutritional compounds was determined in terms of protein content; *in vitro* protein digestibility, amino acid profile and anti-nutritional compounds such as phenolic compounds, condensed tannins and phytic acid were assessed for recommendations in product development. The best levels of substitution were evaluated via descriptive sensory analysis. KNE741 and Snapping green finger millets showed superior qualities in terms of protein and tannin contents. Compositing with precooked cowpea increased *in vitro* protein digestibility in raw flour by about 4-8%. In addition, phenolic compounds, tannin content, and phytic acid content notably decreased by 40%, 18%, and 44%, respectively, after compositing with improved malted finger millet and precooked cowpea at 0%, 10.32%, 21.26%, and 32.75%. Cooking of malted and composited flours resulted in a decrease in total phenolic compounds, condensed tannins and phytic acid, by 22%, 1%, and 13%, respectively, with concomitant increase in *in vitro* protein digestibility. The first three principal components accounted for approximately 75% of the variations in sensory attributes. Among the sensory attributes, astringency and stickiness were more pronounced in KNE741 than Snapping green finger millet variety. Increase in malty flavour and aroma after malting finger millet corresponded to reductions in texture attributes and astringency. In addition, compositing with precooked cowpea flour beyond 21.26% resulted in distinct cooked cowpea flavour. This study shows that, malting of finger millet and compositing it with precooked cowpea has the potential to address protein-energy malnutrition among under five children in sub-Saharan Africa. However, compositing with higher levels of precooked cowpea requires flavour masking.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND ACRONYMS

AACC	Association of American Cereal Chemists
BecA–ILRI	Biosciences eastern and central Africa–International Livestock Research Institute
CESAAM	Centre of Excellence in Sustainable Agriculture and Agribusiness Management
FAO	Food and Agriculture Organisation of the United Nations
GAE	Gallic Acid Equivalent
IVPD	*In vitro* Protein Digestibility
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
KALRO	Kenya Agricultural and Livestock Research Organisation
KNBS	Kenya National Bureau of Statistics
KDHS	Kenya Demographic Health Survey
LC-FLD	Liquid chromatography Fluorescence Detector
PEM	Protein Energy Malnutrition
PDCAAS	Protein Digestibility Corrected Amino Acid Score
UNICEF	United Nations International Children's Emergency Fund
WFP	World Food Programme

CHAPTER 1: INTRODUCTION

1.1: Background information

According to Lutter and Dewey (2003), meeting the nutritional needs of children 6 to 36 month's old children is challenging. The primary cause of more than 2.6 million child deaths each year worldwide is child malnutrition, which represents nearly half of child deaths globally (UNICEF, 2013). One in four of the world's children show stunting disorder, which represents approximately 24% of children's under-five population (De Onis and Branca, 2016). Many affected children survive but suffer lifelong physical and cognitive impairments due to early macronutrient deficiencies (KNBS, 2010). In addition, children lead a poor quality life due to their inability to fight infectious diseases. Kenyan children are no exception. A 2008, health survey showed that 35% of children under-five 5 years old were stunted, 16.1% underweight and 6.7% wasted (KNBS, 2010). A recent survey showed an improved scenario with 26% of children under-five years of age as stunted, 11% underweight and 4% wasted (KNBS, 2014). Though from the statistics, there has been a progressive decline in levels of malnutrition within the country, the indicated levels are still way above World Health Organisation recommendation of less than 10% underweight, less than 20% stunting and less 5% wasting (WHO, 1995). Several interacting factors contribute to malnutrition. These include inadequate dietary intake, recurrent infectious diseases like diarrhoea, and poor food quality (Konyole *et al*., 2012).

Appropriate nutritional intake is important especially for children because it is the most critical stage of human development (Michaelsen *et al*., 2009). Children obtain all the energy and nutrient requirements from breast milk during the first six months of their life. At the age of six months, for them to meet their needs for proper growth and development they require complementary foods in addition to breast milk (Oniang'o *et al*., 2003). The principal food given to children 6 months to 3 years of age in many parts of Kenya is a cereal-based thin porridge (*uji*). Mainly, it is made from sorghum (*Sorghum bicolour* L.), maize (*Zea mays* L.) and finger millet *(Eleusine coracana* L.*)* which are sometimes supplemented when possible with fruit from banana (*Musa acuminate* L.), non-cereals such as pumpkin (*Cucurbita maximai* L.), cassava (*Manihot esculenta* L.*), dagaa* (*Rastrineobola argentea*) sweet potatoes (*Ipomea batatas* L.), or milk (Konyole *et al*., 2012). However, these complementary foods are typically gruels of high bulk, low protein content and quality (Ogbonna *et al*., 2010). High dietary bulk in these gruels makes it hard for children to adequately meet their nutritional requirement when fed, partly because a young child has a less mature

gastrointestinal tract, which results in digestion difficulty as postulated by Owino *et al.* (2007). Therefore, this causes a huge malnutrition implication as it is difficult to meet a child's nutritional needs.

Developing low-cost, protein-dense complementary foods using locally available food ingredients has strongly been recommended by World Health Organisation as a practical and sustainable way to address protein-energy malnutrition problem in developing countries (Konyole *et al*., 2012). Therefore, utilization of traditional food ingredients or introductions of new affordable foods seem crucial to meet the increasing demand for low cost, nutrient-dense complementary food from traditional ingredients (Najdi and Orsat, 2017). Indigenous underutilized cereals and legumes such as sorghum (*Sorghum bicolour* L.), finger millet (*Eleusine coracana* L.), cowpeas (*Vigna unguiculata* L.), chickpea (C*icer arietinum* L.), and mung beans (*Vigna radiata* L.) are nutritious and sustainable crops with great potential to meet the nutritional needs and improve food security among the most impoverished communities (Lutter and Dewey, 2003).

Finger millet is a unique crop because of its ability to tolerate drought, survive variant ecological environments, as well as being a primary source of nutrients especially minerals (Bavec and Bavec, 2007). A challenge though is that finger millet has relatively low protein content and quality, because its major storage protein eleusinin is poor in indispensable amino acid lysine, which accounts for relatively between 3.1% and 3.7% of the protein content (Ramachandra *et al*., 1978). This is low as compared to the recommended level of 5.2% lysine for 6 months to 3 year-old children (WHO/FAO/UNU, 2007). In addition, some finger millet varieties contain relatively higher amounts of anti-nutrients such as polyphenols, condensed tannins and phytic acids. These anti-nutrients although they are important during plant growth, they bind to protein and minerals reducing bioavailability in the human gut (Singh and Raghuvanshi, 2012).

Finger millet sustains one-third of the world's population. Moreover, it represents more than 12% of global millet output, with Africa accounting for a production estimated to be two million tonnes (Siwela, 2009). In Kenya, production increased from 37,000 tonnes in 1981 to 67,000 tonnes in 2012, with annual production estimated to be approximately 60,000 tonnes (FAOSTAT, 2017). Approximately 60% of finger millet produced in Kenya is consumed at a household level, 30% sold locally and the remaining 10% treated as seed (Orr *et al*., 2016). It contains relatively high amounts of sulphur and aromatic containing amino acids and

minerals, especially calcium, manganese, iron, phosphorus, copper, sodium and, a significant proportion of carbohydrates (Mbithi-Mwikya *et al*., 2000).

Cowpea is critical food legume in sub-Saharan Africa. It is a drought-tolerant crop and an inexpensive source of protein. On average cowpea contains about 24% crude protein, and sustains approximately 200 million people (Ojwang, 2012). Storage proteins in cowpea, unlike in finger millet, are globulins, which are relatively rich in lysine (approximately 5 g lysine per 100g protein) (Anyango, 2009). In addition, cowpea is also vital to rural farmers who are unable to afford commercial fertilizers as it is able to biological fix nitrogen into the soil through the action of rhizobia bacteria (Ojwang, 2012). However, it also contains oligosaccharides, verbascose and stachyose, associated with flatulence, but these are reduced significantly through cooking (Onyenekwe *et al*., 2000). Therefore, cowpea provides a good alternative to complement protein-deficient millet diets, which on average, contain 7.3% protein (Siwela *et al.,* 2007).

The present research focused on utilizing malting technique that employs the use of inherent grain hydrolase enzymes in converting insoluble protein to soluble protein and hydrolysis of tannin-protein complex of improved finger millet genotypes developed and grown in Kenya, in the formulation of complementary food. To upgrade the overall protein content and quality of the complementary food, in terms of protein content, protein digestibility and amino acid score, the research used a precooked cowpea in compositing with improved malted finger millet. The objective of precooking was to reduce the protein inhibitors and other anti-nutrient compounds in cowpea such as flatulence-causing oligosaccharides. Malting was aimed at hydrolysing insoluble protein to soluble protein and acting on tannin-protein complex. These food-processing techniques would help improve the protein digestibility and bioavailability thereby leading to the introduction of a reliable and affordable alternative to the present proprietary complementary formulas.

1.2: Statement of the problem

The major contributory factor in protein-energy malnutrition among children under-five years of age in developing countries is the consumption of cereal based complementary food. These foods include finger millet and have low protein content and quality. Recent demographic and health surveys have shown cereal-based complementary food as the major weaning food among these children. These poor complementary practices have resulted in 26% of children less five years of age having stunting disorder and of 39 deaths per 1000 live birth children

mortality (KNBS, 2014). Therefore, an introduction of a culturally acceptable cereal and legume has potential of being a sustainable and practical way to improve finger millet protein content and quality and in addressing protein-energy malnutrition. Although finger millet and cowpea possess many essential nutrients, these grains contain considerable amounts of anti-nutrient compounds such as phytic acid and condensed tannins (Chandra *et al*., 2016).These anti-nutritive compounds especially condensed tannins, bind with dietary protein making it less digestible in the human gut (Hejazi and Orsat, 2016). Significant availability of these anti-nutrients in these two crops may negatively influence bioavailability and quality of the nutrients especially protein, in formulated products. Therefore, it was essential to study the effect of compositing improved malted finger millet with precooked cowpea on protein content and quality of a complementary food formula.

1.3: General objective

To contribute to the reduction of protein-energy malnutrition through compositing improved malted finger millet flour with precooked cowpea for complementary food formulation to improve nutritional status of children in Kenya.

1.4: Specific objectives

i. To determine the effect of compositing improved malted finger millet flour with precooked cowpea on the protein quality of formulated complementary food.
ii. To determine the effect of compositing improved malted finger millet flour with precooked cowpea on anti-nutrient contents of formulated complementary food.
iii. To determine the effect of compositing improved malted finger millet flour with precooked cowpea on the sensory properties of formulated complementary food.

1.5: Hypotheses

i. Compositing improved malted finger millet flour with precooked cowpea has no significant effect on protein quality of formulated complementary food.
ii. Compositing improved malted finger millet flour with precooked cowpea has no significant effect on anti-nutrient contents of formulated complementary food.
iii. Compositing improved malted finger millet flour with precooked cowpea has no significant effect on sensory properties of formulated complementary food

1.6: Justification of the study

The World Health Organisation recommends the use of traditional ingredients in complementary food formulation. There is diversity in food ingredients, such as finger millet and cowpea suitable for ecological conditions in Kenya. Proprietary formulas usually are of high protein quality, acceptable and safe to infants. However, they are cost-prohibitive hence unaffordable for the low-income rural populations. Finger millet and cowpea grains are readily available underutilized food ingredients. Indigenous processing techniques such as malting, can enhance protein-starch digestibility, reduce anti-nutrients especially condensed tannins and improve on mineral bioavailability. The use of culturally acceptable and affordable traditional crops would result in high uptake of the developed product and enhance utilization of improved finger millet genotypes developed and grown in Kenya.

CHAPTER 2: LITERATURE REVIEW

The chapter consists of four sections. A first section provides a review on current trends on developing of baby weaning foods. It highlights previous investigations used in baby weaning food formulations. Second section provides a review on finger millet grain. It mainly discusses morphological characteristics of finger millet grain, protein composition and lastly processing techniques that affect its protein digestibility. Third section provides a review on cowpea, its chemical composition and it highlights also different anti-nutrients the cowpea contains. Finally, there is also a review of analytical procedures for evaluating polyphenols content, phytic acid and sensory analysis.

2.1: Complementary foods

The art of introducing liquid semi-solid or solid food to child life or complete discontinuation of breast milk by the introduction of semi-solid or solid food to the diet is called weaning. It involves expanding the infant's diet to include other drinks and food rather than infant formula or breast milk (Sajilata *et al.,* 2002). During this dietary transition, the nutritional requirements for the brain and growth of the infant are high. Alexander (1983) observed that, among developing countries, the weaning period commences at the age of 4-6 months, which continues till the age of 2-3 years. During weaning, the transitional phase from liquid to solid or semi-solid is critical to a child's life, since a nutritional deficiency in the form of protein-energy malnutrition may likely occur. Based on the reviewed guidelines, given by the Protein Advisory Group, the desired complementary food should contain 10-20% protein content, with protein providing 15% of calories (Najdi and Orsat, 2017). Therefore, introducing complementary foods with right protein quantity and quality at the right stage and in right amount may reduce protein-energy malnutrition cases in Kenya.

2.1.1: Current trends in the development of complementary foods in Kenya.

In formulating a well-balanced complementary food, different food ingredients have been used. Konyole *et al*. (2012) in their research exploited the use of amaranth grain (*Amaranthus retroflexus)*, edible termites (*Macrotermes subhylanus*) and *dagaa* fish (*Rastrineobola argentea*) in formulating of complementary food for communities living in western Kenya. Other authors have used pigeon peas (*Cajanus cajan*), common bean (*Phaseolus vulgaris*) and bambara nuts (*Vigna subterranean*) in formulating cereal-legume complementary food (Asma *et al.,* 2006). A more advanced formulation: corn-soy-blend *plus* (CSB+) and corn-

soy-blend (CSB) and were promoted by the World Food Programme to manage acute malnutrition in sub-Saharan Africa (WFP, 2010).

Many local rural mothers use local ingredients to formulate complementary foods for their children. These complementary foods fail to meet the nutritional requirements of children since they are of low nutrient density and contain considerable amount of anti-nutrients such as polyphenols that limit protein digestibility. Unlike, the elite and economically affluent population, who are able to meet the growing, needs of infants through the commercially proprietary food formulas that are considered nutritious, acceptable and safe.

One of the Kenya's Big 4 agenda is to contribute to food security and nutrition and increase employment opportunities among Kenyans (The Presidency, 2019). This can be realised through initiatives such as flour blending based on nutritious but under-utilised food ingredients grown locally. The research used a cereal (finger millet) and a legume (cowpea) in formulating a protein-dense complementary food thereby improving nutritional status of children in Kenya.

2.2: Finger millet grain morphology

The common name for finger millet grain is African millet, bird's foot or coracana in English. In Eritrea and Ethiopia it is known as *dagusha*, *wimbi* in Swahili, *bulo* in parts of Uganda, *ragi* in India, while, in Nigeria it is called *pwana* or *tamba*. Finger millet plant can grow to a height of 0.4–1.0 m while the length of its panicles varies between 3 and 13 cm (Bavec and Bavec, 2007). The crop is well adapted to arid, marginal land probably due to its C4 photosynthetic nature since it can use water efficiently, hence making it suitable for low socio-economic farmers who inhabit these areas. Moreover, it can be stored for long, approximately 10 years (Bavec and Bavec, 2007). Morphologically, the seeds vary from spherical to oval and dimension range between 1 to 2 mm, with the weight of 1000 kernels on average being 2.5 g. Its colour spectrum ranges from white to brown to black (Obilana and Manyasa, 2002; Siwela *et al*., 2007). Unlike most cereals such as rice, maize wheat and rye, which are caryopsis meaning the pericarp adheres tightly to the seed coat, finger millet grain is a utricle, and therefore, it has pericarp like sac which encloses its seed (Siwela, 2009). The underlying kernel structure consists of the following anatomical parts; endosperm, embryo (germ) and pericarp, as represented in Figure 1.

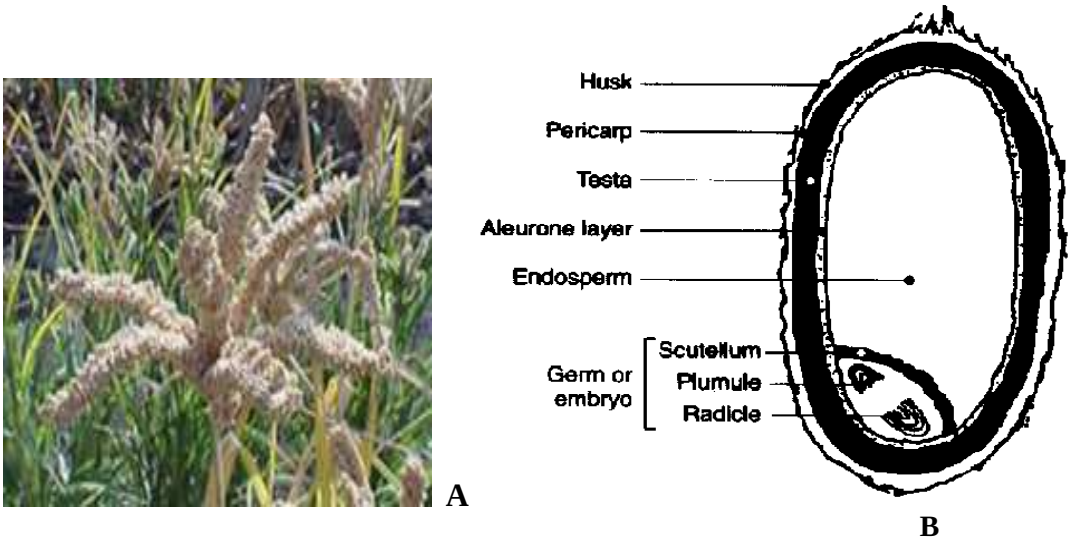


Figure 1: A-Finger millet crop, **B**-Finger millet grain morphology

Source: Ramashia (2015**)**

2.2.1: Finger millet pericarp

Finger millet pericarp consists of a fruit coat, which comprises three layers (epicarp, mesocarp, and endocarp) and five-layered testa seed coat. The testa has varying levels of pigmentation ranging from purple to red (McDonough *et al*., 2000). Since finger millet is a utricle, its pericarp can easily be detached from testa through washing or rubbing. McDonough *et al*. (2000) highlighted that the pericarp appeared to have several layers of tissues. Occurrences of condensed tannins in finger millet are influenced by variety (Siwela et al., 2007) and are located mostly in the testa. These authors concluded that finger millet comprising of thick testa had a significant amount of tannins as compared to those with thin testa. The condensed tannins are important during plant growth as they help protect finger millet grains against birds, and fungal attack. However, they are linked with reduced protein and mineral bioavailability in human gut (Siwela, 2009).

2.2.2: Finger millet endosperm

Endosperm comprises a significant portion of the finger millet kernel. It contains three distinct starchy sections namely corneous, peripheral and floury and aleurone layer as noted by McDonough *et al*. (2000). The aleurone layer, according to McDonough *et al.* (2000) is comparable to that of other cereals such as maize and sorghum. Starchy endosperm is surrounded with one thick layer of cells. The peripheral endosperm consists of tightly packed small cells. It also has protein bodies embedded in protein matrices that associate with simple starch granules and compound starch granules ranging approximately 8.0-16.5 μm in

diameter. The corneous endosperm is the most significant part of the endosperm and it consists of cells of different sizes. It has compound starch granules of diameter ranging between 3.0-19.0 μm, with simple starch granules running between them. The starch granule associates with patches of the protein matrix. McDonough *et al.* (2000) noted that the floury endosperm consists of compound starch granules ranging approximately 11-21 μm in diameter. Variety significantly influences the composition of each of the endosperm components (Obilana and Manyasa, 2002).

2.2.3: Finger millet Germ

Finger millet embryo is approximately 5% of the total seed weight; majorly it consists of grain lipid (Obilana and Manyasa, 2002). The size is small ranging between 270 and 980 μm and is located in a depression that is enclosed in a characteristic ridge (McDonough *et al.*, 2000). The scutellum epidermis separates the scutellum from the floury endosperm. The scutellum contains protein bodies, which act as prolamin storage.

