EVALUATION OF QUALITY OF PUMPKIN AND SOY BEAN-SEED BLENDED MAIZE FLOUR

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EXTENDED ABSTRACT

Maize is a major food staple in most of the sub-Saharan African countries. Maize flour is mostly rich in carbohydrates, which provide energy to the human body. Nutritionally, maize flour is deficient in the other major diet requirements of proteins, vitamins and essential minerals. In this study, maize flour was enriched using pumpkin and soybean seed flours and the sensory and nutritional qualities of the resulting composite flours were evaluated.

Four formulations of maize, soybean and pumpkin seeds flours were preparedby compositing various proportions of each ingredient. The flours were produced by grinding the seeds using a hammer mill to a particle size fine enough to pass through a 1 mm sieve size. The composite flours were then used to prepare stiff porridges (*ugali*), which is the popular format of preparation for this meal. Nutritional and sensory quality of the flours and stiff porridges were evaluated. The nutritional quality parameters included crude protein, which was found to increase from 8.1% (w/w) in the plain maize flour to up to 24.09% in the blended flour formulations; crude fibre increased from 7.3% to13.45%; crude fat from 3.6% to 32.26%; ash from 1.09% to 3.44%; and moisture content was increased from 8.1% to10.29%. Micronutrient parameters evaluated included Vitamin A which was found to increase from 0.00µg/g to 78.82 µg/g; Folic acid from 2.5 µg/g to 5.33 µg/g; Vitamin C from 0.00 mg/100g to 12.23 mg/100g; Zinc from 1.8 mg/100g to 3.43 mg/100g; and Iron from 3.5 mg/100g to up to 20.81 mg/100g, in the formulations. Sensory attributes evaluated for the composite flour *ugali* were aroma, color, texture, general appearance, smell, flavour, hardness, springiness, oiliness, taste and general acceptability. A 9-point hedonic scale was employed in the sensory evaluation, alongside instrumental evaluation using a laboratory texture analyzer. There was no significant difference in smell, appearance, texture, flavour, hardness, springiness across the four formulations among the panelists. The mean score was above 5 for all parameters, which is in the middle of the 9- point hedonic scale. These findings indicate that generally all the formulations were accepted. Formulation F4 (8.47%) had the highest score among the formulations. The instrumentally evaluated results indicated significant differences in hardness, cohesiveness and springiness in the stiff porridge samples. From the human sensory evaluation, all samples were accepted regardless of their instrumentally evaluated differences, indicating that the differences were not enough to substantially influence the human sensory organs. The effect of cooking was established by evaluating the vitamin A and B9 losses. The vitamin A losses after cooking were established to vary in the range from a minimum of 0.06% to a maximum of 0.88% among the formulations. The vitamin B9 losses after cooking were established to vary by a minimum of 0.05% to a maximum of 1.04% among the formulations.

Pumpkin and Soy bean seeds flour improved the nutritional quality of the stiff porridge composite flours. The micronutrients compositions were increased due to the added ingredients of soy bean and pumpkin seeds flour. The sensory evaluation of stiff porridge from all formulations and control sample indicated that all formulations were acceptable. Therefore the inclusions of pumpkin and soy bean seeds flour in the levels used in this study improved maize flour stiff porridge but did not significantly affect the product acceptability.

DECLARATION

I, Daza, Roina Deus do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted to any other institution for a Degree award.

_____ _____

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The above declaration is confirmed;

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Prof. B. Chove Date

(Supervisor)

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TABLE OF CONTENTS

LIST OF TABLES

APPENDICES

LIST OF ABREVIATIONS AND ACRONYMS

B12	Calabamin
B6	Pyridoxine
B9	Folic Acid
DFSAP	Department of Food Science and Agro Process
DM	Dry Matter
FAO	Food Agricultural Organization
ML	Milliliter
MNM	Micronutrient Malnutrition
PUFA	Poly Unsaturated Fatty Acid
RDA	Recommended Daily Allowances
SUA	Sokoine University of Agriculture
TBS	Tanzania Bureau of Standard
TFNC	Tanzania Food and Nutrition Centre
USDA	United states Department of Agriculture
WHO	World Health Organization
µ g	Micrograms

CHAPTER ONE

1.0INTRODUCTION

1.1Background Information

Fortification is the practice of deliberately increasing the content of an essential micronutrient, i.e. vitamins and minerals (including trace elements) in a food, so as to improve the nutritional quality of the food supply and provide a public health benefit with minimal risk to health (WHO, 2016). Food fortification refers to the addition of micronutrients to processed foods. Fortification of food with micronutrients is a valid technology for reducing micronutrient malnutrition as part of food-based approaches when and where existing food supplies and limited access fail to provide adequate levels of the respective nutrients in the diet. In such cases, food fortification reinforces and supports ongoing nutrition improvement programmes and should be regarded as part of a broader, integrated approach to prevent micronutrient malnutrition (MNM) thereby complementing other approaches to improve micronutrient status. Food fortification has a long history of use in industrialized countries for the successful control of deficiencies of vitamins A and D, several B vitamins (thiamine, riboflavin and niacin), iodine and iron. (WHO, 2016).

Multiple micronutrient deficiencies including iron, folate and vitamin A are key contributors to morbidity and mortality globally. In Tanzania, anaemia affects 40% of women of reproductive age, with deficiencies in iron and vitamin A measuring 30% and 36%, respectively (Noor *et al.,* 2017). There are widespread vitamin and mineral deficiency problem in Tanzania with known deficiencies of at least vitamin A, iron, folate and zinc, resulting in lasting negative consequences especially on maternal health and cognitive development. Consequently, the nation has resolved to implement a mandatory fortification of maize and wheat flour program. Food fortification is described as the single most cost-effective public health strategy for preventing and controlling micronutrient deficiencies.

1.1.1 Food-to-food fortification

Defined as the addition of micronutrient-dense food/s to a recipe (household level) or food formulation (food industry level), or the replacement of micronutrient-poor/ant nutrient-rich ingredients, to substantially increase the amount of bioavailable micronutrients, with the aim of improving the micronutrient status of populations where the intake of bioavailable micronutrients is inadequate (Krugel *et al.,* 2020). Food-to-food fortification is an approach that uses an interesting (contain useful amounts of micronutrients), available, and accessible local resource (plant or animal) to fortify another food. Though, it is difficult to find an interesting resource that meets the availability and affordable accessibility conditions (Teye *et al.,* 2020). It is also necessary that the fortificant food may not affect sensory properties of the food that needs to be fortified. In food- to-food fortification, the main objective is to improve the nutritional quality of the fortified food without losing sight of the acceptability criteria (mainly the food organoleptic quality) (Chadare *et al.,* 2019).

1.1.2 Benefits of food-to-food fortification

Food-to-food fortification aims at fighting against malnutrition. It helps to improve nutritional, sensory, biological, and physical gaps. Food-to-food fortification usually provides energy, proteins, fat, fiber, carbohydrates, phosphorus, iron, zinc, potassium, manganese, sodium, calcium, and vitamin C (Chadare *et al.,* 2019).

1.1.3 Maize flour

Maize flour is among the main vehicles in fortification programs, for it is the staple food in many African countries, thus increased possibilities and success of nutritional interventions (WHO, 2016). Since maize flour is mainly rich in carbohydrate, there is need to improve the nutritional status of products made from it. As a result, maize flour can be fortified with several micronutrients, such as iron, folic acid and other B-complex vitamins, vitamin A and zinc. Some are used for restitution of nutritional contents and others for preventing micronutrient deficiencies of public health significance (WHO, 2016). Despite fortification being a great tool towards mitigating malnutrition and more so to the vulnerable groups, the use of chemical fortificants is a short-term measure that targets reducing the level of micronutrient malnutrition. It is also necessary to have access to, and to use, fortificants that are well absorbed yet do not affect the sensory properties of foods. Therefore, there is need to think about long term and sustainable strategies. In this research a food to food blending approach was employed as one of the long-term and sustainable strategies in which pumpkin, soybean seeds and maize grains can be blended to form a composite flour.

Maize flour blending with pumpkin and soybean seed flours is a preventive food-based approach to improve micronutrients status of the population by mitigating mineral and vitamins deficiencies identified as public health problems. Maize flour can be fortified by several micronutrients including Iron, Folic acid- Vitamin B-9, Vitamin A, Vitamin B-12, Iron and Zinc which recognized to be public health significance in developing countries.