2.2.4: Finger millet protein composition

The protein content of finger millet is quite variable ranging between 5.0 -12% as reviewed by McDonough *et al*. (2000). This variability is mainly due to variations in water availability, genotype, environmental conditions and soil fertility as reviewed by Siwela *et al*. (2007). A study done on different finger millet genotypes grown in Eastern Kenya revealed that high yielding genotypes had a low protein content as compared to low yielding ones (Shibairo *et al*., 2014). Mbithi-Mwikya *et al*. (2000) noted that white coloured finger millet varieties had higher protein content when compared to brown coloured finger millet varieties, and they attributed this to a high amount of eleusinin a prolamin fraction in white varieties than brown coloured varieties. Finger millet protein of 7.3% compares favourably to rice protein of 7.9% and also it is similar or lower to that of sorghum 8% and wheat 12% (Siwela *et al*., 2007). However, finger millet protein is inversely related to its fat content, as varieties rich in fat content are low in protein content and vice versa (Shibairo *et al*., 2014).

The major protein fraction, based on Osborne's classification which fractionates cereal protein based on solubility, is the prolamins followed by the glutelins, representing 47% and 41%, respectively, as reviewed by Dharmaraj and Malleshi (2011). From the same review, the prolamins were within protein bodies. Other protein fractions in the finger millet are albumins and globulins that occur at 8% and 4%, respectively (Dharmaraj and Malleshi, 2011). The major protein fraction eleusinin is rich in proline, glutamic acid, valine alanine,

isoleucine, phenylalanine, and leucine (Table 1). However, it has low amounts of arginine, lysine, and glycine as compared to other protein fractions (Mbithi-Mwikya *et al*., 2000). Finger millet contains relatively high amount of methionine at approximately 5% of the protein. However, just like other cereals lysine and tryptophan are the major limiting essential amino acids (Table 1).

Table 1: Amino acids composition of finger millet proteins, values expressed as g/100g protein

Amino acid	Total protein in grain	Prolamin fraction	Glutelin Fraction	Albumin-Globulin	[a]WHO standard
Lysine	3.1- 3.7	0.66	7.69	5.56	5.2
Histidine	2.6- 2.8	3.08	3.88	2.21	1.8
Threonine	5.1- 5.2	5.23	4.85	5.16	2.7
Valine	7.9- 8.2	7.56	5.32	5.35	4.2
Methionine	2.6- 4.5	3.01	1.64	0.76	2.6[b]
Isoleucine	5.1- 5.2	5.13	4.17	3.24	3.1
Leucine	11.7- 13.5	12.24	7.98	6.40	6.3
Tryptophan	1.3	4.65	3.26	3.05	0.7
Phenylalanine	6.1- 6.2	7.55	4.44	2.79	4.6[c]
Arginine	4.9- 5.2	2.08	8.67	8.96	
Aspartic acid	7.2- 7.9	4.38	7.53	9.01	
Serine	6.6- 6.9	6.30	5.60	6.45	
Glutamic acid	24.2- 27.1	32.24	19.02	16.70	
Proline	6.7-7.6	10.40	6.85	5.35	
Glycine	4.5- 4.8	1.65	4.29	6.20	
Alanine	7.2- 8.0	6.76	6.46	8.34	

Source: Ramachandra *et al*. (1978) and Siwela (2009)

[a] Based on the WHO requirements for essential amino acid composition of a 1 to 3-year-old child (WHO/FAO/UNU, 2007).

[b]Methionine + cysteine

[c]Phenylalanine + tyrosine. Tyrosine and cysteine not essential amino acids, but they can spare the requirement for phenylalanine and methionine, respectively.

Generally, the proteins in finger millet are more nutritionally balanced (Table1). (Mbithi-Mwikya *et al*. (2000) noted that finger millet grain contains relatively high amounts of sulphur-containing amino acids as well as aromatic amino acids that are important in human

growth when compared to other popular cereals such as maize. However, they noted a negative correlation between the protein content in the grain and lysine levels, which they attributed to a severe deficiency of lysine in the eleusinin protein fraction (Mbithi-Mwikya *et al*., 2000). Landraces or local finger millet varieties grown in Kenya are rich in essential amino acids when compared to improved varieties produced by research organizations such as ICRISAT, which have high total crude protein (Shibairo *et al*., 2014).

2.3: Protein nutritional value of finger millet

A high quality protein should contain all the essential amino acids at the level higher than or equal to the reference levels as outlined in WHO/FAO/UNU (2007). Moreover, its digestibility should be comparable to egg white or milk proteins.

2.3.1: Finger millet protein digestibility

An indicator of protein nutritional value is amino acid composition. Finger millet just like other cereals, when compared to legumes, is a poor source of lysine. Therefore, supplementation of finger millet diets with legumes could help to alleviate this problem, which is of particular importance for infants who have a high requirement of essential amino acids (Siwela, 2009).

An indicator of protein availability is protein digestibility. It involves measuring how protein is susceptible to proteolytic enzymes. A high protein digestibility means after proteolysis with proteolytic enzymes it is able to provide more amino acids for absorption in the gut. Plant storage proteins, especially in cereals and legumes have poor protein digestibility than animal protein. This is attributed to various factors such as protein conformation resulting in low solubility, inhibition of digestive enzymes by protease inhibitors and association of cell components with protein, resulting in lower enzyme accessibility (Becker and Yu, 2013). Several studies have shown a contradiction concerning raw and cooked finger millet flour. In some cases, protein digestibility may be higher or lower after cooking (Ravindran, 1992). This author found that raw finger millet, proso millet, and foxtail millet had *in vitro* protein digestibility of 72.3%, 71.3% and 77.1%, which increased after cooking to 85.5%, 88.6% and 91.6%, respectively.

2.4: Processing techniques that affect finger millet protein digestibility

2.4.1: Malting of finger millet

Malting/germination/sprouting is a three-step process. Frist there is steeping (absorption of water) of the grains, Secondly, the steeped grains are sprouted at controlled temperature and then dried or kilned at a controlled temperature. In developing countries, it is a traditional technique that has been employed over the years. Malting reduces bulk (viscosity) and enhances the overall nutritional quality of formulated food. For example, *in vitro* protein digestibility has been shown to increase after malting finger millet (Hejazi and Orsat, 2016). Through the process of germination, soluble protein compounds are enhanced and the level of lysine increased through the action of hydrolytic enzymes. Mbithi-Mwikya *et al*. (2000) supported the claim and attributed it to breakdown of insoluble storage protein and making them available for pepsin hydrolysis therefore increasing protein digestibility. Saleh *et al*. (2013) studying the effect of duration and temperature on the nutritional enhancement found a direct positive correlation between the nutrition composition and duration of germination. Additionally, with increase in germination time there is also an increase in protein content as noted Swami *et al*. (2013).

During malting, tannin-protein complexes reduce significantly and thus enhances protein bioavailability (Mbithi-Mwikya *et al*., 2000). A recent study showed that after 48 h germination at 30^{o}C, *in vitro* protein digestibility of finger millet grain improved by 17%, while oxalate, phytic acid, and tannins decreased by 29%, 45%, and 46%, respectively (Hejazi and Orsat, 2016). Generally, malting improves protein digestibility and bioavailability. Through the process, starch granules near the cell wall are broken down into simpler compound, exposing the proteinaceous matrices. An inherent hydrolase enzyme acts on the protein converting them into simple peptides and amino acids. Therefore, in formulating of nutritious weaning product with improved protein digestibility malting of finger millet is necessary.

2.4.2: Fermentation of finger millet

Over the centuries, fermentation of cereals to improve nutritional, shelf life and sensory attributes of either thin porridge or thick porridge has been performed (Sajilata *et al*., 2002). A lot of work has carried out to determine how fermentation affects finger millet digestibility. For example, Shobana *et al.* (2013) noted that there were significant reductions in phytate (20%), tannins (52%), during fermentation of finger millet for 24 h using grain micro-flora.

Germination and fermentation of finger millet showed similar results, as there was an enhancement in soluble protein and *in vitro* protein digestibility after 24 h malting followed by fermentation (Mbithi-Mwikya *et al.*, 2000; Saleh *et al.*, 2013). From these reviews, it is obvious that fermentation improves the protein quality, probably through the reduction of phytate-protein complex and tannin-protein complex enhancing protein solubility, through the action of lactic acid (Saleh *et al.*, 2013).

2.5: Cowpea

Cowpea belongs to *Leguminosae* or *Fabaceae* family. It is widely grown in sub-Saharan Africa (Anyango, 2009). It can improve health and prevent various diseases (Ojwang, 2012). It is a dicotyledonous seed that can be oval, kidney or globular shaped (Anyango *et al*., 2011a). Furthermore, it is a heat tolerant crop that can survive arid or semi-arid soil conditions experienced in sub-Saharan-Africa. In Kenya, where there are many low socio-economic subsistence farmers, cowpea could be a suitable alternative to expensive sources of protein such as meat and fish, because of its unique attributes and low agronomical requirement in terms of fertilizer (Ojwang, 2012). As indicated, poor subsistence farmers are more prone to malnutrition because quality proteins from animal sources are rather expensive. Therefore, to improve their nutritional status, less costly, nutritious plant sources such as pulses can be a source of cheap protein.

Cowpea leaves are consumed as a fried vegetable, boiled, fermented or steamed. In West Africa for example, traditional recipes, which employ cowpea paste and soup are *akara* (deep-fried de-hulled cowpea paste), *moin-moin* (steamed cakes), *gbegiri* (cowpea soup) and *kpejigaou* (a griddled cowpea-paste) (Anyango, 2009). In East Africa, young shoots of the cowpea (*kunde*), are often consumed (Ojwang, 2012). The above illustrations underscore the importance of cowpea in many African cuisines in providing essential nutrients and energy. However, among the urban population, they consider cowpea grain as poor man's food, a stigma that undermines the use of this valuable protein, despite its excellent nutrient profile (Table 2).

2.5.1: Chemical composition of cowpea seed as related to protein quality

Nutritional properties and chemical composition of cowpea grain vary depending on environmental condition, genotype, season, agronomic practices and soil fertility. Varietal differences also affect the nutritional balance of cowpea. The major cowpea seed proteins are

the globulins, they contain significant amount of lysine but relatively low amounts of sulphur-containing amino acids (Ojwang, 2012).

Table 2: Nutrient composition and amino acid profile of selected legumes

Nutrients (g/100g)	**Legume**			
	Cowpea	Chickpea	Lentil	Green pea
Crude protein	29.9	21.9	25.4	24.9
Crude fat	2.9	5.4	2.1	1.5
Ash	4.2	3.6	2.8	3.6
Amino acids				
Arginine	7.5	8.3	7.8	7.2
Leucine	7.7	8.7	7.8	7.4
Lysine	7.5	7.2	7.0	8.1
Methionine	2.2	1.1	0.8	1.1
Phenylalanine	7.5	5.5	5.0	5.2
Tryptophan	0.7	0.9	0.7	0.8
Valine	5.0	4.6	5.0	5.0
Histidine	3.1	3.0	2.2	2.4
Isoleucine	4.5	4.8	4.1	4.5
Leucine	7.7	8.7	7.8	7.4
Alanine	4.2	4.97	4.2	5.2
Aspartic acid	10.8	11.0	11.8	11.0
Glutamic acid	17.2	17.3	21.5	17.5
Proline	4.0	3.8	3.5	3.8

Source: Ojwang (2012)

Globulins are approximately from 70% of the total crude protein in the cowpea seed (Anyango, 2009). The major proteins in cowpea cotyledon are albumins and globulins accounting for 45% and 51% of its crude protein composition, respectively (Freitas *et al*., 2004). The globulins are further grouped into two classes, the 11S (legumins) and 7S (vicilin), based on their sedimentation coefficients (Freitas *et al*., 2004). Despite cowpea grain having relatively high protein content, its use has been limited due to the presence of

trypsin inhibitors and indigestible oligosaccharides, such as stachyose and raffinose. Intestinal micro-flora is able to ferment the oligosaccharides resulting in flatulence (Onyenekwe *et al*., 2000).

2.6: Anti-nutrients in finger millet and cowpea grains that affect protein digestibility

As earlier stated, finger millet and cowpea grains contain significant amount of anti-nutritional compounds such as phytates, polyphenols and enzyme inhibitors that adversely affect the absorption of nutrients in the human gut. These anti-nutritional compounds play an integral role in biological functions in plants, however, they bind to proteins and enzymes in case of tannins, thereby reducing protein digestibility.

2.6.1: Polyphenols

Generally, there are three categories of phenolic compounds. These include flavonoids, tannins and phenolic acids (Duodu and Awika, 2018). Derivatives of benzoic or cinammic acids are called phenolic acids; they have hydroxyl and methoxy groups substituted on aromatic ring structure (Siwela, 2009). The same author noted that the phenolic acids can occur as soluble esters, free acids or insoluble esters in outer layers (pericarp) of cereals and legumes. Ferulic acid (3-methoxy-4-hydroxycinnamic acid) is the major bound phenolic acid and is mainly found in the grain endosperm. Flavanoids has two units of C6 fragment from malonyl-coenzyme A and C6-C3 fragment from cinnamic acid as reviewed by Duodu and Awika (2018). In the same review, these authors noted that stalks, leaves, and flowers had anthocyanidins, leucoanthocyanidins, and catechin as major flavonoids. Finger millet, just like sorghum has anthocyanin and anthocyanidin that are responsible for pericarp pigmentation (Siwela, 2009).

Tannins are compounds that are insoluble in nonpolar solvents and because of the polar groups on their structures they readily dissolve in water. They are polymeric phenols of higher molecular weight ranging averagely between 500 and 5000, consisting of hydroxyl groups that allow creation of strong cross-links with hydrophobic amino acids (Siwela, 2009). Since they are able to irreversibly bind to and denature collagen proteins, therefore they have ability to tan leather, hence their name (Duodu *et al*., 2003). There are two categories of tannins distinguished by their action towards hydrolytic agents especially the acids (Duodu and Awika, 2018). Hydrolysable tannins have a polyester structure (phenolic carboxylic acids that are esterified to any sugar), which makes it readily hydrolysed by enzymes or acids into polyhydric alcohols or sugars. On the other hand, procyanidins (condensed tannins), or

proanthocyanidins found in sorghum and millet, in presence of acid do not break, rather they undergo progressive polymerization or condensation to form amorphous tannin reds or phlabaphens (Duodu and Awika, 2018).

Finger millet tannins are located in the pericarp to protect the grain against birds, insects and prevailing high temperatures, which gives it an agronomic advantage (Siwela, 2009). White coloured finger millets have lower phenolic compounds with approximately 0.09 mg GAE/100 mg, while for brown coloured varieties, the average range of 1.09 mg Gallic Acid Equivalent/100 mg (Siwela *et al*., 2007). Chethan and Malleshi (2007) noted a similar observation, they found that polyphenol content was dependent on the colour of pericarp, whereby white coloured finger millet had an approximately 0.3% to 0.5% (GAE), while in brown coloured had approximately 1.2% to 2.3 %. Brown coloured finger millets contained relatively more tannin (0.12-3.47% catechin equivalents) compared to white coloured finger millets (0.04%-0.06% catechin equivalents) (Mbithi-Mwikya *et al.*, 2000).

Condensed tannins are capable of binding and precipitating proteins at least twelve times their own weights at optimal conditions (Duodu *et al*., 2003). This interaction (protein-tannin) is hypothesised to involve non-polar hydrophobic interactions and hydrogen bond according to the same author. Anyango *et al.* (2011a) hypothesised that condensed tannins were able to bind and form complexes with proline-rich protein, forming a hydrophobic interaction. Proline residues not only act as binding sites but they also helped in keeping the protein extended, hence increasing the available surface area for binding. Moreover, concerning lysine, the tannins interact covalently with, a ε-amino group making it less available.

Various researchers have reported a reduction in condensed tannins when cowpeas are soaked and cooked. The loss is due to the formation of water-soluble complexes between the bean tissues molecules, which leach out into cooking liquor or heat degradation of tannin molecules (Ojwang, 2012). Sprouting just like soaking of beans in water has been shown to reduce significantly, the effects of tannins and improve the biological value of protein. The mechanism of reduction as hypothesized by Mbithi-Mwikya *et al*. (2000) is due to leaching of tannin molecules from seed pericarp, or action of hydrolytic enzyme polyphenol oxidase that can hydrolyse condensed tannin. Sprouting or malting also improves amino acid balance of finger millet grains according to the same author.

Low molecular weight phenols, because of their hydroxylation are unable to precipitate proteins as compared to condensed tannins. Apart from molecular weight, protein conformation also determines whether a precipitated protein is digestible or not. Condensed tannin, affect the protein conformation and due to its large size, is able to exert some stearic effect and in the process preventing enzymes from accessing the proteins (Duodu *et al*., 2003).

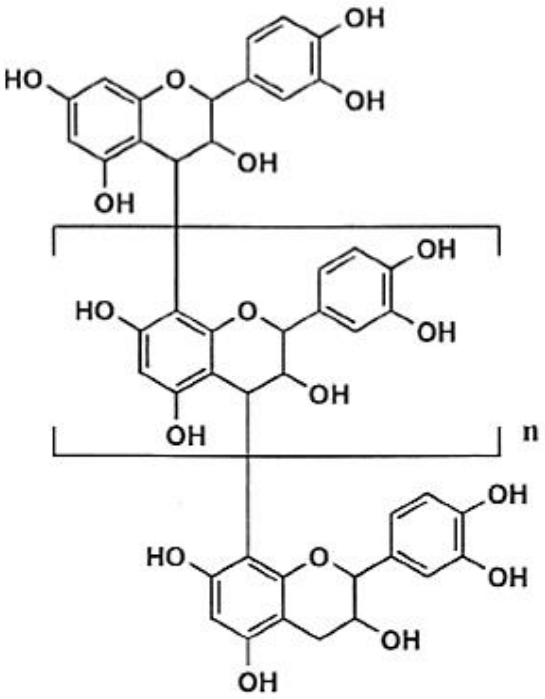


Figure 2: Structure of condensed tannin
Source: Duodu and Awika (2018)

2.6.2: Phytic acid (PA)

Phytic acid or ester of myo-inositol (I) is an organic acid extracted from cereals, legumes, and oilseeds. Phytic acid is the major storage form of phosphorus in grains accounting approximately 75% of the total grain phosphorous (Schlemmer *et al.*, 2009). Metallic cations (K^+, Mg^{2+}, Mn^{2+}, Fe^{2+}, Ca^{2+}, and Zn^{2+}) and proteins are able to bind strongly with highly charged six phosphate groups in PA structure resulting in mixed phytate or phytin. Phytate-to-cation molar ratio, presence of other compounds in the solution, pH, proteins, and individual cation influences the solubility and stability of the protein-phytate or the cation-phytate complexes (Greiner and Konietzny, 2006). It is also the primary storage for inorganic phosphate and inositol ions which are important in energy metabolism during germination of

plants as reviewed by Duodu *et al*. (2003). Malting or sprouting has been hypothesised to significantly reduce the amount of phytates in the plant due to the action of hydrolytic enzyme phytatase (Duodu *et al*., 2003). Phytic acid in finger millet just like sorghum is highly concentrated in the germ while in cowpea it is concentrated within the protein bodies (globulins) of bean embryo and aleurone layers (Schlemmer *et al.,* 2009). Phytic acid has major application in the food industry; it acts as an acidulant for pH adjustment during beverage production.

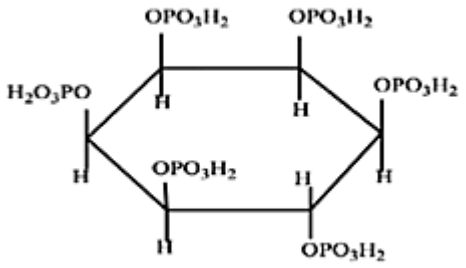


Figure 3: Chemical structure of phytic acid.
Source: Schlemmer *et al.* (2009)

The formation of insoluble complexes results in a reduction of protein and trace minerals bioavailability. Decortication, malting, fermentation, or milling, are some of the processing techniques employed to reduce phytate levels in finger millet.