1.1.4 Pumpkin seeds

Pumpkin seeds (*Cucurbitapepo L*.), refer to the edible seeds of the pumpkin species, are rich in lipids, protein and crude fiber. In addition, the seed is a good source of minerals like phosphorous, sodium, calcium, copper, zinc, magnesium, potassium and iron that are important for human health. These seeds are also rich in protein and oil. In addition, they are good source of mono-unsaturated fatty acids, which are good for heart health. They are also a rich source of lignans, and phytosterols such as delta 7-sterols and delta 5-sterols, essential amino acid like as tryptophan and glutamate. These are beneficial for maintenance of immune system, cell growth and multiplication, eye and skin health, insulin regulation and male sexual functions such as sperm generation and testosterone metabolism (Kumar *et al.,* 2020).

1.1.5 Soybean

Soybean (*Glycine max*) is one of the most important food plants of the World, and seems to be growing in importance. It is a versatile food plant that, used in its various forms, is capable of supplying most nutrients. It can substitute for meat and to some extent for milk. It is a crop capable of reducing protein malnutrition (Ishaq and Ehirim, 2014).Soybean is an abundant and economical source of protein which is apparently cheaper than animal source protein and contains all essential amino acids. High World production, low cost and desirable nutritional and functional properties of soybean make it a substantial contribution to the World’s food protein requirements. Soy protein is normally used as a supplement in different forms of food for the purpose of improving protein quality (Laswai and Kulwa, 2010).

1.2 The Deficiencies in Maize

The deficiencies have been worked out using Tanzania Food Composition Tables (Lukmanji *et al.,* 2008), indicates that the average Maize flour Vitamin A level is 0.00µ g while the micronutrient target level in maize is 1.0mg /kg and the regulatory maximum level is 3.0mg/kg. In the case of Folic Acid the level is 25µ g while micronutrients target level in maize is 1.0mg/kg and regulatory maximum is 3.0mg/kg. For Vitamin B-12 the level is 0.0µ g while micronutrients target level in maize is 0.008mg/kg and regulatory maximum is 0.012mg/kg. For Zinc it is 1.8mg while micronutrients target 30mg/k and regulatory maximum is 45mg/kg. Lastly for Iron it is 3.5mg while micronutrients target 15mg/kg and regulatory maximum level is 25mg/kg.

Table 1.1: Features of Tanzania food composition

Vitamins	A (µgRE)	A-VITA (Mgre)	D	E	C	THIA (Mg)	RIBOFLAVIN (Mg)	NIACIN (Mg)	B6 (mg)	FOLIC (µ g)	B12 (µ g)	PANTO (mg)
Maize, dried, raw	0.0	0.0	0.0	1.0	0.0	0.4	0.2	3.6	0.3	25.0	0.0	0.4
Maize, flour	0.0	0.0	0.0	1.0	0.0	0.4	0.2	3.6	0.3	25.0	0.0	0.4

Source: (Lukmanji *et al.,* 2008).

Table 1.2: Mineral profile for white maize/yellow maize in 100g

Minerals	Ca (mg)	P (mg)	Mg (mg)	K (mg)	Na (mg)	Fe (mg)	MFP-Fe(mg)	Zn (mg)	Cu (mg)	Mn (mg)
Maize, dried, raw	6.0	241.0	127.0	287.0	35.0	3.5	0.0	1.8	0.2	5.0
Maize, flour, white	6.0	241.0	127.0	287.0	35.0	3.5	0.0	1.8	0.2	0.5
Maize, yellow, flour	6.0	241.0	127.0	287.0	35.0	3.5	0.0	1.8	0.2	0.5

Source: (Lukmanji *et al.,* 2008).

Table 1.3: Mineral profiles for pumpkin and soy bean seeds (mg/100)

Minerals	Ca (mg)	P (mg)	Mg (mg)	K (mg)	Na (mg)	Fe (mg)	MFP-Fe(mg)	Zn (mg)	Cu (mg)	Mn (mg)
Pumpkin seeds	97.0	574.0	257.0	301.0	30.0	5.8	0.0	7.6	1.1	1.1
Soybean seed	278.0	705.0	280.0	1798.0	3.0	15.8	0.0	5.0	1.2	2.5

Source: (Lukmanji *et al.,* 2008)

Table 1.4: Vitamin contents for pumpkin and soybean seeds (µ g/100g, mg/100)

Vitamins	Aµ g	Dµ g	Eµ g	C mg	Thia mg	Rib mg	Niacin mg	B6 mg	Folic µ g	B12 µ g	Pant mg
Pumpkin seeds	5.0	0.0	1.0	0.0	0.5	0.1	3.5	0.1	71.0	0.0	0.5
Soybean seeds	3	0.0	0.0	3	3	0.7	1	0.2	133.0	0.0	0.4

Source: (Lukmanji *et al.,* 2008)

1.3 Micronutrient Malnutrition

Micronutrients are nutrients needed by the body in very small amounts including vitamins and minerals. Their impacts on the body health are critical and deficiency in any of them can cause severe and even life-threatening conditions. Micronutrient malnutrition (MNM) can affect all age groups, but young children and women of reproductive age tend to be among those most at risk of developing micronutrient deficiencies (WHO, 2009). Micronutrient malnutrition has many adverse effects on human health, not all of which are clinically evident. Even moderate levels of deficiency (which can be detected by biochemical or clinical measurements) can have serious detrimental effects on human function. Thus, MNM has profound implications for economic development and productivity, particularly in terms of the potentially huge public health costs and the loss of human capital formation. Worldwide, the three most common forms of micronutrient malnutrition are iron, vitamin A and iodine deficiency. These affect at least one third of the world's population, the majority of who are in developing countries. Of the three, iron deficiency is the most prevalent. From a public health viewpoint, micronutrient malnutrition is a concern not just because such large numbers of people are affected, but also because micronutrient malnutrition, being a risk factor for many diseases, can contribute to high rates of morbidity and even mortality. It has been estimated that micronutrient deficiencies account for about 7.3% of the global burden of disease, with iron and vitamin A deficiency ranking among `the 15 leading causes of the global disease burden. According to WHO mortality data, around 0.8 million deaths (1.5% of the total) can be attributed to iron deficiency each year, and a similar number to vitamin A deficiency (WHO, 2009).

The scale and impact of deficiencies in other micronutrients is much more difficult to quantify, although it is likely that some forms of micronutrient malnutrition, including zinc, folate and vitamin D deficiency, make a substantial contribution to the global burden of disease (Denoist *et al*., 2006). The micronutrient content of cereals (especially after milling), roots and tubers is low, so these foods typically provide only a small proportion of the daily requirements for most vitamins and minerals. Fat intake among such groups is also often very low and given the role of fat in facilitating the absorption of a range of micronutrients across the gut wall, the low level of dietary fat puts such populations at further risk of MNM (Denoist *et al*., 2006).

1.4 The Importance of Selected Food Nutrients in Human Body

1.4.1 Proteins

Proteins are essential for human cells and body cells as they form the basic parts within the cells. Proteins are responsible for providing amino acids and nitrogen which is required for non-essential amino acids and nitrogen balance in the body (FAO, 2011). The amino acids are important in linear growth, repair and maintenance of body tissues, formation of antibodies to defend the body against infections, control body electrolytes and fluid balance, regulate acid balance, transport nutrients and provide energy (Rolfes *et al.,* 2014). Protein from animals is complete as they contain all the essential amino acids, while majority of the plant proteins are incomplete. It is recommended that the protein intake of the total energy intake should be between 5-20% of the total energy intake.

1.4.2 Zinc

This is one of the critical micronutrients that is required in the growth and development of young children. Zinc is responsible for cell division and differentiation in children and

therefore inadequate zinc intake predisposes children to stunting (Hambidge *et al.,* 2008). Deficiency also results in reduction in the appetite, poor taste acuity and increased morbidity to childhood illnesses such as diarrhea and respiratory infections as it is important in the development of the innate and acquired immunity in children (Rolfes and Whitney, 2014). The main sources of zinc in the diet are animal source foods such as beef, poultry and organ meats. Legumes and nuts also rich sources of zinc, however the presence of phytate reduces bio-availability of zinc (Akomo *et al.,* 2016).