Different finger millet varieties have different levels of phytic acid content, majorly attributed to variations in an adaptation of these varieties to different prevailing environmental conditions (Shibairo *et al.*, 2014). Research by Sreerama *et al*. (2012) showed that brown coloured cowpea contained averagely 14.0 mg/g phytic acid, while other cultivars had a phytate content of 8.4–9.92 mg/g. Phytic acid contributed 54–59% of the phosphorous in cowpea. Boiling has been reported as a more effective method of phytic acid reduction when compared to steaming according to Giami (2005). This author reported that phytic acid reduced by about 41% when boiled as opposed to 13.5% reduction for steamed cowpea, as more assayable phytates could leach into the cooking liquor.

2.7: Analytical methods for evaluating protein nutrition value

Several factors affect protein nutritional value, therefore it is of great importance to have methods that can determine protein quality. Protein quality estimates are important especially when formulating complementary foods, as it is able to determine required amounts of

essential amino acids needed for growth and development among children (Damodaran, 2008). Protein nutrition value can be determined by several procedures, which include chemical, biological and enzymatic or microbial methods. Each method has its own advantages and short comings (Damodaran, 2008).

2.7.1: Biological methods

Through feeding a protein-containing diet to rats and humans one can derive general knowledge about protein quality of the food. This is the basis of the biological methods of protein quality determination. The use of rats as test animals is preferred as compared to humans because of ethical concerns. However, they are costly and time consuming when determining protein quality and the minimum number of test animals for any statistically meaningful data inferences is nine (Damodaran, 2008). A relatively cheaper and quicker alternative is the use of *in vitro* assays that employ enzymes similar to those found in a gastrointestinal tract of the human with some modifications, to simulate the physiological conditions (Damodaran, 2008). A review by Duodu *et al.* (2003) highlighted characteristics of a good *in vitro* method. The method should be applicable to a varied range of protein samples, simple and accurate.

2.7.2: Enzymatic methods

Several researchers have developed various *in vitro* procedures for determining protein nutrition value and they include both the multiple-enzyme and single enzyme assays. Some researchers have reviewed the use of several multiple enzymes systems in evaluating protein quality such as trypsin-chymotrypsin peptidase, pepsin-trypsin and pepsin-pancreatin (Duodu *et al*., 2003). From this review, the authors recommended the use of multiple-enzyme as compared to a single enzyme, since they could reduce the negative effects of enzyme inhibitors for a single enzyme. Also, when a single enzyme is employed it may not hydrolyse all peptide bonds for proteins having different levels of amino acids, if it is a bond-specific enzyme. However, multiple-enzymes assays more often are expensive, time-consuming and complicated involving numerous times of digestion and washing (Damodaran, 2008). They also employ pH- shift for optimal functioning and are prone to complication if optimal pH for digestion is not achieved (Duodu *et al*., 2003).

Single-enzyme assay (Pepsin digestion)

Single-enzyme methods are simple, rapid and accurate as compared to muitiple-enzymes. They exhibit excellent correlation with *in-vivo* animal models especially with humans

(Anyango, 2009). In this technique, protein digestibility evaluation requires treating the food sample with proteolytic enzymes. Protein digestibility in the sample is determined when there is a change in amino nitrogen or protein solubility of the food sample. First, there is an evaluation of crude protein content in the food, and then sample digested using proteolytic enzymes (pepsin enzyme) under specific set conditions. Thereafter, there is an evaluation of the residual crude protein content in the food and protein digestibility expressed in terms of the original crude protein content (Hamaker *et al*., 1987).

2.7.4: Chemical methods

Amino acids evaluation and its comparison to reference protein can be the quick assessment of the nutritional quality of a protein. Like other cereals, finger millet in comparison with a high animal protein like an egg, is poor source of essential amino acids, as stated elsewhere in previous sections. A food should supply almost all essential amino acids in required amounts for it to be considered of high quality. This is of particular importance for children who have a high requirement for essential amino acids especially lysine for growth and development (Serna-Saldivar and Rooney, 1995).

Essential amino acids are amino acids that human body cannot synthesize. Therefore they must be provided in required levels in the food the human consumes. Each essential amino acid in a food sample has a chemical score. It is a measure of that amino acid in the food as compared the amino acid it reference protein. When all essential amino acids in the test protein are computed, the most limiting essential amino acid is the one with least score. The chemical score of this limiting amino acid provides the chemical score for the test protein (Damodaran, 2008). Chemical score is calculated as follows:

$$\text{Chemical Score} = \frac{\text{mg of amino acid in 1g of test protein}}{\text{mg of same amino acid in 1g of reference protein}} x100 \quad \text{. (Equation 1)}$$

Chemical score facilitates estimation of the amount protein mix or test protein required to meet the daily requirement of the limiting amino acid. Chemical sore method provides several advantages. First, it is simple method to compute when the amounts of essential amino acid in a test protein are known. Secondly, it permits one to determine the complementary effects of proteins in the human diet. Thirdly, it also allows one to develop protein diets of high-quality through compositing different food ingredients. However, to be

used as a quality tool, there should be a correlation with protein digestibility (Damodaran, 2008).

Protein digestibility corrected amino acid score (PDCAAS)

The standard measure of determining how well a protein is digested and absorbed in the human body involves a calculation of its chemical score and *in vitro* protein digestibility commonly referred to as protein digestibility corrected amino acid score (PDCAAS). It is the official method adopted by World Health Organisation in predicting protein nutritional value (WHO/FAO/UNU, 2007).

Protein Digestibility Corrected Amino Acid Score (PDCAAS) is computed as follows:

PDCAAS= protein digestibility (%) × amino acid score. ...…… (Equation 2)

2.8: Analytical methods for determining polyphenol and phytic acid content

Analysing and quantifying phenolic compounds involves several methods. From literature, there are those techniques that analyse total phenolic content and some techniques that target a specific derivative of the phenolic compounds. Folin-Ciocalteu phenol assay as described by Singleton and Rossi (1965) employs quantification of total phenol content in the grains (as total reducing phenolic groups) whereas vanillin-HCl assay as described by Price *et al*. (1978), is for the phenolic compound derivate, specifically catechins. Folin-Ciocalteu phenol assay provides a relatively good and reliable estimate of total reducing phenolic compounds concentration in a test sample. Sample particle size, chemical nature of phenolic compounds, sample particle size and the choice of assay method to be used greatly influence total phenol content assayed results (Naczk and Shahidi, 2004). Principle behind Folin-Ciocalteu phenol assay involves oxidation-reduction reactions during which at basic pH, the phenolate ion is oxidised, while phosphotungstic/phospho-molybdic acid complex is reduced to chromogens (blue coloured solution) (Siwela, 2009). Principle behind vanillin-HCl assay, involves pro-anthocyanidins, as well as leuco-anthocyanidins (catechins), reacting with vanillin reagent in presence of HCl to give a bright red colour as the basis for colorimetric determination (Price *et al*., 1978). Determination of phytic acid content involves phosphorus iron analysis of ferric phytate. The method of analysis can either be direct or indirect determination of residual iron after ferric phytate has precipitated. Trichloroacetic acid and HCl are used for extraction. Phytate is precipitated as ferric salt by using trichloroacetic acid, iron content in the precipitate is then spectrophotometrically determined. Phytate-phosphorous in the precipitate is then calculated using 4 Fe/6 P constant molecular ratios (Makker *et al*., 2007).

2.9: Sensory evaluation

Generally, there are two main types of sensory evaluation methods. First there is an analytical or objective method and second there is a affective or subjective method. Analytical methods include difference, ranking, and quality tests, while hedonic comprises of preference, consumer and market tests (Anyango, 2009). Based on this author, a trained panel is an essential element for any analytical method, and they act as an analytical instrument. This is a stark contrast to hedonic methods, where a large number of untrained individuals are used and their sensory evaluation instinctively influenced. The descriptive sensory analysis involves the identification of food attributes and then quantifying the perception levels using trained human subjects (Anyango, 2009; Meilegard *et al*., 2007). It further provides detailed information on products' sensory attributes. The data obtained can easily be analysed statistically. However, the use of descriptive sensory evaluation analysis in the evaluation of finger millet weaning food in terms of described sensory attributes remains limited in its application.

2.10: Conclusion

Children rely on cereals such as finger millet and other poorly formulated complementary foods as a source of both protein and energy. Finger millet is a nutritious, underutilized crop; however, finger millet has low protein content, low lysine content and intrinsic factors such as condensed tannins affect its protein bioavailability. Locally grown food ingredients such legumes especially cowpea can be utilised to improve finger millet protein content and quality and help in addressing protein-energy malnutrition. Traditional processes, such as malting can improve finger millet protein digestibility by converting insoluble protein into soluble protein and in formulating of nutritious baby-weaning product.

CHAPTER 3: MATERIALS AND METHODS

3.1: Research site

Study was carried out at Guildford Dairy Institute, Department of Dairy and Food Science and Technology, Egerton University. Determinations of protein quality and anti-nutrients were carried out at Kenya Agricultural and Livestock Research Organisation (KALRO) food Laboratory in Njoro, Kenya and Biotechnology Laboratory at Egerton University, Kenya. Amino acid analysis was carried out at BecA-ILRI Mycotoxin and Nutrition Analysis Laboratory, Nairobi, Kenya.

3.2: Materials

Grains used and their sources are presented in Table 3.

Table 3: Grain, variety, description and sources used in research

Grain	Variety	Description	Source
Cowpea	*Kundesoko*	Dual variety, high yielding, with long pods	KALRO Katumani
	Kundefaulu	Brown coloured, with large grains. Has long pods loosely attached to the pedicel.	KALRO Katumani
Finger Millet	U15	Red coloured and high yielding	Egerton University Agro-Science Park
	P224	High yielding but susceptible to blast disease	Egerton University Agro-Science Park
	Snapping green	Easy to harvest and thresh	Egerton University Agro-Science Park
	KNE 741	Super early in maturity and suitable to low altitude	Egerton University Agro-Science Park
	KNE 629	Adaptable to high altitude and blast-resistant	Egerton University Agro-Science Park

3.3: Preparation of malted finger millet flour

Malting of the finger millet grain was done with modification as described by Mbithi-Mwikya *et al*. (2000). Finger millet grains (1 kg) were cleaned three times using tap water

and steeped in 2-L of water at room temperature for 24 h. Water was changed after every 6 h during steeping (maintain good air circulation). After steeping, the grains were then germinated in a forced draft oven at an ambient temperature of 25°C. Tap water was sprinkled regularly onto the grains and mixed to ensure uniform malting. After 48 h, the grains were dried in an oven at 50°C for 24 h, to a moisture content of 10%, and then milled using a microphyte lab disintegrator model Fz102 (Tianjin, China), fitted with 500-µm sieve to give malted finger millet raw flour. Control (unmalted finger millet was also milled using microphyte lab disintegrator model Fz102 to give unmalted finger millet raw flour. The flours were then stored at 10°C until further analyses.

3.4: Preparation of precooked cowpea flour

Dry grains (1 kg) were cleaned by removing foreign matter, broken seeds and immature seeds. The grains were then washed using tap water and placed in a metallic pot. Tap water was then added until the grains were all submerged. They were cooked in boiling water (93°C) for approximately 1 h and then dried in an oven set at 50°C to a moisture content of 10%. Dried grains were then milled using a microphyte lab disintegrator model Fz102 (Tianjin, China), to give precooked cowpea flour (Figure 4). The flours were then stored at 10°C until further analyses.

3.5: Preparation of composite flours

Malted finger millet and precooked cowpea flours were composited at 10.34%, 21.26%, and 32.75% with precooked cowpea (Table 4) to reflect 9-13 g protein requirements per day for infants 1 to 3 years old as outlined by WHO/FAO/UNU (2007). Sample with 0% cowpea (100% unmalted and malted finger millet) were used as controls as presented in Figure 4. The ratios were composited as follows (Table 4),

Table 4: Compositing ratios for different complementary flours

Finger millet flour (malted/unmalted) (g)	Precooked cowpea flour (g)	Total(g)
89.66	10.34	100
78.74	21.26	100
67.25	32.75	100
100	0	100

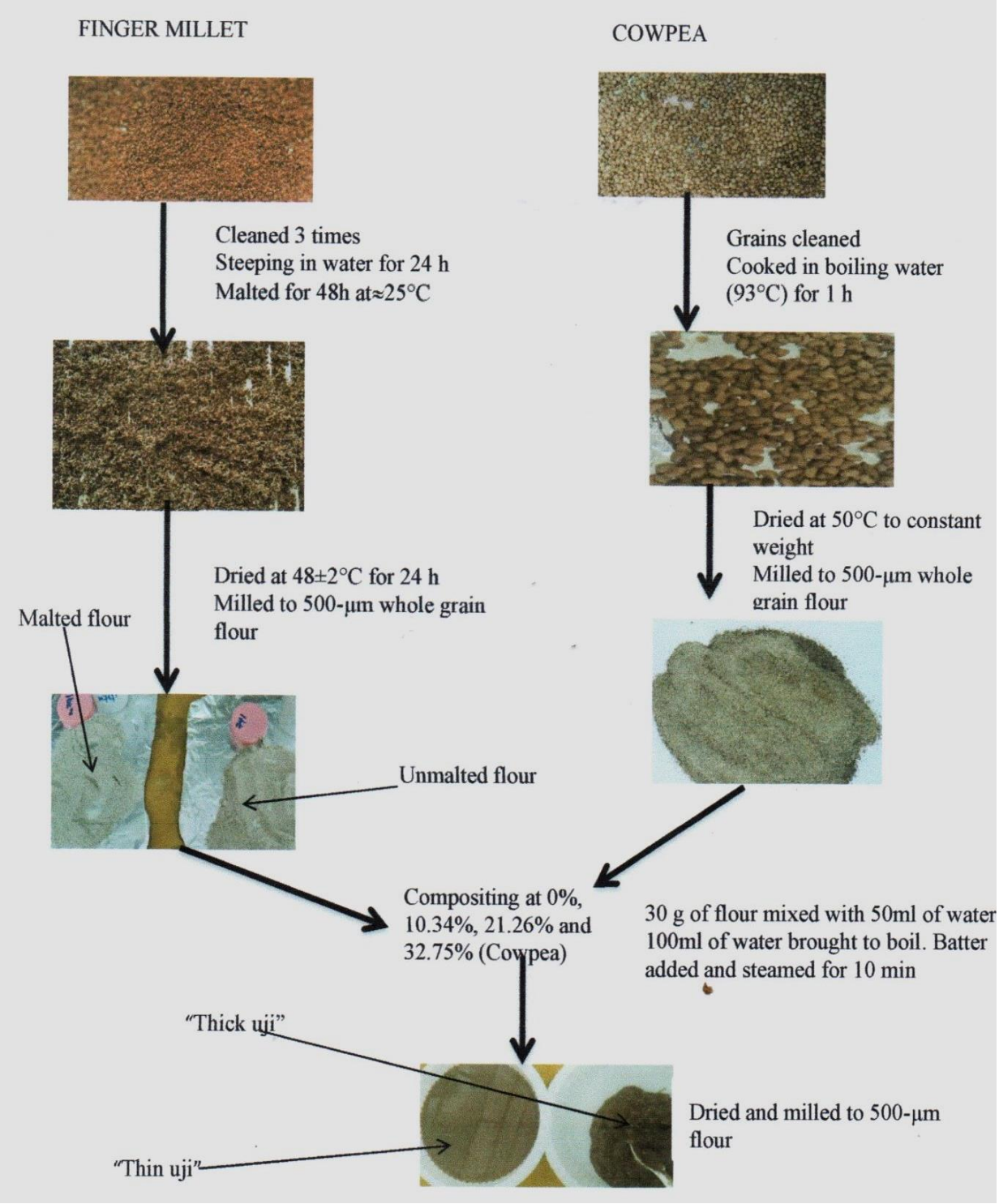


Figure 4: Flow diagram for preparation flour and complementary porridge

3.6 Preparation of porridges and their dried flours

Composite flour (30 g) was mixed with 50-mL cold water to form a thick batter. Water (100-mL) in a metallic pot covered with a lid, was brought to boil and then the batter added with continuous stirring using a cooking stick until the mixture formed a viscous gruel. The

porridge was steamed on low heat for 10 min and then cooled in shallow plates. Compositing unmalted finger millet (control) with precooked cowpea resulting in thicker *uji* as compared to *uji* made from compositing malted finger millet with cowpea. The porridges were then dried in a forced draft oven at 60°C to a moisture content of 10%, then milled using a microphyte lab disintegrator model Fz102 (Tianjin, China), to give porridge flours. The flours were then stored at 10°C until further analyses

3.7: Analyses

3.7.1: Determination of moisture content

Oven-drying method was used in determination of moisture content, according to AACC International (2000), Method 44-15A. Samples were exposed to a single-stage air oven drying set at 105°C for 3 h. Moisture content in the samples was then calculated as the loss in weight and expressed as a percentage of the original weight of a sample.

3.7.2: Determination of protein content

Determination of protein content in the samples involved use of Kjeldahl procedure (AACC International 2000), Method 46-10.01 to obtain percentage nitrogen then converted using a conversion factor of 6.25 to get crude protein. Ground sample (0.3 g), was weighed and transferred into a clean well-labelled digestion tube. Digestion mixture (4-mL) containing selenium powder and concentrated sulphuric acid (2.8 g/800-mL), was carefully added to the samples. Samples were digested using a DKL Heating Digester (Velp Scientifica, Usmate, Italy), for 2 h at 330°C. Thereafter, distilled water (25-mL) and 25-mL 40% sodium hydroxide added, after cooling the samples. The samples were then distilled using a Foss Kjeltec system 1002 distillation unit (Foss Analytical, Hoganas, Sweden). The distillate was collected in a conical flask containing 25-mL of boric acid mixed with 3 drops of mixed indicator (bromocresol green and methyl red) and then titrated against 0.1N HCl until pale pink. A blank sample (digestion mixture) was also run.

Calculation of crude protein was as follows:

$$\% \text{ Nitrogen} = \frac{(T - B) \times N \times 14.007 \times 100}{\text{Weight of the sample in grams}} \quad \text{..... (Equation 3)}$$

$$\text{crude protein} = 6.25 \times \% \text{ Nitrogen}$$

where: T is sample titre, B is blank titre, N- Normality of HCl.

3.7.3: Determination of *in vitro* protein digestibility (IVPD)

Determination of *in vitro* protein digestibility involved used of modified form of single-enzyme assay (pepsin) as described by Hamaker *et al*. (1987) and modified by Anyango *et al*. (2011a). Sample (300 mg), was weighed into a 50-mL centrifuge tube. Exactly 15-mL of 0.1NHCl solution containing 0.02 g/100 g sodium azide and 1.5 mg pepsin from porcine gastric mucosa powder (Sigma P7000-100G; activity ≥250 units/mg solid, Sigma-Aldrich Co, USA) was added. The contents were incubated in a shaking water bath (Model GFL-1083 Gesellschaft, Labortechnikmbh Burgwedel, Germany) maintained at 37ºC for 3 h. Samples were then centrifuged using Eppendorf centrifuge (Model 5804, Eppendorf, Hamburg, Germany) at 2060 ×*g* for 20 min at room temperature and the supernatant was discarded. The pellet (residual) was dried in a forced draft oven at 100ºC for 5 h and nitrogen content determined using the Kjeldahl method according to the AACC International (2000), Method 46-10.01. Protein digestibility of the sample was determined by getting the difference between crude protein in the initial sample and the residual crude protein after single-enzyme digestion (pepsin) divided by crude protein in the initial sample and results expressed as a percentage.