1.4.3 Iron

There are two major sources of iron in the body, the haem and the non-haem sources. Haem sources are those from animal source foods and are highly bioavailable. Non-haem sources are those of plant origin and include majorly green leafy vegetables, legumes, nuts and cereals. The bio-availability of iron in these foods is low. Iron is critical in the process of blood formation. Low intake of dietary iron is associated with increased levels of iron deficiency anemia. Iron deficiency has been implicated for poor cognitive development, low levels of concentration and low productivity in children (Rolfes and Whitney, 2014).

1.4.4 Folic acid (B9)

Are part of the water-soluble B-vitamin its occur naturally in many foods. Are needed by the human body as a coenzyme substrate in many reactions (single carbon metabolism reactions) of amino acids and nucleotides to synthesize DNA, repair DNA, and methylate DNA as well as to act as a cofactor in biological reactions (during rapid cell division and growth, such as in infancy and pregnancy) involving folate (FAO/WHO, 2005). The three main sources of folate and/or folic acid intake in the diet are (a) naturally occurring food folate (b) synthetic folic acid added to food (fortified foods), and (c) supplements containing folic acid (Berry *et al.,* 2010). In many developed countries, due to the importance of folates in the diet especially in the early stages of pregnancy, folates (folic acid) fortification in cereals especially wheat and maize products has become the most common source of folates in the diet. This is because adequate consumption of folic acid before pregnancy and during the early weeks of gestation protects fetuses from developing neural tube defects (Berry *et al.,* 2010).

1.4.5Vitamin C (ascorbic acid)

The biochemical functions of Ascorbic Acid can be divided into four main categories. Antioxidant: It plays a part in the removal of reactive oxygen species (superoxide, singlet oxygen, ozone and hydrogen peroxide) generated during aerobic metabolism and during exposure to some pollutants, herbicides and environmental stress. In the human body it reduces the risk of arteriosclerosis, cardiovascular diseases and some forms of cancer (FAO /WHO, 2005).

Enzyme cofactor: It is a cofactor in a number of hydroxylase enzymes (example prolyl residues) involved in hydroxyproline synthesis (HP) (FAO /WHO, 2005). In the human body, it also acts as an electron donor for several enzymes (FAO/WHO, 2005). The consequences of scurvy such as breakdown of connective tissue fibres (Oguntibeju, 2008) and muscular weakness.

Electron transport: It acts as an in vitro electron donor for photosynthetic and mitochondrial electron transport. In the human body AA aids in the absorption of non-heme iron

(Oguntibeju, 2008). Synthesis of oxalates and tartrate (used as antioxidants, acidity regulators, and emulsifiers in food).

1.4.6 Vitamin A

The term vitamin A is a fat soluble vitamin and to refer to a group of compounds with biologic activity of all trans-retinol. Vitamin A plays an important role in vision, bone growth, reproduction, cell division, and cell differentiation (Ronoh, 2017). There are two categories of vitamin A: Preformed vitamin A from animal sources and pro-vitamin A (carotenoids) from plant sources. In humans, either form of vitamin A when ingested becomes available as retinol (active form). Vitamin A is well known as an anti-infective vitamin because of its role in the immune system (Mangusho *et al*., 2010). Retinol and its metabolites are required for maintaining the integrity and functioning of the skin and mucosal cells (cells lining the surfaces of the respiratory, urinary and intestinal tracts) which form the body's line of defense (Ronoh, 2017). Vitamin A also has a role in the activation of the lymphocytes. The main sources in the diet are dark green leafy vegetables and deep yellow and orange fleshed foods.

1.5 Effect of Cooking on composition of Vitamin B9 (Folic acid) and vitamin A

1.5.1 Folates (B9)

Is a water soluble vitamin, water soluble vitamins are bound to be deficient in the body since they are not stored in appreciable amounts and therefore, provision of these vitamins is essential in the diets and supplementation in cases when diets do not meet the recommended daily needs (Munyaka *et al.,* 2009). Folates losses during harvesting, storage, distribution, and cooking can be substantial as a result of a combination of thermal degradation and leaching of the vitamin into the cooking water. The degree of loss can be influenced by environmental factors, including temperature, pressure, pH, oxygen, light, metal ions, antioxidants and duration of heating (Wawire, 2014). The method of cooking also has effect on the degree of folate losses. In general, cooking methods that minimize the direct contact of food with the cooking water, such as pressure-cooking and microwave cooking have better folate retention than boiling. The presence of reducing agents in the food can increase folate retention during thermal processing by protecting folates from oxidation processes. Furthermore, modification of the thermal processing conditions, example, low temperature long time (LTLT) or high temperature short time (HTST) in combination with acidification, pressure and antioxidants (like ascorbic acid) can help to improve folate bioavailability (Wawire, 2014; Munyaka *et al.,* 2009).