3.7.4: Amino acid analysis

Performic acid oxidation-acid hydrolysis was used to extract amino acid from the test samples (Bidlingmeyer *et al*., 1984). Performic acid was used to oxidise sulphur-containing amino acids cysteine and methionine into cysteic acid and methionine sulphone, respectively. Sodium metabisulphite was added to decompose excess performic acid. Amino acids were liberated from proteins using 6 N HCl. Amino acid hydrosylates were then analysed by pre-column derivatization with O-phthalaldehyde (OPA) and, separated on a reverse-phase Ultra Performance Liquid Chromatography (UPLC) with fluorescence detection. The concentrations of amino acids in the test solution were determined by relating the peak area of the sample to respective individual calibration curves.

3.7.5: Protein Digestibility Corrected Amino Acid Score (PDCAAS)

Protein digestibility corrected amino acid score was determined using *in vitro* protein digestibility, lysine content and lysine requirement pattern of 5.2 g lysine per 100 g protein for children 1 - 3 years old, and calculated based on Equation 2.

3.7.6: Determination of total phenolic content

Modified Folin-Ciocalteu method (Singleton and Rosi 1965) as modified by Siwela *et al*. (2007) was used in total phenol content determination. Flour (300 mg), was extracted with 30-mL of acidified methanol for 1 h at room temperature and then centrifuged at 2060 ×*g* for 20 min using Eppendorf centrifuge (Model 5804, Eppendorf, Hamburg, Germany), decanted and two replicate supernatants obtained. Sample extracts (1-mL) were mixed with 5-mL Folin-Ciocalteu phenol reagent in 50-mL centrifuge tube containing 10-mL distilled water, 7.5-mL 20% (w/v). Sodium carbonate was then added within 8 min after addition of the Folin-Ciocalteu reagent. The contents were then made up to volume with distilled water, stoppered and then thoroughly mixed. Tubes were left to stand at room temperature for 2 h and absorbance read at 760 nm using a UV/VIS Spectrophotometer (model PharmaspecUV-1700, Shimadzu, Japan). Sample blank was included in which distilled water replaced the sample. Catechin was used as standard. Total phenol expressed as mg catechin equivalent per 100 mg sample.

3.7.7: Determination of condensed tannin content

Modified vanillin-HCl in methanol method was used in determining condensed tannins as described by Price *et al*. (1978). Ground samples (0.25 g) were weighed into a 100-mL conical flask, and then 10-mL 4% HCl in methanol (v/v) was added and the content shaken for 20 min using Ratek Orbital Incubator (Boronia, Victoria, Australia). Samples were centrifuged at 2060 ×*g* for 20 min at room temperature using Eppendorf centrifuge (Model 5804, Eppendorf, Hamburg, Germany). Sample extracts (1-mL) were then mixed with 5-mL of the vanillin-HCl reagent in a clean test tube. The specific reagent (vanillin-HCl) for the determination was prepared just before use by mixing equal volumes of 1% vanillin in methanol (w/v) and 8% conc. HCl in methanol (v/v). Absorbance was read at 500 nm using UV/VIS Spectrophotometer (model Pharmaspec UV-1700 Shimadzu, Japan) exactly after 20 min. Sample blanks in which 4% HCl in methanol replaced vanillin reagent were included. For zero setting of the colorimeter, 1-mL of a blank (1% HCl in methanol) was used. Catechin was used as standard. Condensed tannin was expressed as mg catechin equivalent per 100 mg sample.

3.7.8: Determination of phytic acid content

Phytic acid analysis was based on precipitation of phytate, as described by Makkar *et al*. (2007) with some modifications. Sample (500 mg) was accurately weighed and phytate

extracted using 50-mL of 3% trichloroacetic acid (TCA) by shaking on Ratek Orbital Incubator (Boronia, Victoria, Australia) for 40 min. The suspension was then centrifuged at (3000 ×*g*, 10 min) using Eppendorf centrifuge (Model 5804, Eppendorf, and Hamburg, Germany), a 10-mL aliquot of the supernatant was transferred to a 50-mL centrifuge tube and 4-mL of $FeCl_3$ solution added rapidly. The contents in the tubes were then heated in boiling water for 45 min, then centrifuged at 3000×*g* for 10 min using Eppendorf centrifuge (Model 5804, Eppendorf, Hamburg, Germany) and the clear supernatant decanted. The precipitate was then washed twice by dispersing in 25-mL 3% TCA, and then heated in boiling water for 10 min, then centrifuged at (3000 ×*g*, 10 min) using Eppendorf centrifuge (Model 5804, Eppendorf, Hamburg, Germany), and washed again with 20-mL distilled water. The precipitate was then dispersed in 5-mL of distilled water and 3-mL 1.5N NaOH added, then topped up to 30-mL with distilled water and heated for 30 min in boiling water. The contents were then filtered using Whatman No. 2 filter paper with a pore size of 8 µm and then washed with 70-mL hot distilled water. The precipitate was transferred and dissolved into the 100-mL volumetric flask containing 40-mL hot 3.2N HNO_3. The filter paper was washed using distilled water, and the washings collected in the one flask. The flask was cooled to room temperature and the volume made to 100-mL with distilled water. An aliquot (5-mL) was transferred to another 100-mL volumetric flask and mixed with 65-mL distilled water, 20-mL 1.5M potassium thiocyanate (KSCN) then added. The volume made to 100 mL with distilled water, and absorbance read at 480 nm using a spectrophotometer (model pharmaspec UV-1700 Shimadzu, Japan) within 1 min. Reagent blank in which distilled water replaced the sample was included. A calibration curve was made from iron (III) nitrate solution stock solution. Iron (in micrograms), present in test solution was determined from the calibration curve and phytate P calculated as follows,

Phytate P mg/100 g sample = [Fe (µg) × 15]/Weight of sample (g)…….... (Equation 4)

3.7.9: Preparation of porridge for descriptive sensory evaluation

Composite flour (100 g) was mixed with 200-mL cold water to form a thick batter. Water (350-mL), was brought to boil and then the batter added with continuous stirring using a cooking stick until the mixture formed a viscous gruel. The porridge was steamed on low heat for 10 min. The porridge was then placed in well-labelled jars, and then maintained at 50º C in a water bath.

3.8.1: Recruitment and screening of the panel

Students and academic staff of Egerton University, Department of Dairy and Food Science and Technology, who were willing to consume finger millet porridge, and had some experience of descriptive sensory evaluation and did not suffer from any food allergies, were invited through telephone, emails, and notices to apply to participate in descriptive sensory analysis. Twenty individuals responded, signed consent form (Appendix 6) and attended an introduction session. Introduction involved familiarisation with food ingredients used in porridge formulation. Ten persons had some experience in sensory evaluation, nine confirmed their availability. Screening for sensory acuity of the ten panellists took place, using standard screening method which involved testing their ability to differentiate different sensory taste (sweet, bitter, salty, umami and sour) and lexicon identification that describe aftertaste, taste, aroma and flavour of different finger millet complementary porridges. Nine previously trained and one recruit constituted the final selected panel of judges, which comprised of six female and four males.

3.8.2: Training of the panel of judges

The panel was trained for 4 days, in 2 h sessions per day and was according to generic descriptive method described by Meilgaard *et al.* (2007). During the training, porridge was repeatedly described to ensure consistency among the panellists. For purposes of descriptive sensory evaluation, lexicons (descriptors) and scale anchors for the descriptors were developed by the panel, defined and agreed on.

3.8.3: Descriptive sensory evaluation of Porridge

Cooked porridge (20-mL portions) was served in 50-mL glass tumblers kept warm in a water bath at 50°C. Descriptive sensory evaluation of the complementary porridge was carried out in a Sensory Evaluation Room with individual booths in the Food Chemistry laboratory situated at the Department of Dairy and Food Science and Technology, Egerton University. Panellists evaluated all samples in duplicate during the final day of sensory evaluation. Each panellist received six samples of porridge in glass tumblers and serviette. For purposes of rinsing the mouth before and between tasting of the porridge each panellist was given filtered tap water in plastic disposable cup with two plastic tablespoons. Three samples were first tasted and after a 30 min break, the other three samples kept warm in a water bath at 50°C, were tasted to avoid fatigue among the panellists. The samples were coded with three Arabic numerals and order of presentation randomised for each panel. Seventeen descriptive terms

were used by the panellists, grouped under flavour, texture, aroma and sensation after swallowing the sample (aftertaste) attributes as represented in Table 5. The first to be evaluated was aroma using short sniffs. Thereafter, texture and flavour attributes were analysed by chewing a spoon full of porridge. Aftertaste sensory properties were analysed by the panellists after swallowing the porridge. Lexicons identified and developed by the panellists are described in Table 5.

Table 5: Terms used by descriptive sensory panel to describe the sensory attributes of the complementary porridge

Descriptor`	Definition	Reference	Rating scale
Aroma			
Overall aroma intensity	Intensity of the aroma of porridge		1-Less intense 7- Very intense
Malty porridge aroma	Intensity of aroma associated with malted finger millet	7 Aroma of malted finger millet after 2 days of malting Snapping variety	1-Less intense 7 strong malty aroma
Cooked cowpea aroma	Intensity of aroma associated with cooked cowpea	7-Aroma of boiled whole cowpea *kundefaulu* variety (Boiled for 60 min)	1-Low cooked cowpea aroma 7 strong cooked cowpea aroma
Finger millet aroma	Intensity of aroma associated with millet	7-Aroma of finger millet porridge with 25% solids	1-Less intense 7-Very intense
Texture			
Coarseness	Extend to which grittiness or granules of porridge caused by small particles		1-Less coarse 7-Very coarse
Viscosity	Force required to draw a liquid from a spoon over the tongue	7-Finger millet porridge with 25% solids	1-Thin 7-Thick
Stickiness	Ability of porridge to stick on the wall of glass		1-Less sticky 7-Too sticky
Flavour			
Overall Flavour intensity	Overall flavour intensity of the porridge		1-Less intense 7- Very intense

Table 5: Continued….

Descriptor`	Definition	Reference	Rating scale
Flavour			
Cooked cowpea flavour	Intensity of the flavour of cooked cowpea	7-Flavour of boiled whole cowpea (Boiled for 60 min)	1-Less intense 7-Strong intense
Millet flavour	Intensity of flavour of cooked raw finger millet	7-Flavour finger millet porridge with 25% solids	1-Less intense 7-Very intense
Malty flavour	Intensity of flavour of cooked malted finger millet	7-Flavour of malted finger millet after 2 days of malting Snapping variety	1-Less intense 7-Very intense
Burnt flavour	Intensity of flavour of porridge associated with burnt porridge residues	7-Flavour malted finger porridge having 25% solids burnt preparations	1-Not intense 7-Very intense
Aftertaste			
Malty aftertaste	Intensity of cooked malted finger millet porridge flavour perceived in the mouth after swallowing	7-Aftertaste of malted finger millet after 2 days of malting Snapping variety	1-Less intense 7-Very intense
Cowpea aftertaste	Intensity of cooked cowpea flavour perceived in the mouth after swallowing	7-Aftertaste boiled whole cowpea (Boiled for 60 min in excess water)	1-Less intense 7-Very intense
Millet after taste	Intensity of aftertaste associated with cooked millet porridge perceived after swallowing porridge	7-Aftertaste of finger millet porridge with 25% solids	1-Less intense 7-Very intense
Astringency	Dry feeling in the mouth after swallowing		1-Less intense 7-Very intense
Presence of residue	Leaves particles of the grain in mouth and teeth		1-Low 7-High

To measure the strength of each of the sensory attribute of the porridge, a seven-point line scale was used. One was the minimum point denoted less intense, thin and less coarse. While seven the maximum value denoted very intense, thick and strong flavour.

3.9: Experimental design and statistical analysis

This experiment employed a completely randomized design (CRD) in a 2 × 2 × 4 factorial arrangement. The first factor was two finger millet genotypes Snapping green and KNE741 after screening process. The two cowpea varieties (*kundesoko* and *kundfaulu*) represented the second factor, while 0%, 10.34%, 21.26% and 32.75% level of compositing represented the third factor.

The statistical model:

$$\mathbf{Y_{ikjlm} = \mu + \alpha_i + \beta_j + \gamma_k + \alpha\beta_{ij} + \alpha\gamma_{ik} + \beta\gamma_{jk} + \alpha\beta\gamma_{ijk} + R_l + \varepsilon_{ijklm}}$$

Where Y_{ijkm} is the observation on the response variable; μ is the overall mean; α_i is the effect due to the i^{th} finger millet variety; β_j is the effect due to j^{th} cowpea variety; γ_k is the effect due to k^{th} level of flour compositing; $\alpha\beta_{ij,}$ is the interaction effect between i^{th} finger millet variety and jth cowpea variety, $\alpha\gamma_{ik}$ is the interaction effect between i^{th} finger millet variety and k^{th} level of flour compositing, $\beta\gamma_{jk}$ is the interaction effect between jth cowpea variety and k^{th} level of flour compositing, $\alpha\beta\gamma_{ijk}$ is the interaction effect between i^{th} finger millet, jth cowpea variety and k^{th} level of flour compositing; R_l is the effect due to l^{th} replication and ε_{ijkm} is the random error associated with Y_{ijkm}.

Effects of malting finger millet, compositing and cooking on the protein quality, anti-nutrient content, and sensory properties of the complementary porridge were analysed using two-way analysis of variance (ANOVA) at $p< 0.05$. Significant differences between means were determined using Tukey's honestly significant difference (HSD). Calculations were performed using Statistical Analysis System (SAS Institute Inc, 2006) version 9.3 based on 5% level of significance. Furthermore, to study sample relationships, principal component analysis (PCA) was performed.

CHAPTER 4: RESULTS AND DISCUSSION

To establish the effect of compositing improved malted finger millet with precooked cowpea with on protein quality and anti-nutrients content of complementary porridge, several parameters were determined. They included protein content, *in vitro* protein digestibility, total phenols, condensed tannins, and phytic acid. The anti-nutrients bind with finger millet protein thereby reducing content and protein quality (Serna-saldivar and Rooney, 1995). Moreover, various studies have shown that cooking has a positive effect on the protein quality of legumes (Anyango *et al*., 2011a).

4.1: Effect of compositing improved malted finger millet flour with precooked cowpea on the protein content of flour and complementary porridge

Effects of malting, compositing and cooking of improved finger millet flour with precooked cowpea on protein content of flour and complementary porridge were analysed. Malting resulted in approximately 6% and 29% increase in protein content after malting Snapping green and KNE741 respectively. There was an approximately 22% increase in protein content when improved malted finger were composited with the two precooked cowpea varieties. Cooking had an effect on protein content of complementary porridge composited using improved malted finger millet

4.1.1: Effect of malting improved finger millet on protein content of flour

Protein content of finger millet grains varied from 6% to 11%, with an average of 8.5% (Table 6). Before malting, Snapping green had significantly high protein content ($p<0.05$), of 10.87%, followed by KNE741 with 10.13%, showing a varietal influence in the difference in nutritional composition of finger millet. This is in line with results reported by Shibairo *et al*. (2014), which showed that high yielding varieties had low protein content as compared to low yielding ones. Also, part of the difference is attributed to the effect of the environment in different finger millet varieties. Since all varieties were grown in a low altitude region (plants were stressed) because of low nitrogen content characterising these regions. Snapping green variety was probably able to accumulate more heat-stable proteins because of low photosynthesis (Bavec and Bavec 2007) as compared to other finger millet varieties. After malting, KNE741 had the highest protein content of 13.09% and the lowest was P224 at 7.29% (Table 6). Malting resulted in between 4 and 30% increase in protein content (Table 6), with KNE741 showing the highest increase of the five finger millet varieties after malting. The results were in agreement with what has been reported in literature (Mbithi-Mwikya *et*

al., 2000; Singh and Raghuvanshi, 2012; Hejazi and Orst, 2016). Mbithi-Mwikya *et al.* (2000) for example, reported an approximately 30% increase in protein content after malting finger millet for 96 h at room temperature. Swami *et al*. (2013) showed after malting finger millet grains for 24 h there was a linear increase in protein content of approximately 25%. In the present study, after malting, there was a positive relationship with protein content. The chemical reactions coupled with physical changes that probably caused the observed increase in protein content availability can strongly be attributed to plant amylolytic activity on the morphology of the finger millet seed. This is because a significant portion of the finger millet kernel is comprised of the endosperm, with three distinct parts corneous, peripheral and floury endosperm and aleurone layer (McDonough *et al.*, 2000). Unlike other proteins prolamins (eleusinin), are found within protein bodies. Endosperm consists of starch granules that are embedded in a protein matrix. During malting, there is an enhancement of hydrolytic enzymes, which results in starch granules breakdown in endosperm by amylase enzyme releasing the packed protein in the protein bodies found in the endosperm increasing protein content. Snapping green (high protein and low tannin content) and KNE741 (high protein and high tannin content) in Table 6, were chosen for subsequent process in compositing with precooked cowpea flour.

Table 6: Effects of malting on protein content and anti-nutrient content of finger millet flour

Finger Millet	Malting	Protein content (g/100g,(db)	Condensed tannins as mg CE /100 mg,(db)
U15	Unmalted(Control)	6.77^{h}±0.18	0.82^{c}±0.05
	Malted	7.29^{g}±0.18	0.48^{f}±0.05
P224	Unmalted(Control)	6.50^{h}±0.38	0.72^{d}±0.01
	Malted	7.29^{g}±0.17	0.38^{g}±0.01
Snapping green	Unmalted(Control)	10.87^{c}±0.18	0.61^{e}±0.05
	Malted	11.62^{b}±0.21	0.37^{g}±0.02
KNE629	Unmalted(Control)	8.41^{f}±0.35	1.17^{b}±0.06
	Malted	9.70^{e}±0.17	0.53^{f}±0.04
KNE741	Unmalted (Control)	10.13^{d}±0.18	1.27^{a}±0.02
	Malted	13.09^{a}±0.18	0.64^{e}±0.01

Values of a parameter in a column, followed by different superscript letters are significantly different at $p \leq 0.05$ n=3. Values are means ±standard deviations.

4.1.2: Effect of compositing improved malted finger millet with precooked cowpea on protein content of flour and complementary porridge

There was a significant difference in protein content ($p<0.05$) when malted finger millet flour was composited with precooked cowpea flour (Table 7). It increased protein content by between 6% and 39%. As expected, compositing at 32.75% showed the highest amount of protein content in both finger millet varieties. After compositing malted KNE741 (Table 7) with 32.75% precooked *kundefaulu* cowpea it resulted in approximately 25% increase in protein content (Table 7). Malted Snapping green with 32.75% precooked *kundefaulu* had the highest increase at 39%. Addition of protein content from the two cowpea varieties, precooked *kundesoko* and precooked *kundefaulu,* had no significant difference in the composite flours and porridges as it resulted in almost same increase in protein content (Table 7). The two-cowpea varieties contain significantly high amount of protein content as compared to finger millet, which resulted in a relatively increase in protein content of the raw composite flours. The results are similar to Pelembe *et al.* (2002) and Anyango *et al*. (2011b) who noted a linear increase in protein content of flours composited with cowpea, although, these authors worked with sorghum and not millet flour in compositing with cowpea.

4.1.3: Effect of cooking improved malted finger millet composited with precooked cowpea on protein content of flour and complementary porridge

Protein content of raw malted flours increased by approximately 2.3% after it was cooked into porridge flours (Table 7). This appears counter-intuitive as nitrogen cannot be destroyed nor be created under the cooking conditions employed in the current study. Since the results were on dry matter basis, the increment could probably be attributed to possible source of errors such as over titration. Porridge flours formulated with precooked cowpea at 21.75% and 32.75% showed a greater increase in protein content with cooking as compared to 0% and 10.34% (Table 7). The increase was probably due to the addition of more digestible proteins (globulins) in precooked cowpea, as hypothesised by Anyango *et al.* (2011a).

Protein requirements for children 1 to 3 years old according to WHO/FAO/UNU (2007) vary between 9 and 13 g protein per day. Also, based on the same report children between 1-3 years of age averagely weigh between 10-15 kg (WHO/FAO/UNU, 2007), therefore a complementary food with (21.26% and 32.75%) precooked cowpea, with a serving size of about 50 g complementary food(db) could therefore, provide averagely 80% of the required protein.