1.5.2 Vitamin A

Is of public health importance in Tanzania. Vitamins can be lost during cooking in two ways. First, by degradation, this can occur by destruction or by other chemical changes such as oxidation, and secondly by leaching into cooking medium (Kailembo, 2011). Processing and cooking conditions cause variable losses of vitamins. Losses vary widely according to cooking method and type of food. Degradation of vitamins depends on specific conditions during the cooking process, e.g., temperature, presence of oxygen, light, moisture, pH, and, of course, duration of heat treatment (Kailembo, 2011). The major cause of carotenoids destruction during food processing and storage is enzymatic and non-enzymatic oxidation. Isomerization of trans-carotenoids to cis-isomers, particularly during heat treatment, alters their biological activity and discolors the food, but not to the same extent as oxidation (Gibson *et al,* 2006). In many foods, enzymatic degradation of

carotenoids may be a more serious problem than thermal decomposition. In home preparation, losses of carotenoids generally increase in the following order: microwaving, steaming, and boiling. Deep-frying, prolonged cooking, combination of several preparations and cooking methods of carotenoids (Gibson *et al.,* 2006). Carotenoid retention decreases with longer processing time, higher processing temperature. Mostly losses in cooking are very minimal.

Table 1.5: Nutritional Composition of White Maize (Value per 100 g)

Nutrient	Unit	Value per 100 gram of edible portion
Water	Gram	76
Protein	Gram	3.27
Total Fat(lipid)	Gram	5.17
Fiber,total	Gram	2
Sugar, total	Gram	6.26
Carbohydrate	Gram	18.7

Source: USDA National Nutrient, database 2019

Table1.6: Minerals composition of White Maize

Nutrient	Unit	Value per 100 gram of edible portion
Calcium, Ca	Milligram	2
Iron, Fe	Milligram	0.52
Magnesium,Mg	Milligram	37
Phosphorus, P	Milligram	89
Potassium, K	Milligram	270
Sodium, Na	Milligram	15
Zinc, Zn	Milligram	0.46

Source: USDA National Nutrient, database 2019

Table 1.7: Nutrition Goals for Each Age Group

	Source of goal	Age 1-3yrs	Age 4-13yrs	Age 14-30+years
Protein g	RDA	13	19-34	46-56
Dietary fibre g	14g/1000	14	16-25	28-30
Total fat	%Kcal AMDR	30-40	25-35	20-30
Vitamin A mg	RDA	300	400-600	700-900
Vitamin C mg	D, 1U RDA	600	600	600
Folate mg	RDA	150	200-300	300-400
Zinc mg	RDA	3	5-8	9-11
Iron mg	RDA	7	8-10	11-18

Source: 2010- 2015 Dietary Guidelines USDA Food Pattern

1.6 Anti-Nutrition Factors

Anti-nutritionals are important metabolites produced by grains which often serve as a natural defense against attacks from insects, animals and other pathogens (Samitiy *et al.,* 2020). However, they can be harmful to human health as they affect digestions and bioavailability of nutrients when consumed. Several types of anti-nutritional factors with toxic potential have been measured in foods and shown to be heat stable or heat-labile(Elhady,2005). These factors include saponins, tannins, phytic acid, gossypol, lectins, protease inhibitors, amylase inhibitors, anti-vitamin factors, metal binding ingredients,

goitrogens. Nutrition-related problems and harmful effects to human health are raised by these factors, which are present in the seeds of cereals and legumes (Jati, 2018).

1.6.1 Phytate

Phytate or phytic acids occur naturally in the plant kingdom. Phytic acid is a secondary compound, which concentrates naturally in plant seeds, mainly in legumes, peanuts, cereals, and oilseeds and generally found in all plant-based foods. Phytic acid hinders the activity of enzymes, which are necessary for protein degradation in the small intestine and stomach (Samitiy *et al.,* 2020). These phytate tend to bind to certain minerals, including iron, decreasing their absorption by the body. Generally, phytic acids affect the bioavailability of minerals and has a strong effect on infants, pregnant and lactating women when large portions of cereal-based foods are consumed (Jati, 2018). One of the properties of these compounds is that they can precipitate proteins.

1.6.2 Tannin

Tannins are phenolic compounds, Tannins usually affect protein digestibility and lead to reduction of essential amino acids by forming reversible and irreversible tannin-protein complexes between the hydroxyl group of tannins and the carbonyl group of proteins (Samitiy *et al.,* 2020).Tannins accumulates mainly in the bran section of the legumes. When ingested, tannins form complexes with proteins, which cause inactivation of many digestive enzymes and decrease protein digestibility (Jati, 2018).Tannins usually affect protein digestibility and lead to reduction of essential amino acids by forming reversible and irreversible tannin-protein complexes between the hydroxyl group of tannins and the carbonyl group of proteins (Hidvegi, 2002).

1.6.3 Strategies used to reduce anti-nutrientslevels in food

Anti-nutrients cause adverse effects on diet value by reducing nutritional significance of food. These anti-nutrients could cause toxicity when present in higher amounts in the diet. Due to this reasons, reduction in the anti-nutritional content of foods is of great interest (Elhady, 2005). Different traditional methods and technological processing ways such as soaking, milling, debranning, roasting, cooking, boiling, germination and fermentation have been used for reducing these anti-nutritional components (Samitiy *et al.,* 2020).

1.7Problem Statement and Justification

Maize is the staple food in Tanzania with others being rice and wheat. The protein quality in these cereals is inadequate, especially in essential amino acids. Milling of maize involves removal of bran. This further reduces the nutrient density of the end product since fibre, some vitamins and minerals are lost in the process. Some of the micronutrients affected are zinc, calcium and iron (Bonatti *et al.,* 2021). The restoration of niacin, riboflavin and thiamine in maize flour should remain a regular practice in fortification. In rural populations, cases of repeated consumption of this staple food on all the three daily meals in a household over prolonged periods are common. Although they provide the body with the required energy, they are largely deficient in some of the nutrients, therefore the need to diversify our meals. It is therefore important to stress the need to educate families to exploit locally produced foods to produce nutritionally adequate products (WHO and FAO, 2006).

Tanzania has good potential for increased soya bean production. The crop can be grown almost everywhere particularly where common beans and maize are grown. In the year 2004/2005 the total of 15 tons of soy bean was harvested (Ronner, 2013). The two proposed food based fortificants crops are widely available in the country. The production of soy beans in Tanzania was 68 859 tons in 2019 and is estimated to change by an average of 7.65%National Horticulture Development Strategy 2012 – 2021: was drawn by Horticultural Development Council of Tanzania (HODECT), and sets a road map for transforming horticulture sector in Tanzania through achieving the seven pillars of its strategic initiatives including the promotion of horticulture; expanding long-term financing and investment; addressing land, policy and infrastructure bottlenecks; expanding production base and improve quality; strengthen industry linkages and mobilize human resources which are expected to directly address the most critical constraints in the industry and provide the catalyst for expanding the market for Tanzania horticulture for the year 2012- 2016 export trends and production was pumpkin seeds net weight 62 tons (2014year), customer value Tanzania shillings 219 967 641 (Match maker, 2017).

Existing efforts for food fortification largely depend on adding specific minerals. However, the amounts added may either be too little or above the required limit set by regulatory authorities such as the Tanzania Bureau of Standards and intake guidelines recommended by institutions such as the Tanzania food and Nutrition Centre (TFNC). This might lead to other unknown health problems since fortification of maize flour is still at its infancy in this country. Moreover, compared to blended foods, chemical fortificants may pose a threat to the health of consumers if not added in the right amounts (Syliviah, 2019).

1.8Objectives

1.8.1 Overall objective

The overall objective of the study is to improve the nutritional quality of a maize based stiff porridge (*Ugali*) by blending maize flour with pumpkin and soybean seed flours.