Table 7: Effects of compositing improved malted finger millet with precooked cowpea on protein content and *in vitro* protein digestibility (IVPD) of flour and complementary porridge

		Protein content (g/100 g,db)				*In vitro* Protein digestibility (%)			
		Flours		Porridge		Flours		Porridge	
Millet variety	Composite flours	Unmalted +Cowpea	Malted+ Cowpea	Unmalted +Cowpea	Malted+ Cowpea	Unmalted +Cowpea	Malted+ Cowpea	Unmalted +Cowpea	Malted+ Cowpea
Snapping	0%(Control)	10.87^{k}±0.18	11.29^{j}±0.36	11.06^{i}±0.19	11.60^{k}±0.36	72.28^{f}±4.14	79.06^{h}±0.00	73.40^{e}±2.09	79.81^{g}±3.81
	10.34%PKS	11.50^{i}±0.18	13.31^{h}±0.18	11.59^{g}±0.69	13.64^{i}±0.00	75.11^{d}±1.96	85.66^{d}±1.69	75.60^{d}±0.00	85.88^{d}±1.66
	21.26% PKS	12.78^{g}±0.18	14.23^{f}±0.36	12.79^{e}±0.37	14.23^{g}±0.48	77.60^{b}±1.76	84.50^{d}±1.57	77.41^{c}±1.77	85.60^{d}±3.13
	32.75% PKS	13.54^{e}±0.37	15.08^{e}±0.36	13.89^{c}±0.31	15.60^{c}±0.49	78.66^{a}±1.68	86.42^{cd}±0.00	78.66^{b}±0.00	85.47^{de}±1.47
	10.34%PKF	11.18^{j}±0.49	12.97^{i}±0.18	11.39^{h}±0.18	13.50^{i}±0.48	77.37^{b}±1.39	81.78^{f}±0.00	77.13^{bc}±0.00	81.70^{f}±1.44
	21.26% PKF	13.09^{f}±0.37	14.31^{f}±0.18	13.10^{d}±0.18	14.65^{f}±0.55	78.13^{ab}±1.72	85.82^{cd}±0.00	80.31^{a}±0.00	86.02^{cd}±0.00
	32.75% PKF	14.39^{c}±0.67	15.66^{c}±0.36	14.45^{b}±0.18	15.71^{c}±0.65	78.81^{a}±0.00	84.05^{e}±1.41	79.86^{a}±0.00	84.54^{e}±0.00
KNE741	0%(Control)	10.13^{l}±0.18	13.09^{i}±0.18	10.31^{j}±0.19	13.17^{j}±0.48	73.28^{e}±0.00	86.64^{c}±0.00	79.58^{ab}±0.00	86.96^{c}±3.35
	10.34%PKS	11.82^{h}±0.32	13.92^{g}±0.18	11.89^{f}±0.18	14.07^{h}±0.31	74.67^{d}±3.77	85.28^{de}±3.2	77.03^{c}±1.80	86.81^{cd}±1.55
	21.26% PKS	13.81^{d}±0.36	15.29^{d}±0.55	13.92^{c}±0.18	15.57^{c}±0.56	76.00^{cd}±1.89	86.69^{c}±1.46	77.36^{c}±1.60	90.72^{a}±0.00
	32.75% PKS	14.90^{a}±0.49	16.50^{a}±0.18	15.00^{a}±0.18	17.59^{a}±0.19	76.52^{bc}±1.51	86.75^{bc}±1.34	77.73^{bc}±0.00	87.92^{bc}±0.00
	10.34%PKF	12.66^{g}±0.18	14.26^{f}±0.37	13.78^{c}±0.19	14.95^{e}±0.19	75.11^{d}±1.76	85.50^{d}±0.00	79.84^{a}±0.00	85.91^{d}±3.06
	21.26% PKF	13.43^{e}±0.18	15.11^{e}±0.32	12.92^{e}±0.19	15.28^{d}±0.32	76.04^{cd}±0.00	88.42^{a}±0.00	75.75^{d}±0.00	88.66^{b}±0.00
	32.75% PKF	14.68^{b}±0.18	16.34^{b}±0.18	14.90^{a}±0.18	16.39^{b}±0.54	76.17^{c}±1.53	88.40^{a}±1.36	77.59^{c}±0.00	83.81^{e}±0.00

Values of a parameter in a column, followed by different superscript letters are significantly different at $p \leq 0.05$, n=3, PKS- Precooked *Kundesoko*, PKF- Precooked *Kundefaulu*. Values are means ±standard deviations.

4.2: Effect of compositing improved malted finger millet flour with precooked cowpea on *in vitro* protein digestibility (IVPD) of flour and complementary porridge

Effects of malting, compositing and cooking of improved finger millet flour with precooked cowpea on *in vitro* protein digestibility of flour and complementary porridge were investigated. Malting resulted in approximately 10% and 18% increase in IVPD after malting Snapping green and KNE741 finger millet varieties respectively. There was an approximately 4% increase in *in vitro* protein digestibility when improved malted finger varieties were composited with the two precooked cowpea varieties. Cooking resulted in a 1% increase in *in vitro* protein digestibility of complementary porridge composited using improved malted finger millet after cooking respective raw flours.

4.2.1: Effect of malting improved finger millet on IVPD of flour and complementary porridge

KNE741 (0%) showed a higher IVPD value 73.28% as compared to Snapping green (0%) at 72.28% (Table 6). A relatively low IVPD value was recorded for the controls ranging between 69% and 73% (Table 7). This was in agreement with Omary *et al*. (2012) who reported approximately 63% IVPD in native finger millet grains (unmalted). Malting resulted in approximately between 10% and 18% increase in IVPD (Table 7) with malted KNE741 showing the highest increase of 18.23%. The increase in IVPD after malting has been reported in the literature (Singh and Raghuvanshi, 2012; Hejazi and Orsat 2016). During the malting process, after steeping, complex enzymatic reactions break down macromolecules into simpler units that are more digestible (Hejazi and Orsat, 2016). Mbithi-Mwikya *et al*. (2000) reported that there is also partial proteolysis where proteins are broken down into smaller chain peptides that are more digestible, resulting in an improved IVPD.

4.2.2: Effect of compositing improved malted finger millet with precooked cowpea on IVPD of flour and complementary porridge

The different precooked cowpea ratios had a significant effect on IVPD of the composite flours, as there was an overall increase in IVPD in the complementary flours (Table 7). Compositing resulted in approximately 5% and 8% increase in IVPD for the unmalted KNE741 and unmalted Snapping green complementary flours respectively (Table 7). Moreover, compositing the improved malted finger with precooked cowpea resulted in approximately 4% increase in IVPD. The apparent improvement in IVPD after compositing

with precooked cowpea can be attributed to increase in more digestible globulin proteins, inactivation of trypsin inhibitors through thermal denaturation of cowpea proteins coupled with a proportionate decrease in less digestible finger millet prolamins. Cowpeas are rich in globulins (Anyango *et al*., 2011a) which are more digestible as compared to finger millet eleusinin (prolamins), and they become more digestible after heat treatment probably due to hydrogen bonds cleavage, which exposes more sites for a proteolytic attack. This is because enzymes function optimally in an aqueous environment and prolamins (eleusinin) are relatively hydrophobic proteins and are believed to be less susceptible to an attack from preotelytic enzymes as compared to globulins proteins found in cowpea. In addition, low digestibility of eleusinin proteins was probably due to partial folding which may have resulted in a reduction in protein-water interfacial area. It is, therefore, evident that the increase in IVPD is accompanied by an increase in cowpea globulins (more digestible). Also, there is a decrease in finger millet prolamins (less digestible) with cowpea globulins predominating during the compositing levels, to yield the net increase in IVPD (Table 7). Moreover, by reducing tannin content through compositing improved malted finger millet with precooked cowpea, a high IVPD is realized as in case of Snapping green (32.75%) and KNE741 (21.26%), probably due to reduction of proteins-tannin complexes (Table 6). Emmambux and Taylor (2003) from their study they noted that with an increase in tannin concentration there was also increase in protein-bound tannin-protein complex. Tannins are able to bind to protein and form complexes as previously discussed thereby reducing their digestibility (Taylor *et al*., 2007). Therefore, malting and compositing with precooked cowpea results in a reduction in tannin-protein complexes, which results in an improved of IVPD of raw flours formulated using malted finger millet.

4.2.3: Effect of cooking improved malted finger millet composited with precooked cowpea *on IVPD* of flour and complementary porridge

In-vitro protein digestibility of raw malted flours increased by approximately 1% after it was cooked into porridge flours (Table 7). This underscores the importance of thermal denaturation, making the peptides more susceptible to proteolytic attack. This apparent increment was probably due to further thermal denaturation of protein inhibitors in cowpea such as kunitz and bowman-birk and enzyme inhibitors in finger millet, which resulted in an improvement in IVPD. Some inconsistencies were observed in IVPD values of improved malted finger millet with precooked porridge flours, after cooking of the raw flours, which could be explained by different finger millet varieties used. Various researchers working on

various composites have reported similar increase in IVPD. Examples include), *injera* (fermented flatbread), *ugali* (unfermented thick porridge) and *uji* (unfermented thin porridge) made from composites of African sorghum and cowpea (Anyango *et al*., 2011b), porridge made from sorghum-cowpea (Vilakati *et al*., 2015).

4.3: Effect of compositing improved malted finger millet flour with precooked cowpea on lysine content, amino acid score, and Protein Digestibility Corrected Amino Acid Score of flour and complementary porridge

The first limiting indispensable amino acid in cereals is lysine (WHO/FAO/UNU, 2007). Unmalted Snapping green finger millet variety showed a low amount of lysine as compared to KNE741 (Table 8). The recommended level of lysine for 1-3 year-old child is 5.2 g per 100 g of protein (WHO/FAO/UNU, 2007). After malting, lysine content significantly increased ($p<0.05$) with KNE741 showing the highest amount of lysine content (Table 8). Lysine values were apparently significantly higher than value reported elsewhere in literature probably due to the following reasons. First, the assay method used as which was based on matching of peaks retention time on LC-FLD. There could be also a drift in the retention time as drifts of greater than 2.5% would result in false identities, maybe due to sample matrix effect. Nevertheless, Inclusion of precooked cowpea substantially increased lysine content of the baby weaning foods. The increment was a result of high protein lysine content found in cowpea. This was reflected in high values of PDCAAS (Table 8).

4.4: Effect of compositing improved malted finger millet flour with precooked cowpea on total phenolic content of flour and complementary porridge

Effects of malting, compositing and cooking of improved finger millet flour with precooked cowpea on total phenol content of flours and complementary were determined. Malting resulted in approximately 41% and 34% reduction in total phenolic content after malting KNE741 and Snapping green finger millet varieties, respectively (Table 9). There was an approximately 40% reduction in total phenol content when improved malted finger were composited with the two precooked cowpea varieties. Cooking resulted in 22% reduction in total phenol content of complementary porridge composited using improved malted finger millet.

Table 8 : Effects of compositing improved malted finger millet with precooked cowpea on lysine content, amino acid score and Protein Digestibility Corrected Amino Acid Score of flour and complementary porridge

Flours	Composite flours + PKS	Protein content [a]	Lysine content [b]	Amino acid score [c]	IVPD [d]	PDCAAS [e]
Flour						
KNE741	0%	10.13	9.77	1.88	73.28	1.38
	32.75%	14.9	N/A	N/A	76.52	N/A
MKNE741	0%	13.09	22.99	4.42	86.64	3.83
	32.75%	16.5	N/A	N/A	86.75	N/A
Snapping	0%	10.87	1.66	0.32	72.2	0.23
	32.75%	13.54	N/A	N/A	78.66	N/A
MSnapping	0%	11.29	18.87	3.63	79.06	2.87
	32.75%	15.08	N/A	N/A	86.42	N/A
Porridge						
KNE741	0%	10.31	12.22	2.35	79.58	1.87
	32.75%	15	26.33	5.06	77.73	3.94
MKNE741	0%	13.17	18.15	3.49	86.96	3.03
	32.75%	17.59	24.33	4.68	87.92	4.11
Snapping	0%	11.06	20.89	4.02	73.4	2.95
	32.75%	13.89	33.62	6.47	78.66	5.09
MSnapping	0%	11.6	17.50	3.37	79.81	2.69
	32.75%	15.6	21.03	4.04	85.6	3.46

Key:

[a] Protein content values from Table 7 expressed as g/100 g,(db)

[b] Lysine content values calculated as follows: (100 g protein × lysine content per 100g)/protein content per 100 g sample

[c] Lysine scores (mg lysine in 1 g protein /mg of lysine requirement pattern): lysine requirement pattern is 52 for 1-3 year old (WHO/FAO/UNU, 2007)

[d] *In vitro* protein digestibility(IVPD) values obtained from Table 7

[e] PDCAAS = lysine score × % IVPD

M-Malted

PKS-Precooked *kundesoko*

4.4.1: Effect of malting improved finger millet on total phenolic content of flour and complementary porridge

Unmalted KNE 741 (control), had the highest amount of total phenols (condensed tannins, flavonoids, and phenolic acids) at 2.32 mg CE /100 mg, (db) as compared to unmalted Snapping green (control) with 1.26 mg CE /100 mg, (db) (Table 9). These results are fairly comparable to those of red tannin sorghum reported by Dlamini *et al*. (2007). The current results were significantly different ($p<0.05$), denoting varietal influence in the phenol content of the finger millet varieties (Table 9). Polyphenols act as anti-feedants, phytoalexins, antioxidants and contributors to plant pigmentation; therefore, they have an important role in cereals such as finger millet and sorghum (Naczk and Shahidi, 2004). This explains the high levels observed in these two pigmented varieties of finger millet. In addition, because of their astringency especially condensed tannins, they can reduce bird damage during immature stages of crop growth, thus they play a critical role in plant growth. However, these positive agronomic attributes of polyphenols are accompanied by nutritional disadvantages to consumers especially infants, as they bind to protein reducing protein bioavailability. After malting, there was a significant reduction of total phenols in the two-finger millet varieties (Table 9). About 38% reduction in phenol was noted and it was similar to what has been reported in literature by Singh and Raghuvanshi (2012). Malted KNE741 showed a substantial reduction as compared to Snapping green variety. This reduction in polyphenols can be attributed to a number of reasons. First, there could be leaching of water-soluble compounds especially the free bound phenolic acid. Secondly, there could also be the formation of insoluble complexes of phenolic compounds, especially with dietary protein. Lastly, the breakdown of condensed tannins to lower molecular compound through the action of inherent hydrolytic enzyme polyphenol oxidase may contribute to further reduction.

4.4.2: Effect of compositing improved malted finger millet with precooked cowpea on total phenolic content of flour and complementary porridge

Addition of precooked cowpea, which had a low amount of phenolic compounds and other anti-nutrients, significantly ($p<0.05$) reduced of assayable phenols in the composites flours (Table 9). This is illustrated by the high reduction in phenols of approximately 40% when malted Snapping green finger millet variety was composited with cowpea at 10.34% and 32.75% (Table 9).

4.4.3: Effect of cooking improved malted finger millet composited with precooked cowpea on total phenolic content of flour and complementary porridge

Cooking resulted in a significant reduction of phenol content ($p<0.05$) of up to 22% in extractable phenol of the porridge flours (Table 9). This is probably due to structural binding of phenols to other macromolecules during cooking which resulted in low assayable phenols. Sample particle size and extraction methods greatly influence assayable phenols in the samples. The method used involved oxidation-reduction reactions during which at basic pH, the phenolate ion is oxidised, while phosphotungstic/phospho-molybdic acid complex reduced to chromogens (blue coloured solution) (Waterman and Mole, 1994). Therefore, assayable phenols may have decreased either through the interference of reducing substances or apparent binding of phenols to other macromolecules such as protein at a basic pH during cooking of the complementary porridge (Naczk and Shahidi, 2004).

4.5: Effect of compositing improved malted finger millet flour with precooked cowpea on condensed tannin content of flour and complementary porridge

Effects of malting, compositing and cooking of improved finger millet flour with precooked cowpea on condensed tannin content flours and complementary porridge were analysed. Malting resulted in approximately 49% and 44% reduction in condensed tannin content after malting KNE741 and Snapping green finger millet varieties respectively (Table 9). There was an approximately 18% reduction in condensed tannin content when improved malted finger were composited with the two precooked cowpea varieties. Cooking resulted 1% reduction in condensed tannin content of complementary porridge composited using improved malted finger millet as compared to respective raw flours.

4.5.1: Effect of malting improved finger millet on condensed tannins content of flour and complementary porridge

Control KNE741 (with 0% cowpea) had the highest amount of tannins, which was higher than even the corresponding control Snapping green (with 0% cowpea) (Table 9). This may also be attributed the influence of variety on condensed tannins of finger millet varieties. Tannins are located mostly in the testa, and their occurrence is influenced by finger millet variety (Siwela *et al.,* 2007). These authors working on different finger millet varieties concluded that finger millet comprising of thick testa has a significant amount of tannins as compared to those with thin testa and they help protect finger millet grain against birds, insects, and fungal attack.

Table 9: Effects of compositing improved malted finger millet with precooked cowpea on total phenolic content of flour and complementary porridge

		Condensed tannins as mg CE /100 mg,(db)				Total Phenols as mg CE /100 mg,(db)			
		Flours		Porridge		Flours		Porridge	
Millet variety	Composite flours	Unmalted +Cowpea	Malted+ Cowpea	Unmalted +Cowpea	Malted+ Cowpea	Unmalted +Cowpea	Malted+ Cowpea	Unmalted +Cowpea	Malted+ Cowpea
Snapping	0%(Control)	0.61^{fg}±0.06	0.34^{h}±0.04	0.61^{e}±0.03	0.35^{fh}±0.00	1.26^{i}±0.09	0.83^{h}±0.03	1.09^{b}±0.03	1.07^{b}±0.09
	10.34%PKS	0.62^{f}±0.06	0.35^{gh}±0.02	0.62^{e}±0.04	0.33^{h}±0.03	1.44^{k}±0.03	1.30^{c}±0.05	1.03^{cd}±0.03	1.01^{c}±0.09
	21.26% PKS	0.56^{h}±0.04	0.37^{f}±0.06	0.56^{f}±0.02	0.38^{ef}±0.05	1.50^{j}±0.00	1.22^{e}±0.05	0.92^{e}±0.05	0.87^{e}±0.26
	32.75% PKS	0.53^{i}±0.02	0.40^{e}±0.04	0.53^{g}±0.03	0.39^{e}±0.05	1.54^{i}±0.03	1.10^{g}±0.03	0.85^{f}±0.04	0.80^{f}±0.11
	10.34%PKF	0.59^{g}±0.06	0.33^{h}±0.09	0.55^{g}±0.05	0.33^{h}±0.06	1.76^{g}±0.03	1.49^{b}±0.09	1.05^{c}±0.08	1.00^{c}±0.10
	21.26% PKF	0.55^{hi}±0.02	0.32^{h}±0.03	0.46^{h}±0.04	0.34^{fh}±0.07	1.70^{h}±0.03	1.23^{e}±0.07	1.13^{a}±0.00	1.11^{a}±0.27
	32.75% PKF	0.50^{j}±0.03	0.32^{h}±0.11	0.47^{h}±0.07	0.36^{f}±0.00	1.68^{h}±0.03	1.17^{f}±0.07	1.11^{ab}±0.03	1.02^{c}±0.19
KNE741	0%(Control)	1.27^{a}±0.02	0.64^{b}±0.02	1.11^{a}±0.03	0.62^{b}±0.03	2.32^{a}±0.05	1.36^{c}±0.05	1.00^{d}±0.03	0.92^{d}±0.03
	10.34%PKS	1.11^{c}±0.05	0.69^{a}±0.03	1.00^{b}±0.04	0.62^{b}±0.02	2.24^{b}±0.05	1.71^{a}±0.09	1.14^{a}±0.03	1.09^{ab}±0.03
	21.26% PKS	0.98^{d}±0.04	0.64^{b}±0.03	0.94^{c}±0.03	0.61^{bc}±0.02	2.10^{d}±0.03	1.47^{b}±0.19	1.04^{c}±0.05	1.02^{c}±0.03
	32.75% PKS	0.84^{e}±0.07	0.59^{c}±0.10	0.76^{d}±0.03	0.59^{c}±0.00	2.09^{d}±0.09	1.28^{d}±0.05	0.94^{e}±0.03	0.93^{d}±0.05
	10.34%PKF	1.15^{b}±0.06	0.65^{b}±0.03	0.94^{c}±0.03	0.65^{a}±0.02	2.16^{c}±0.09	1.33^{c}±0.05	1.03^{cd}±0.12	1.03^{c}±0.03
	21.26% PKF	0.96^{d}±0.03	0.61^{c}±0.03	1.12^{a}±0.04	0.60^{bc}±0.03	2.01^{e}±0.04	1.33^{c}±0.07	0.88^{f}±0.03	0.87^{e}±0.05
	32.75% PKF	0.86^{e}±0.04	0.56^{d}±0.02	0.77^{d}±0.04	0.54^{d}±0.04	1.97^{f}±0.03	1.28^{d}±0.05	0.98^{d}±0.03	1.00^{c}±0.03

Values of a parameter in a column, followed by different superscript letters are significantly different at $p \leq 0.05$ n=3, PKS- Precooked *Kundesoko*, PKF- Precooked *Kundefaulu*, CE- Catechin equivalent. Values are means ±standard deviations.

This possibly, explains the difference in tannin content of the two-finger millet varieties however, this needs to be confirmed (Table 9). Condensed tannins can form an indigestible protein-tannin complex, via hydrophobic interactions and hydrogen bonding. Finger millet prolamins (eleusinin) just like sorghum kafirins are rich in amino acid, proline. Therefore, they are able to strongly bind with protein reducing protein digestibility as reviewed by Anyango *et al.* (2011a). There was a significant reduction ($p<0.05$) of up to approximately 47% in tannin content after malting the two-finger millet varieties (Table 9). Reduction in tannin after malting has widely been reported in the literature (Singh and Raghuvanshi, 2012). Hejazi and Orst (2016) working with finger millet malted for different durations (24 h, 48 h, and 72 h) were able to show a positive correlation between tannin content reduction and germination duration. The reduction is mainly attributed to the steeping step of the finger millet grains. During steeping, there is leaching of tannin into the sprouting medium (Mbithi-Mwikya *et al.*, 2000). In addition, during malting tannin reduction is hypothesized to be via the high activity of polyphenol oxidase that hydrolyses tannin molecule into lower molecular weight compounds, flavanols (Sripriya *et al*., 1997). It is therefore important to note that a reduction in condensed tannins would result in the formation of less indigestible protein-tannin complexes. Furthermore, the low molecular weight compounds formed (flavanols), after the breakdown of condensed tannins, are unable to exert steric effects to proteins and in the process would increase protein quality.

4.5.2: Effect of compositing improved malted finger millet with precooked cowpea on condensed tannins content of flour and complementary porridge

Compositing resulted in an average of 21% reduction in condensed tannins when unmalted finger millet was composited with precooked cowpea at 10.34%, 21.26%, and 32.75% levels (Table 9). Compositing had also a compound effect when malted finger millet was composited with precooked cowpea as it resulted in an approximately 18% reduction in condensed tannins (Table 9). The reduction was probably due to the addition of precooked cowpea that had a low amount of tannins as compared to uncooked cowpea. Also, malting resulted in a low amount of tannins in porridge flours due to similar reasons as discussed in section 4.5.1. Cooking of the complementary foods resulted in significant decrease ($p<0.05$) in assayable tannin content by approximately 1% when raw malted flour was cooked (Table 8). This is in agreement with previous studies. For example, Anyango *et al*. (2011a) working on sorghum foods composited with cowpea, observed a substantial tannin reduction of between 18% and 69%. Hypotheses, surrounding these reductions include the structural

organization of tannins resulting in the interaction of tannins with grain macromolecules like carbohydrates and protein, forming less extractable complexes (Emmambux and Taylor, 2003), thereby lowering tannin assayed in the complementary foods. Secondly, during cooking of the porridges, there could be thermal degradation of tannins (Awika *et al.,* 2003) probably resulting in simpler flavanol compounds as seen in sorghum tannin.

There were inconsistencies in some of the condensed tannin values for the porridge flours as compared to the flour, especially raw flour made of improved malted finger millet with precooked cowpea. These apparent inconsistencies may be attributed to the principle behind the vanillin-HCl method, which is specific to dihydrochalcones, flavan-3-ols and proanthocyanidins, because of the single bond they possess at 2, 3-position and a free hydroxy group (Naczk and Shahidi, 2004). The shortcoming with this assay method is that it is more sensitive to polymeric proanthocyanidins rather than monomeric flavan-3-ols. During cooking, assayable tannins may have decreased due to the binding of leuco-anthocyanidins to macromolecules such as protein or depolymerisation, degradation, and polymerization of the proanthocyanidins (Taylor and Duodu, 2015).

4.6: Effect of compositing improved malted finger millet flour with precooked cowpea on phytic acid content of flour and complementary porridge

Effects of malting, compositing and cooking of improved finger millet flour with precooked cowpea on phytic acid content of flours and complementary porridge were analysed. Malting resulted in approximately 44% and 41% reduction in phytic acid content after malting KNE741 and Snapping green finger millet varieties respectively. There was an approximately 44% reduction in phytic acid content when improved malted finger varieties were composited with the two precooked cowpea varieties. Cooking resulted in 13% reduction in phytic acid content of complementary porridge composited using improved malted finger millet.

4.6.1: Effect of malting improved finger millet on phytic acid content of flour and complementary porridge

Snapping green (Control) showed a significant higher ($p<0.05$) amount of phytate as compared to KNE741 (Control) variety (Table 10) suggesting that variety had a significant effect on phytic acid in the grains as suggested by Shibairo *et al*. (2014). The high values seen for phytate in the unmalted samples may be attributed to the area where they were grown. The low altitude environments are characterized by high temperatures and low rainfall, which have a great influence on phytic acid synthesis. Phytic acid synthesis involves

enzyme myo-inositol-3-phosphate synthase (MIPS), which converts D-glucose to myo-inositol phosphate, which later undergoes various phosphorylation steps to form phytic acid. The optimum temperature for MIPS activity in plants is 35^{o}C and a pH, between 7.0 and 7.5 (Ngure *et al*., 2016). These conditions could, therefore, be responsible for high phytate levels in the unmalted grains. After malting, KNE741 showed a significant reduction ($p<0.05$) of phytate content by approximately 44% (Table 10). This is probably due to increment in endogenous phytatase which significantly degraded phytate into inorganic phosphorous and inositol (Traore *et al*., 2004). In the present study, the apparent decrease was lower as compared to a reported 67% reduction reported by Traoré *et al*. (2004) who used ionic chromatography to determine inositol-6-phosphate (phytate). The difference is probably due to different assay used in the analysis.

4.6.2: Effect of compositing improved malted finger millet with precooked cowpea on phytic acid content of flour and complementary porridge

When unmalted Snapping green was composited with precooked cowpea, it showed significantly higher ($p< 0.05$) values of phytate as compared to KNE741. Unmalted KNE741 composited with 32.75% precooked *kundesoko* at 32.75% had the lowest amount of phytate (Table 10). Compositing with precooked cowpea resulted in significant reduction ($p< 0.05$) of phytate by a factor of 44% in the raw malted flours as compared to raw unmalted flours (Table 10), probably because of the low amount of phytate in precooked cowpea. Cooking resulted in 6% and 20% significant reduction ($p< 0.05$) of phytate in the complementary porridge (Table 10). These apparent low phytate values of the porridge flours may be due to structural binding of phytates to other cell components because phytic acid is a strong chelating agent of minerals, proteins and other macromolecules (Traoré *et al*., 2004). Therefore, during cooking, may be heat was a strong promoter of chelation between phytate and other macromolecules reducing assayable phytates.

4.7: Effect of compositing improved malted finger millet flour with precooked cowpea on sensory properties of complementary food

Effects of malting, compositing and cooking of improved finger millet with precooked cowpea on sensory attributes of complementary food were determined. Malting resulted in a positive change in textural attributes (coarseness, stickiness, and viscosity) of the complementary food. Compositing at higher levels beyond 21.26% resulted in a significant cooked cowpea flavour, which significantly influenced principal component three.

Table 10: Effects of compositing improved malted finger millet with precooked cowpea on phytic acid content of flour and complementary porridge

Phytic acid as Phytate P mg/100g (db)					
		Flour		Porridge	
Millet variety	Composite flours	Unmalted +Cowpea	Malted+ Cowpea	Unmalted +Cowpea	Malted+ Cowpea
Snapping	0%(Control)	1252.14^{a}±62.44	741.15^{ab}±40.5	1090.60^{b}±24.25	667.41^{b}±46.33
	10.34%PKS	1186.65^{b}±40.50	720.76^{b}±23.60	1110.08^{a}±24.68	644.88^{c}±23.82
	21.26% PKS	1129.51^{c}±47.20	727.65^{b}±61.36	1016.13^{c}±23.82	646.65^{c}±61.86
	32.75% PKS	1071.13^{d}±23.82	754.65^{a}±23.38	908.16^{f}±23.17	692.36^{a}±24.03
	10.34%PKF	1139.88^{c}±23.82	687.15^{c}±84.31	969.94^{d}±22.73	552.15^{f}±46.77
	21.26% PKF	1047.76^{e}±23.60	673.65^{cd}±23.38	921.70^{ef}±22.52	552.15^{f}±23.38
	32.75% PKF	1029.88^{f}±23.82	627.29^{ef}±23.17	969.94^{d}±22.73	553.61^{f}±41.63
KNE741	0%(Control)	1047.76^{e}±23.60	584.51^{g}±70.80	1016.13^{c}±23.82	466.79^{i}±23.17
	10.34%PKS	1038.15^{ef}±23.38	611.76^{f}±23.60	934.70^{e}±22.52	568.43^{e}±39.75
	21.26% PKS	876.15^{h}±23.38	633.15^{e}±46.77	822.15^{h}±40.50	534.88^{g}±23.82
	32.75% PKS	761.64^{j}±23.60	667.41^{d}±46.33	748.01^{j}±40.87	516.60^{h}±0.00
	10.34%PKF	1034.14^{ef}±40.88	666.26^{d}±0.00	1015.59^{c}±24.46	622.99^{d}±48.06
	21.26% PKF	979.64^{g}±23.60	720.76^{b}±23.60	866.60^{g}±48.50	633.15^{cd}±23.38
	32.75% PKF	843.39^{i}±47.20	727.65^{b}±46.77	775.26^{i}±23.60	680.79^{b}±23.17

Values of a parameter in a column, followed by different superscript letters are significantly different at p≤ 0.05 PKS- Precooked *Kundesoko*, PKF- Precooked *Kundefaulu*, Values are means ±standard deviations, n=3.

4.7.1: Effect of malting improved finger millet on sensory attributes of complementary food

F-values from the analysis of variance showed a significant difference ($p<0.05$) for all the 17 sensory attributes of the complementary porridge (Table 11), showing that the panellists were able to describe and differentiate porridge prepared from improved malted finger millet composited with precooked cowpea. There was a significant difference ($p<0.05$) in textural

attributes (coarseness, stickiness, and viscosity) and astringency aftertaste between the unmalted and malted complementary food, with the former, showing a high intensity for the above four attributes. Since hydrolytic enzymes break large molecules especially starch, reducing the amount available for gelatinization, thereby resulting in less viscous porridge (Hejazi and Orsat, 2016). This could explain the difference between malted and unmalted complementary porridges. Astringency is defined as the sharp, constricting sensation felt in the mouth tissues as one consumes the porridge. This is mostly caused by the high levels of bitter and strong tannins and polyphenols (Awika *et al*., 2003), which are found in unmalted millet flour. As the millet is malted, the level of tannins and polyphenols reduces due to the action of the enzyme phytatase activated in malting conditions as already described in previous sections. This could explain the low values exhibited by malted complementary porridge.

4.7.2: Effect of compositing improved malted finger millet with precooked cowpea on sensory attributes of complementary porridge

Samples composited at 21.26% and 32.75% had significant malty and cooked cowpea flavour characteristics with the latter predominating as the ratio increased. There was also a concomitant reduction in astringency aftertaste as more cowpea was added. Cooked cowpea, instead of characteristic beany flavour associated with most legumes; contain a roasted nut flavour due to action of sugars and protein when exposed to drying temperatures after cooking (Kayitesi *et al*., 2013). This probably caused the significantly higher cooked nut flavour in complementary porridge composited at 32.75% than 21.26% (Table 11).

4.7.3: Principal component analysis of sensory attributes of complementary porridge

Principal component analysis was used to extract important information from the heterogeneous data, and reduce a set of correlated variables to uncorrelated measures (principal components) without loss of original information as suggested by (Mwove *et al.*, 2018). The first three principal components accounted for approximately 76% of the total variation observed (PC1=54%, PC2=12%, PC3=10%, (Figure 4). Various researchers have identified different principal components. Anyango *et al*. (2011b) were able to extract two major principal components that accounted for over 96% of the total variation in seventeen sensory attributes of thick porridge.

Table 11: Effects of compositing improved malted finger millet with precooked cowpea on sensory attributes of complementary porridge

Composite flours	Aftertaste					Flavour				
	Malty aftertaste	Astringency	Cooked cowpea	finger millet	Presence of residue	Cooked cowpea	Finger millet	Malty flavour	Overall flavour	Burnt flavour
KNE741(0%)	1.4^{f}±0.89	5.4^{b}±0.89	1.8^{g}±0.45	6.0^{b}±0.71	2.4^{e}±0.89	1.2^{h}±0.45	4.6^{d}±0.89	1.4^{h}±0.89	2.8^{e}±0.84	1.0^{g}±0.00
KNE741(21.26%)	1.0^{f}±0.00	3.6^{f}±0.55	4.8^{d}±0.45	4.6^{c}±1.34	1.8^{f}±0.45	4.8^{c}±0.84	3.8^{e}±0.84	1.4^{h}±0.89	4.0^{d}±0.71	1.2^{g}±0.45
KNE741(32.75%)	3.4^{d}±1.14	4.4^{d}±0.89	6.0^{a}±1.22	4.0^{d}±0.71	3.4^{d}±0.89	4.2^{de}±0.45	3.8^{e}±0.45	3.8^{e}±0.84	1.8^{f}±0.45	3.6^{cd}±0.89
MKNE741(0%)	5.8^{a}±0.84	2.8^{gh}±0.84	3.6^{ef}±0.55	3.8^{d}±1.10	4.4^{c}±0.89	2.6^{g}±0.55	1.8^{h}±0.45	6.6^{a}±0.55	5.6^{b}±0.89	4.6^{a}±1.14
MKNE741(21.26%)	4.4^{bc}±0.55	1.6^{i}±0.55	3.4^{f}±0.55	4.4^{cd}±0.89	3.8^{d}±0.45	4.0^{e}±0.71	3.4^{f}±0.89	5.6^{b}±0.89	4.8^{cd}±1.10	3.8^{c}±1.10
MKNE741(32.75%)	4.4^{bc}±0.55	2.0^{h}±0.00	4.6^{d}±0.55	4.4^{cd}±0.89	4.2^{cd}±0.84	5.4^{b}±0.55	2.4^{g}±0.55	4.8^{d}±1.10	4.8^{cd}±1.10	4.4^{ab}±0.89
SNAPP(0%)	2.6^{e}±0.89	6.0^{a}±0.71	2.0^{g}±0.00	6.8^{a}±0.45	6.2^{a}±0.84	2.6^{g}±0.55	6.8^{a}±0.45	2.6^{g}±0.89	4.8^{cd}±1.10	2.6^{e}±0.89
SNAPP(21.26%)	3.2^{d}±0.45	5.0^{c}±0.71	3.8^{e}±0.45	6.0^{b}±0.71	4.4^{c}±1.34	4.2^{de}±1.10	5.2^{c}±0.84	3.2^{f}±0.45	3.6^{d}±0.55	1.8^{f}±0.45
SNAPP(32.75%)	4.0^{c}±0.71	4.0^{e}±0.71	5.6^{b}±0.55	4.8^{c}±0.45	5.0^{b}±1.00	5.8^{a}±0.45	6.0^{b}±0.71	3.8^{e}±0.45	5.6^{b}±0.55	3.6^{cd}±0.55
MSNAPP(0%)	5.2^{a}±0.84	3.0^{g}±0.00	1.8^{g}±0.55	3.6^{de}±0.55	2.4^{d}±0.89	3.0^{f}±0.71	3.4^{f}±1.14	6.6^{a}±0.55	6.2^{a}±0.89	4.4^{ab}±0.89
MSNAPP(21.26%)	5.6^{a}±0.55	3.0^{g}±0.71	5.0^{cd}±0.71	3.2^{e}±0.45	3.4^{d}±0.55	4.4^{d}±0.55	3.6^{ef}±0.55	5.6^{bc}±0.89	5.2^{bc}±0.84	4.2^{b}±0.45
MSNAPP(32.75%)	4.6^{b}±0.89	2.0^{h}±0.00	5.2^{c}±0.45	2.6^{f}±0.55	4.2^{cd}±0.84	4.8^{c}±0.84	3.2^{f}±0.45	5.4^{c}±0.55	5.0^{c}±0.00	3.4^{d}±0.55

Values are means± standard deviations. Values in a column followed by different letter notations are significantly different at $p \leq 0.05$ n=5. SNAPP- Snapping finger millet variety, M-Malted.

Table 11: Continued…..

	Aroma				Texture		
Composite flours	Overall intensity	Malty	cooked cowpea	finger millet	Coarseness	Viscosity	Stickiness
KNE741(0%)	$1.4^{f}\pm0.55$	$1.20^{g}\pm0.45$	$2.0^{f}\pm0.71$	$4.8^{c}\pm0.45$	$5.6^{b}\pm0.89$	$7.0^{a}\pm0.00$	$7.0^{a}\pm0.00$
KNE741(21.26%)	$1.4^{f}\pm0.55$	$1.20^{g}\pm0.45$	$4.4^{c}\pm1.14$	$4.0^{e}\pm1.41$	$3.4^{e}\pm0.55$	$7.0^{a}\pm0.00$	$7.0^{a}\pm0.00$
KNE741(32.75%)	$2.4^{e}\pm1.34$	$1.8^{f}\pm0.84$	$4.6^{c}\pm0.89$	$4.6^{cd}\pm0.55$	$4.8^{c}\pm1.10$	$6.6^{b}\pm0.55$	$6.6^{b}\pm0.55$
MKNE741(0%)	$1.4^{f}\pm0.55$	$5.8^{b}\pm1.10$	$4.2^{cd}\pm1.10$	$4.6^{cd}\pm0.89$	$3.8^{de}\pm1.10$	$2.0^{g}\pm0.00$	$3.0^{d}\pm1.00$
MKNE741(21.26%)	$5.4^{b}\pm1.52$	$5.2^{c}\pm0.84$	$5.6^{a}\pm0.89$	$4.4^{d}\pm0.89$	$3.4^{e}\pm0.89$	$2.4^{f}\pm0.89$	$1.4^{f}\pm0.55$
MKNE741(32.75%)	$4.6^{c}\pm0.89$	$3.6^{d}\pm0.89$	$4.4^{c}\pm0.55$	$4.4^{d}\pm0.55$	$2.4^{f}\pm1.34$	$3.4^{d}\pm0.89$	$3.2^{d}\pm0.45$
SNAPP(0%)	$5.4^{b}\pm1.34$	$2.4^{e}\pm0.89$	$4.4^{c}\pm0.55$	$6.0^{a}\pm0.00$	$4.6^{cd}\pm0.89$	$6.8^{ab}\pm0.45$	$7.0^{a}\pm0.00$
SNAPP(21.26%)	$4.0^{d}\pm0.71$	$2.6^{e}\pm0.89$	$3.2^{e}\pm0.84$	$5.6^{b}\pm0.55$	$6.0^{a}\pm1.0$	$6.6^{b}\pm0.55$	$6.4^{b}\pm0.55$
SNAPP(32.75%)	$3.8^{d}\pm1.30$	$3.4^{d}\pm0.55$	$5.0^{b}\pm0.71$	$5.6^{b}\pm0.55$	$4.6^{cd}\pm0.89$	$6.2^{c}\pm1.10$	$5.6^{c}\pm0.89$
MSNAPP(0%)	$6.0^{a}\pm1.00$	$5.8^{b}\pm0.84$	$3.6^{de}\pm0.89$	$3.6^{f}\pm0.55$	$2.4^{f}\pm0.45$	$2.2^{fg}\pm0.45$	$2.0^{e}\pm0.00$
MSNAPP(21.26%)	$5.8^{ab}\pm0.45$	$6.2^{a}\pm0.84$	$3.4^{de}\pm0.89$	$4.0^{e}\pm0.71$	$4.2^{d}\pm1.30$	$1.8^{g}\pm0.45$	$1.6^{f}\pm0.55$
MSNAPP(32.75%)	$5.4^{b}\pm0.89$	$5.0^{c}\pm0.71$	$3.8^{d}\pm0.84$	$4.0^{e}\pm1.0$	$3.2^{e}\pm0.45$	$2.8^{e}\pm0.45$	$3.2^{d}\pm0.45$

Values are means± standard deviations. Values in a column followed by different letter notations are significantly different at $p \leq 0.05$ n=5, SNAPP- Snapping finger millet variety, M-Malted.

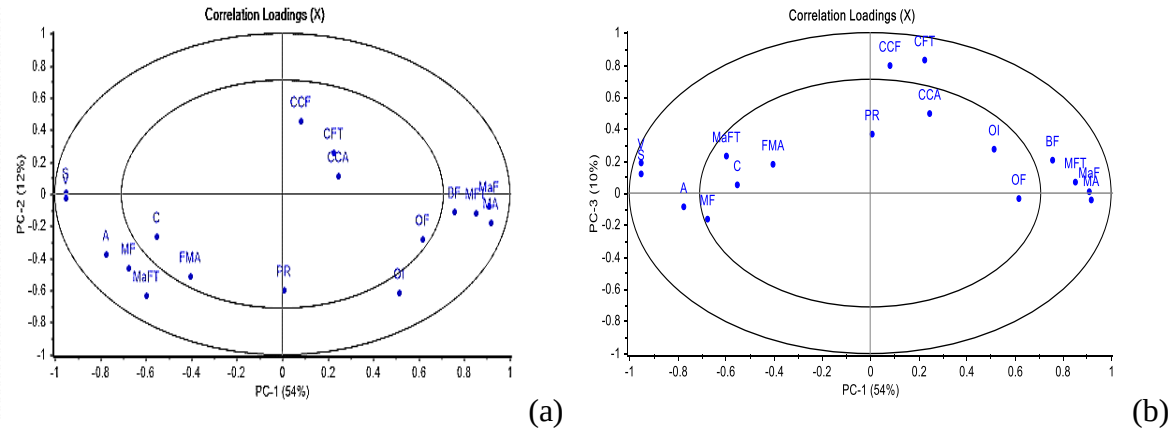
Kayitesi *et al*. (2013) working on sensory attributes of pre-conditioned cowpea subjected to micronisation, were able to extract three major principal components that accounted for over 93% of the total variation. Aroma property (malty), texture properties (viscosity, coarseness, and stickiness), flavour properties (overall flavour, malty and burnt), and aftertaste (malty and astringency), characterised PC1. The second PC was defined by aroma attributes (overall aroma intensity and finger millet aroma), millet aftertaste and presence of residue, while the third PC was characterized by cowpea aftertaste and cooked cowpea flavour. Malting resulted in the antagonistic relationship among sensory attributes (Table 11). There was a positive relationship (Table 11) associated with a malty aroma, malty flavour, and malty aftertaste, and a negative relationship in texture attributes (stickiness and viscosity) and astringency aftertaste. This implies that the malting process had a positive effect on sensory attributes of the complementary food a desirable malty flavour and aroma properties, as described in results under mean values. This was in agreement with the correlation coefficient as malty aroma and malty aftertaste were significant at 0.53 and 0.54, respectively, to the overall flavour. Moreover, in PC1 astringency was negatively correlated with a malty flavour, this further explains the importance of malting and compositing on complementary porridges. Astringency is because of high levels of bitter condensed tannins and polyphenols found in unmalted millet flour as has been suggested by Awika *et al.* (2003). When finger millet is malted level of tannins and polyphenols are reduced by the action of inherent hydrolytic enzymes such as polyphenol oxidase to lower molecular weight compounds that are less bitter (Hejazi and Orsat, 2016). Additionally, compositing malted millet flour with precooked complementary finger millet porridge.

An attribute that was uniquely prominent in PC3, was roasted nut flavour (described by panellists as cooked cowpea flavour) and it was associated with compositing precooked of cowpea. It had a positive loading of approximately 0.80 that influenced the overall flavour and aroma intensity of complementary finger millet porridge. The attribute seemed to be an unfamiliar one to the panellists, as indicated by its low correlation coefficient (-0.10), which was not substantial enough to affect the overall flavour. The findings were in agreement with Kayitesi *et al*. (2013) who observed that roasted nut flavour was undesirable to consumers when pre-conditioned cowpeas were subjected to micronisation. Various researchers have also reported beany flavour as a hindrance to the acceptability of cereal-legume composited foods (Asma *et al.*, 2006; Anyango, 2009). Anyango *et al*. (2011b) for example, was able to identify beany flavour typical with legumes, when sorghum was composited with 30%

cowpea. As suggested by these authors, proper cooking could result in thermal destruction of lipoxygenase enzymes that catalyse the formation of odorous compounds such as 2-pentenyl furan, resulting in a beany flavour (Anyango *et al.*, 2011b). This probably explains the absence of beany flavour in the complementary porridge prepared in the current study.

Correlations loading plot for the complementary sensory attributes for the first three PCs is presented in Figure 4. Measurement close to each other are positively related, while those that are negatively related are separated by 180°, and those that are separated by 90° are independent and therefore they would be loaded on different PCs (Mwove *et al*., 2018). This, therefore, means that interpretation and measurement of PCs were according to the correlations between each parameter and each PC's. In Figure 4a, stickiness, and viscosity of complementary porridge had a negative loading and were placed far in PC1. They were critical parameters that defined PC1, and their negative loading had a huge implication, in that, a decrease would increase PC1. Similarly, burnt flavour, malty aftertaste, malty flavours were far in PC1, with a positive loading. It, therefore, meant an increase in these sensory attributes would increase PC1.

Millet flavour and millet aftertaste were closely located having a positive correlation but negatively correlated to cooked cowpea aroma and cowpea aftertaste, located in the opposite quadrant, which can be attributed to varying level of precooked cowpea flour used.



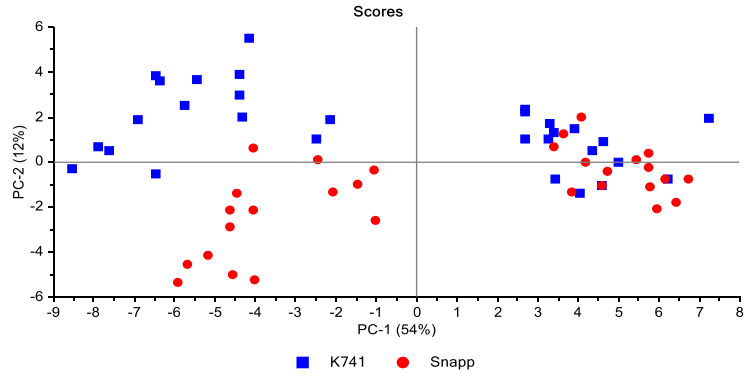
(a) (b)

Figure 5. Biplot for (a) PC1 and PC 2, and (b) PC1 and PC3

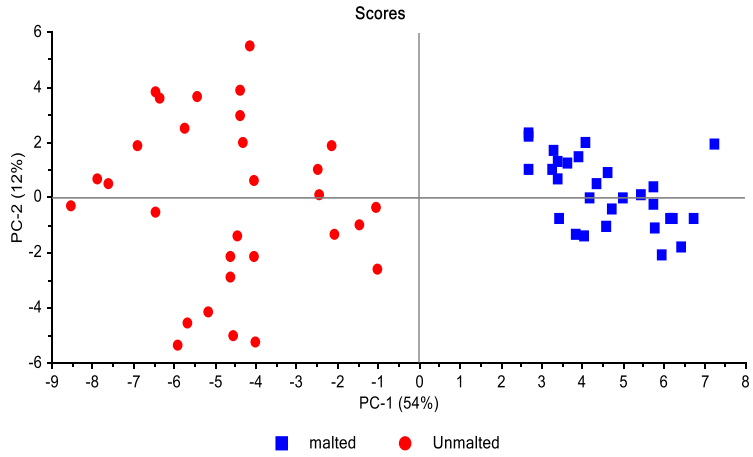
Key: OI-overall intensity, MA- malty aroma, CCA-cooked cowpea aroma, FMA-finger millet aroma, C-coarseness, V-viscosity, S-stickiness, OF-overall flavour, CCF-cooked cowpea flavour, MF-millet flavour, MAF-malty flavour, BF-burnt flavour, MFT-malty aftertaste, CFT-cowpea aftertaste, MAFT-millet aftertaste, PR-Presence of residue, A-astringency.

Malty flavour, malty aftertaste, and malty aroma were located close to the overall flavour and aroma sensory attributes, showing that the three parameters were important in defining the flavour of the complementary porridge. A negative correlation of viscosity and stickiness in Figure 5a, to malty flavour and aroma, showed the importance of the malting process in formulating complementary baby weaning food. In Figure 5b, cowpea, aftertaste and cooked cowpea flavour were negatively correlated to astringency with the latter being close to PC3. Its closeness to PC3 showed its importance in influencing PC3 loadings as compared to neither cooked cowpea flavour nor cowpea aftertaste.

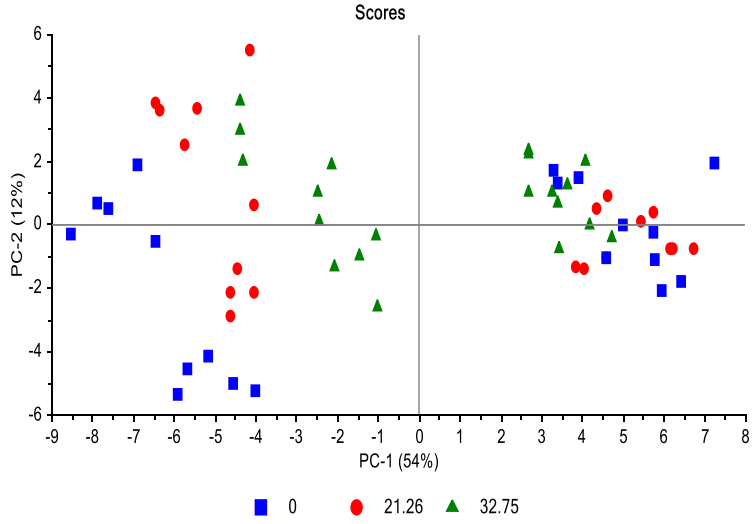
Figure 6 shows the scores biplot for PC1 versus PC2 for KNE741, Snapping, unmalted, malted, 0%, 21.26%, and 32.75%. According to Mwove *et al*. (2018) score plot represents loadings of sensory attributes in the multivariate space of two PC score vectors. From the figures, two samples were clearly distinguished based on the sensory attributes. Two varieties used had distinguishable sensory attributes (Figure 6a) with KNE741 associated with a high level of stickiness and astringency because of a high amount of polyphenol in the grains (Table 9) than Snapping green variety. In Figure 6b, unmalted samples were located on the left side near (stickiness, viscosity, astringency); while after malting process the samples were located on the right side near malty flavour and malty aroma. This shows that malting influenced sensory attribute of the composite flour. In Figure 6c, composites containing 32.75% precooked cowpea were associated strongly with cooked cowpea flavour and aroma, this meant that although they were less viscous and sticky they contained a distinctive cooked cowpea flavour (roasted nut flavour), that influenced PC3 loading.



(a)



(b)



(c)

Figure 6: Scores biplot for (a) PC1 versus PC2, for KNE741 and Snapping green (b), PC1 versus PC2, for Malted and Unmalted (c), PC1 versus PC2, for 0%, 21.26% and 32.75%.

Where; K741 –KNE741 and Snapp-Snapping green

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

5.1: Conclusions

I. Protein quality of complementary foods in terms of protein content and *in vitro* protein digestibility increases through malting and compositing improved finger millet with precooked cowpea.

II. Compositing of malted finger millet with precooked cowpea results in a reduction in anti-nutrients; phenolic compounds, condensed tannins, and phytic acid.

III. A reduction in texture attributes and astringency corresponds to increase in malty flavour and aroma after malting finger millet. In addition precooked cowpea flour addition beyond 21.26% results in a significantly distinct cooked cowpea flavour.

5.2: Recommendations

Based upon the findings of the study, the following recommendations are relevant.

I. Malting and compositing of improved finger millet with precooked cowpea is necessary to increase protein quality of complementary food.

II. Malting and compositing of improved finger millet with precooked cowpea is necessary to reduce anti-nutrient content of complementary food.

III. Higher precooked cowpea addition beyond 21.26% will require masking of cooked cowpea flavour through commercial flavouring.

5.3: Further research

1. Follow-up studies to correlate results found on protein quality with *in vivo* animal models and human intervention studies using malted finger millet composited with precooked cowpea porridge is required.
2. Safety and shelf-life studies are recommended to know spoilage microorganisms and also the shelf life of the product in the market.
3. From descriptive sensory evaluation data, compositing malted finger millet with 21.26% precooked cowpea needs to be subjected to consumer acceptance tests to encourage scale-up and uptake of the product in the market.

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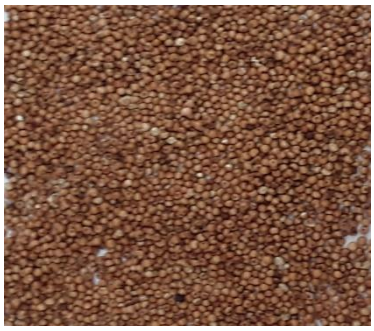
APPENDICES


U15


P224


KNE629


Kundesoko


KNE741


Kundefaulu


Snapping green early

Plate 1: Cowpea and finger millet phenotypes investigated in this study

Appendix 1 selected statistical outputs

Table 1: Anova table of mean square errors for the different sources of variation for the different finger millet varieties and the malting process used in screening process.

SOV	D.o.F	MC	Protein	Tannins	Phenols	IVPD
Variety	4	14.77***	30.06***	0.24	0.53***	30.45***
Process	1	30.91***	12.03***	1.46	4.55***	671.85***
Variety*process	4	5.01***	1.47***	0.05	0.28***	10.18***
Rep	2	0.05ns	0.08ns	0.00	0.00ns	1.79ns
Error	18	0.08	0.05***	0.001	0.002	4.86***
R^2	-	***98.67***	***99.35***	***99.06***	***99.46***	***90.54***
CV	-	***4.42***	***2.44***	***5.30***	***3.23***	***2.89***
MSD	-	***0.50***	***0.39***	***0.06***	***0.08***	***3.85***

Key: S.O.V = Source of variation, DoF = Degrees of freedom, M.C = Moisture Content, R^2 = Coefficient of determination, CV = Coefficient of variation MSD = Minimum Significant Difference, * = Significant at $p < 0.05$, *** = highly significant at $p < 0.001$ and ns = not significant at $p<0.05$

Table 2: Anova table of mean square errors for the different sources of variation for the different composite flour samples for different variables

S.O.V	**DoF**	**M.C**	**Protein**	**Tannins**	**Phenols**	**IVPD**
Millet	1	1.85^{***}	13.15^{*}	2.90^{***}	2.96^{***}	1.83^{ns}
Cowpea	1	0.18^{***}	10.63^{*}	0.03^{***}	0.02^{*}	0.45ns
Substitution	3	1.75^{***}	41.55^{*}	0.06^{***}	0.22^{***}	35.82^{***}
Process	1	7.97^{***}	54.47^{*}	2.04^{***}	5.97^{***}	1113.75^{***}
Replication	2	0.22^{ns}	0.06^{ns}	0.00^{ns}	0.02^{ns}	0.00ns
Millet*Cowpea	1	0.01^{ns}	0.01^{ns}	0.00^{ns}	0.62^{***}	11.72ns
Millet*substitution	3	0.30^{***}	0.24^{ns}	0.04^{***}	0.10^{***}	15.98**
Cowpea*substitution	2	0.77^{***}	0.16^{ns}	0.00^{ns}	0.01^{ns}	5.67ns
Millet*Cowpea*substitution	2	1.15^{***}	1.64^{***}	0.01^{ns}	0.02^{*}	2.67ns
Millet*Process*cowpea*substitution	13	3.01^{***}	0.37^{***}	0.03^{***}	0.10^{***}	10.05**
Error	54	0.02^{***}	0.11^{***}	0.00^{***}	0.00^{***}	3.09***
R^2	-	***98.64***	***97.29***	***97.66***	***98.23***	***94.49***
C.V	-	***1.57***	***2.42***	***7.76***	***4.02***	***2.18***
MSD	-	***0.05***	***0.14***	***0.02***	***0.03***	***0.96***

Key: S.O.V = Source of variation, DoF = Degrees of freedom, M.C = Moisture Content, R^2 = Coefficient of determination, CV = Coefficient of variation MSD = Minimum Significant Difference, * = Significant at $p < 0.05$, *** = highly significant at $p < 0.001$ and ns = not significant at $p<0.05$

Complementary porridge flour samples for different variables.

S.O.V	DoF	M.C	Protein	Tannins	Phenols	IVPD
Millet	1	3.02***	16.84***	2.27***	0.01^{ns}	42.07***
Cowpea	1	3.94***	11.75***	0.02***	0.03*	0.26ns
Substitution	3	0.89***	44.52***	0.05***	0.02*	26.94***
Process	1	0.24***	66.98***	1.48***	0.02^{ns}	904.98***
Replication	2	0.01^{ns}	0.69***	0.00^{ns}	0.01^{ns}	1.44ns
Millet*Cowpea	1	72.14***	0.01^{ns}	0.01*	0.14***	20.56**
Millet*substitution	3	5.14***	0.33*	0.03***	0.04***	16.83***
Cowpea*substitution	2	5.48***	0.46*	0.01*	0.11***	5.55ns
Millet*Cowpea*substitution	2	53.94***	1.89***	0.00^{ns}	0.01^{ns}	1.63***
Millet*Process*cowpea*substitution	13	16.24***	0.50***	0.02***	0.01*	6.37*
Error	54	0.01***	0.11***	0.00***	0.00***	2.45***
R^2	***-***	***99.92***	***97.57***	***98.32***	***69.51***	***94.76***
C.V	***-***	***0.97***	***2.42***	***6.02***	***7.96***	***1.92***
MSD	***-***	***0.03***	***0.15***	***0.02***	***0.03***	***0.85***

Aftertaste sensory properties

S.O.V	DF	Malty	Cowpea	Millet	Residues	Astringency
Millet	1	9.600***	2.817*	29.400***	13.067***	4.267***
Process	1	84.400***	0.817^{ns}	64.067***	0.267^{ns}	81.667***
Substitution	2	1.550^{ns}	39.267***	0.450^{ns}	3.650*	8.267***
Replication	4	0.233	0.083	0.333	0.692	0.058
Millet*Process	1	4.267*	2.017*	4.267**	45.067***	0.000^{ns}
Millet* Substitution	2	3.050**	1.267^{ns}	1.550^{ns}	0.117^{ns}	3.267***
Process* Substitution	2	10.050***	1.867*	5.517***	2.517*	0.867^{ns}
Millet*Process* Substitution	2	0.617^{ns}	3.267**	0.217^{ns}	5.717***	0.200^{ns}
Error	44	0.588	0.483	0.533	0.737	0.440
R^2		0.836	0.821	0.830	0.724	0.852
C.V		20.177	17.750	18.257	22.594	18.601
MSD		0.399	0.362	0.380	0.447	0.345

Flavour properties

S.O.V	DF	Overall Flavour	Cooked cowpea Flavour	Millet Flavour	Malty Flavour	Burnt Flavour
Millet	1	18.150***	0.417^{ns}	0.017^{ns}	5.400***	0.817^{ns}
Process	1	33.750***	0.150^{ns}	43.350***	141.067***	50.417***
Substitution	2	1.716^{ns}	49.400***	6.067***	1.317^{ns}	5.067***
Replication	4	0.308	0.442	0.642	1.475	1.025
Millet*Process	1	7.350***	0.817^{ns}	16.017***	2.400*	3.750*
Millet* Substitution	2	5.150***	2.067**	0.867^{ns}	0.450^{ns}	2.067*
Process* Substitution	2	1.350^{ns}	3.200**	4.200**	13.817***	8.867***
Millet*Process* Substitution	2	6.050***	7.467***	1.067	1.950*	0.800^{ns}
Error	44	0.681	0.351	0.605	0.520	0.534
R^2		0.748	0.892	0.764	0.892	0.798
C.V		18.272	14.993	17.225	17.042	22.720
MSD		0.429	0.308	0.405	0.375	0.380

Aroma sensory properties

S.O.V	DF	Overall aroma	Malty aroma	Cooked cowpea aroma	Millet Aroma
Millet	1	79.350***	18.150***	1.350^{ns}	1.667^{ns}
Process	1	43.350***	150.417***	0.817^{ns}	13.067***
Substitution	2	2.067^{ns}	0.817^{ns}	4.200**	0.317^{ns}
Replication	4	1.485	1.642	0.942	0.567
Millet*Process	1	2.017^{ns}	1.350^{ns}	10.417***	13.067***
Millet* Substitution	2	15.200***	1.050^{ns}	8.600***	0.317^{ns}
Process* Substitution	2	8.600***	8.117***	3.267*	0.617^{ns}
Millet*Process* Substitution	2	2.467^{ns}	0.350^{ns}	1.667^{ns}	0.217^{ns}
Error	44	0.940	0.496	0.705	0.567
R^2		0.819	0.900	0.625	0.569
C.V		24.756	19.125	20.736	16.247
MSD		0.505	0.367	0.437	0.392

Texture sensory properties

S.O.V	DF	Coarseness	Viscosity	Stickiness
Millet	1	1.067^{ns}	1.667*	2.400**
Process	1	38.400***	273.067***	264.600***
Substitution	2	1.317^{ns}	0.517^{ns}	2.450***
Replication	4	0.567	0.392	0.208
Millet*Process	1	0.600^{ns}	0.000^{ns}	0.266^{ns}
Millet* Substitution	2	10.517^{ns}	0.417^{ns}	0.150^{ns}
Process* Substitution	2	1.850^{ns}	3.517***	6.950***
Millet*Process* Substitution	2	2.450^{ns}	0.150^{ns}	1.517**
Error	44	0.939	0.346	0.290
R^2		0.644	0.949	0.958
C.V		24.030	12.884	11.970
MSD		0.504	0.306	0.280

Explained variance

	PC-1	PC-2	PC-3	PC-4	PC-5	PC-6	PC-7	PC-8
Eigenvalues	24.3173	5.27041	4.39763	2.537585	1.76913	1.268425	1.132001	0.992921
Explained variance %	53.64912	11.62765	9.70211	5.59844	3.90309	2.79841	2.49743	2.1906
Cumulative %	53.64912	65.27677	74.97888	80.57732	84.48041	87.27882	89.77625	91.96685

Correlation Loadings

	Abbr	PC-1	PC-2	PC-3
Overall intensity aroma	OI	0.517351	-0.61846	0.273641
Malty aroma	MA	0.920837	-0.18367	-0.04525
Cooked Cowpea aroma	CCA	0.246683	0.110276	0.493355
Finger millet aroma	FMA	-0.40358	-0.51117	0.179904
Coarseness	C	-0.55254	-0.26753	0.052044
Viscosity	V	-0.95027	-0.02184	0.185636
Stickness	S	-0.95425	0.010498	0.117874
Overall Flavour	OF	0.619298	-0.28051	-0.03369
Cooked Cowpea flavour	CCF	0.083561	0.451199	0.797476
Millet Flavour	MF	-0.67769	-0.4621	-0.16098
Malty flavour	MaF	0.912611	-0.07891	0.011275
Burnt Flavour	BF	0.757515	-0.10923	0.201769
Malty aftertaste	MFT	0.851591	-0.12023	0.07131
Cowpea aftertaste	CFT	0.22591	0.256695	0.827028
Millet aftertaste	MaFT	-0.59613	-0.63366	0.230607
Presence of residue	PR	0.006876	-0.59988	0.369141
Astringency	A	-0.77614	-0.37528	-0.08861

Appendix 2: Amino acids results for selected composite flour samples

biosciences africa

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Method used: Performic acid-HCL hydrolysis and OPA derivatization prior to Ultra High-Performance Liquid Chromatography-Fluorescence Detection

Sample Specifications		Amino acids concentration in (g/100g)																							
Lab code	Client code	Aspartic acid	sd	Glutamic acid	sd	Serine	sd	Arginine	sd	Alanine	sd	Tyrosine	sd	Valine	sd	phenyl alanine	sd	Isoleucine	sd	Leucine	sd	lysine	sd
19_284	7	0.24	0.05	1.83	0.29	0.29	0.04	0.61	0.05	0.62	0.11	0.53	0.04	0.44	0.05	0.60	0.06	0.78	0.13	0.82	0.02	0.99	0.06
19_285	8	0.03	0.01	0.75	0.04	0.03	0.01	0.36	0.01	0.08	0.00	0.08	0.01	0.06	0.01	0.13	0.01	0.07	0.00	0.20	0.02	0.18	0.02
19_286	10	0.66	0.03	2.08	0.13	0.64	0.07	2.43	0.12	1.05	0.09	0.72	0.04	0.51	0.02	2.20	0.09	0.67	0.05	1.99	0.12	3.95	0.30
19_287	11	0.17	0.06	1.22	0.22	0.33	0.04	1.38	0.15	0.44	0.05	0.38	0.06	0.28	0.02	0.89	0.08	0.27	0.09	1.04	0.10	1.26	0.08
19_288	18	0.46	0.03	2.13	0.24	0.66	0.02	1.94	0.05	0.81	0.08	0.53	0.04	0.44	0.01	2.12	0.07	0.59	0.01	1.72	0.12	3.01	0.13
19_289	24	0.72	0.03	2.22	0.32	0.36	0.05	2.22	0.13	0.52	0.04	0.41	0.01	0.56	0.03	2.78	0.14	0.76	0.12	0.93	0.06	4.28	0.17
19_290	25	0.41	0.01	1.34	0.16	0.32	0.04	1.73	0.29	0.74	0.08	0.64	0.04	0.44	0.07	2.44	0.21	0.53	0.07	1.99	0.11	2.39	0.04
19_291	66	0.20	0.05	1.15	0.09	0.23	0.03	0.72	0.04	0.34	0.08	0.25	0.03	0.16	0.03	0.50	0.09	0.21	0.02	0.45	0.07	4.67	0.18
19_292	67	0.26	0.05	2.07	0.34	0.41	0.04	1.18	0.96	0.53	0.08	0.31	0.02	0.48	0.08	1.75	0.24	0.38	0.06	0.97	0.10	2.31	0.15
19_293	74	0.33	0.02	1.68	0.17	0.35	0.02	1.37	0.15	0.59	0.06	0.51	0.04	0.37	0.02	1.83	0.13	0.49	0.03	1.32	0.14	2.13	0.01
19_294	79	0.44	0.09	1.89	0.05	0.18	0.02	1.91	0.26	0.35	0.01	0.64	0.02	0.46	0.03	1.80	0.16	0.23	0.04	0.62	0.04	3.28	0.15
19_295	81	0.40	0.09	1.60	0.06	0.36	0.05	2.10	0.20	0.63	0.11	0.62	0.12	0.41	0.08	1.45	0.20	0.51	0.07	1.50	0.21	2.03	0.25
19_296	143	0.26	0.01	0.51	0.13	0.18	0.03	1.81	0.16	0.58	0.01	0.37	0.01	0.65	0.05	1.78	0.16	0.72	0.12	0.79	0.03	1.32	0.03
19_297	144	0.25	0.04	0.63	0.14	0.28	0.05	1.91	0.11	0.45	0.01	0.31	0.02	0.58	0.02	0.78	0.06	0.24	0.03	0.50	0.08	1.33	0.16

Key : sd- standard deviation

Remarks:

1. These results relate only to the samples received as is.
2. These results are for research purposes only.

Appendix 3: Selected amino acid chromatograms

Figure 1a for amino acid standard mix and Figure 2a chromatogram for unmalted KNE 741 finger millet.

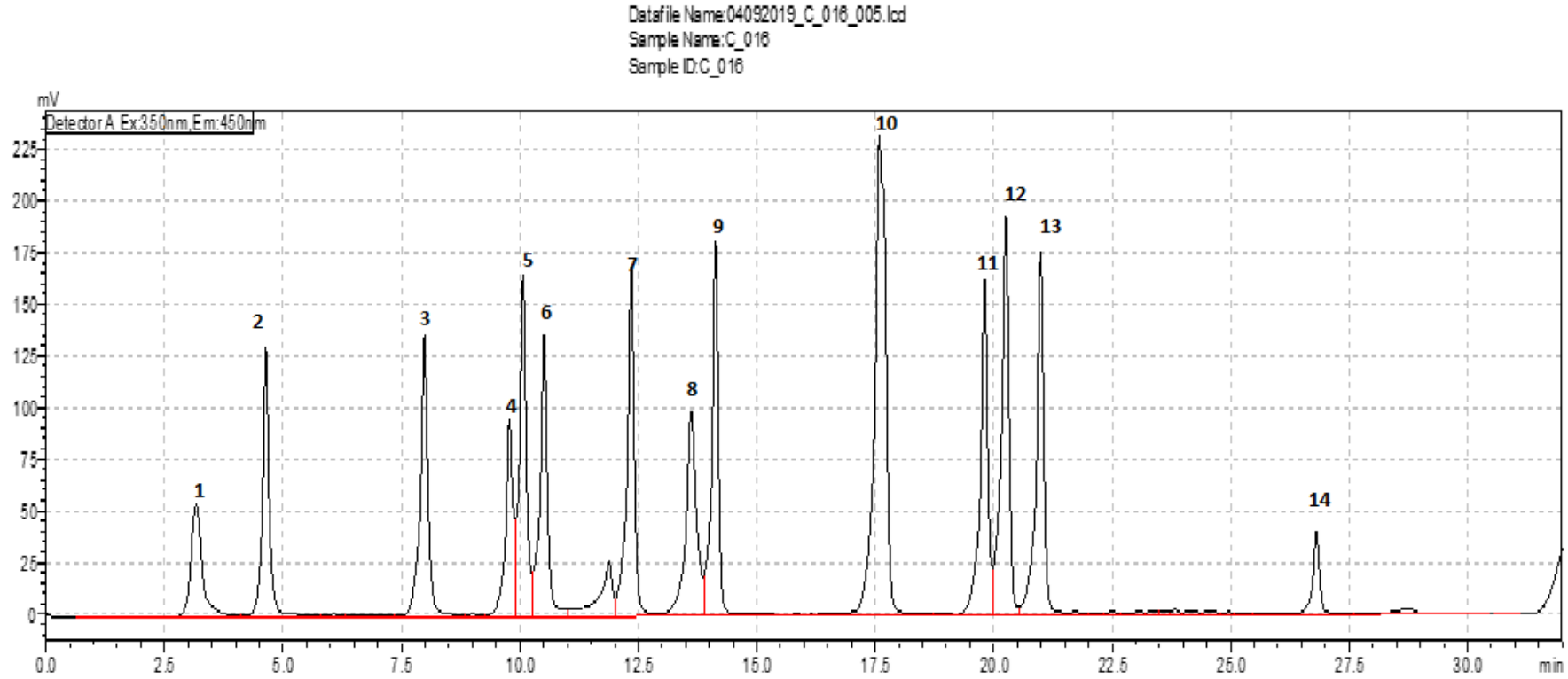

Figure I a: Amino acid standard mix

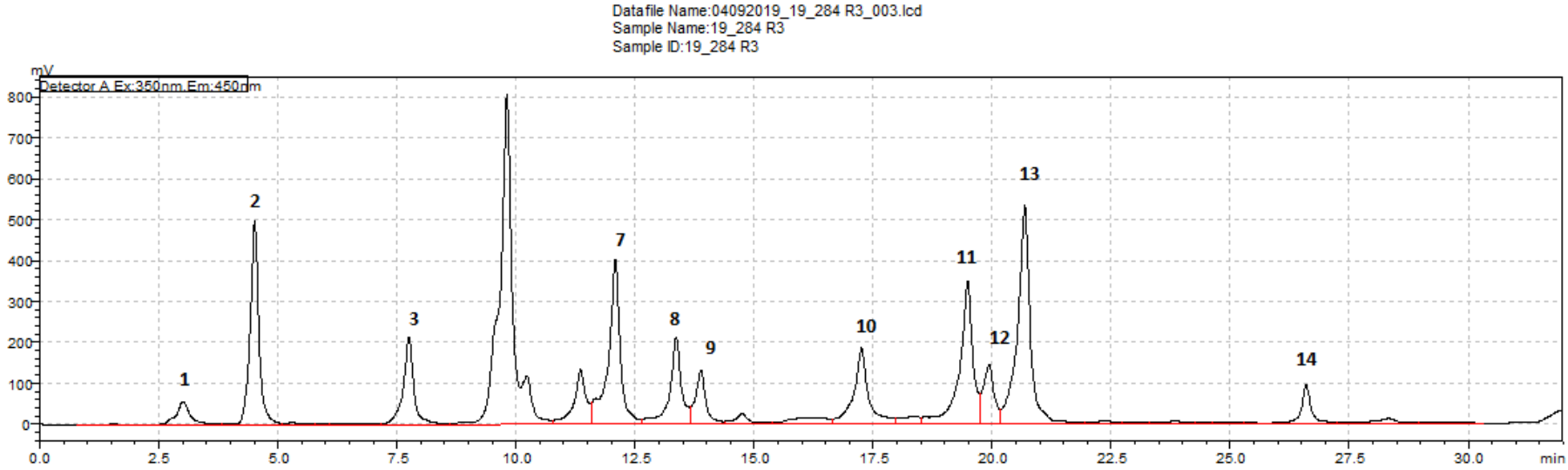

Figure 2 : Sample 19_284 Amino acid standard separation chromatogram

Peak number	Retention time (Mins)	Peak ID
1	3.175	Aspartic acid
2	4.596	Glutamic acid
3	7.085	Serine
4	9.016	Histidine
5	9.784	Glycine
6	10.511	Threonine
7	12.381	Arginine
8	13.599	Alanine
9	15.149	Tyrosine
10	18.615	Valine
11	19.761	Phenyl alanine
12	20.229	Iso-Leucine
13	20.993	Leucine
14	26.829	Lysine

Appendix 4: Research output

Effect of Compositing Precooked Cowpea with Improved Malted Finger Millet on Anti-Nutrients Content and Sensory Attributes of Complementary Porridge

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Abstract

Protein energy malnutrition remains a huge burden in Sub-Saharan Africa. Principally, it is due to children being fed on millet gruels which are high in carbohydrates, and low protein. Moreover, they contain significant amounts of anti-nutrients such as phytates, phenols and tannins. Compositing of malted finger millet flour with other flours has potential for improving the nutritional quality and sensory attributes of these foods. The objective of this study was to determine the effect of compositing malted finger millet flour with cowpea on the anti-nutritional contents and sensory properties of formulated baby weaning food. Mixing selected improved finger millet varieties with precooked cowpea flour was based on WHO recommended levels. There was a significant ($p < 0.05$) reduction in total phenolic content, tannin content and phytic acid by 41%, 50%, and 44%, after compositing with malted finger millet and precooked cowpea at 10.32%, 21.26% and 32.75%, respectively. Cooking process significantly reduced amount of trypsin inhibitors, and other anti-nutrients both in cowpea and complementary porridge. Loadings from principal component analysis (PCA) of 17 sensory attributes of porridge showed that approximately over 80% of the variations in sensory at-

Appendix 5: Manuscript under review

Protein quality of finger millet complementary porridge as affected by compositing precooked cowpea with improved malted finger millet

Cyprian O. Syeunda[1], Joseph O. Anyango[1]†, Abdul K. Faraj[1], Paul K. Kimurto[2]

Abstract

Protein-energy malnutrition is one of the leading causes of death for children under-five in developing countries and Kenya is no exception. These children rely on starchy weaning foods such as finger millet (*Eleusine coracana*) porridge for nutrient supply, which are limiting in indispensable amino acids and have poor protein digestibility. Cowpea (*Vigna unguiculata*), a locally available nutritious legume, could be an excellent complement to lysine-deficient millet diets. The present study aimed at innovatively improving the protein quality of baby weaning food, by evaluating the effect of malting on improved finger millet genotypes (U15, P224, KNE741, KNE629 and Snapping) to enable selection of the best varieties with superior nutritional credential post process. Blending of selected finger millet with precooked cowpea flour followed the WHO recommended level at 10.32%, 21.26% and 32.75% with 0% as control. KNE741 and Snapping showed superior qualities in terms of protein content and tannin content. Total phenol content and tannin content notably decreased by 44% and 47%, respectively after malting finger millet varieties. In addition, compositing with precooked cowpea increased protein content and in vitro protein digestibility in flour by about 6-39%. Cooking resulted in 5% increase in in vitro protein digestibility in the complementary porridge. This study indicates that malting of finger millet and compositing it with precooked cowpea has the potential to address PEM among under five children in sub-Saharan Africa because it results in reduced anti-nutritional content with a concomitant improvement in protein quality of the baby weaning food.

Keywords: Finger millet, malting, protein quality, in vitro protein digestibility

Appendix 5: NACOSTI Research authorization

NACOSTI

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

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When replying please quote

NACOSTI, Upper Kabete
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P.O. Box 30623-00100
NAIROBI-KENYA

Ref: No. **NACOSTI/P/19/75028/28749**

Date: **26th March, 2019**

Cyprian Omondi Syeunda
Egerton University
P.O. Box 536-20115
NJORO

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"Protein, anti-nutrient and sensory qualities of baby weaning porridge made from malted finger millet (Eleusine Coracana L.) and Cowpea (Vigna Unguiculata L.) composite flour"*** I am pleased to inform you that you have been authorized to undertake research in **Nakuru County** for the period ending **25th March, 2020.**

You are advised to report to **the County Commissioner and the County Director of Education, Nakuru County** before embarking on the research project.

Kindly note that, as an applicant who has been licensed under the Science, Technology and Innovation Act, 2013 to conduct research in Kenya, you shall deposit **a copy** of the final research report to the Commission within **one year** of completion. The soft copy of the same should be submitted through the Online Research Information System.

GODFREY P. KALERWA MSc., MBA, MKIM
FOR: DIRECTOR-GENERAL/CEO

Copy to:

The County Commissioner
Nakuru County.

The County Director of Education
Nakuru County.

National Commission for Science, Technology and Innovation is ISO9001: 2008 Certified

Appendix 6: Descriptive sensory evaluation consent form

Contract

I …….. Agree to be a panellist for **DESCRIPTIVE QUALITY SENSORY ANALYSIS OF BABY WEANING for** a period of one week.

I agree to comply with terms and conditions.

Signature …… Date……