1.8.2 Specific objectives

i. To determine optimum mixing ratios of maize flour, pumpkin seeds and soybean flour for nutritional quality.
ii. To assess and evaluate micronutrients of the composite flour.
iii. To determine the effect of cooking on Vitamin A and B9 losses in the stiff porridge.
iv. To perform sensory evaluation of stiff porridge to determine consumer acceptability.

1.9 List of Manuscripts

i. Sensory Quality of Stiff Porridge *(Ugali*) prepared from Pumpkin and Soy bean seed blended Maize flour.
ii. Nutritional Quality of Pumpkin and Soy bean seeds blended Maize flour.

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CHAPTER TWO

Paper One

2.0 Sensory Quality of Stiff Porridge (*Ugali*) Prepared from Pumpkin and Soybean Seed fortified Maize Flour

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CHAPTER THREE

Manuscript One

3.0 Nutritional Quality of Pumpkin and Soybean-seed blended Maize Flour

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Abstract

Maize is a major food staple in most of the sub- Saharan African countries. Maize flour is mostly rich in carbohydrates which provide energy to the human body. Nutritionally, maize flour is deficient in the other major diet requirements of proteins, vitamins and essential minerals. In this study, maize flour was enriched using pumpkin and soybean seed flours and the nutritional qualities of the resulting composite flours and the stiff porridge (*ugali*) prepared using the blended flours were evaluated. Four formulations of maize, soy bean and pumpkin seeds flours were prepared by compositing various proportions of each ingredient. The flours were produced by grinding the seeds using a hammer mill to a particle size fine enough to go through a 1 mm sieve size. The nutritional composition of the flours was observed to change after the blending process as follows; crude fibre from7.3% in plain maize flour to a maximum of 13.45% in the composite flours; crude fat 3.6% -32.26%, ash 1.09% -3.44%, moisture 6.82% -10.29% and Crude protein8.1%-24.09%. In terms of micronutrients, the content of zinc increased from 1.8mg/100g in plain maize flour to a maximum of 3.43mg/100g in the blends, the corresponding contents for iron were 3.5mg/100g -20.81mg/100g, Vitamin A 0.00µg/g- 78.82 µg/g, Folic acid 2.5 µg/g-5.33 µg/g, and Vitamin C 0.00mg/100g -12.23mg/100g, respectively. The effect of cooking was established by evaluating the vitamin A and B9 losses in the prepared *ugali*. The vitamin A losses after cooking were decreased from a minimum of 0.06% to a maximum of 0.88% among the formulations. The vitamin B9 losses after cooking were decreased from a minimum of 0.05% to a maximum of 1.04% among the formulations. Pumpkin and Soy bean seeds flour improved the nutritional quality of the composite flours and the corresponding stiff porridges prepared from the composite flours. The micronutrients compositions were increased due to the added ingredients of soy bean and pumpkin seeds flour. Therefore the mixing of maize, pumpkin-soy bean seed milled flour contributed to the improved nutritional and micronutrients quality of the composite flour stiff porridge.

Keywords: Maize, pumpkin and soy bean seeds flour, Nutrients compositions, micronutrients, *ugali*, stiff porridge, blended maize flour.

3.1 Introduction

Food nutrients for Human consumption are divided into six categories namely: carbohydrates, proteins, fats/lipids, vitamins, minerals and water. All these are further subdivided into macro-and micro- nutrients Macro nutrients include: carbohydrates, proteins and fats/lipids. They are called macro because they are needed in large amounts in the body due to their functions in the body. The second group is micro-nutrients; these are important nutrients but needed in the body in relatively small amounts. The third group is macro-elements; these include Calcium, Phosphorus, Iron, Iodine and Magnesium. These are important elements needed in large amounts. The fourth group is micro-elements which include Selenium, Manganese, Chromium, Vanadium, Molybdenum, Copper and Zinc; these are needed by the body in trace amounts but are very important in the normal physiological functions of the body Tanzania, like other developing countries in sub-Saharan Africa, are faced with challenges of both under- and over nutrition (Lukmanji *et al.,* 2008). Under nutrition comprises of a number of nutritionally related conditions such as protein-energy malnutrition and micronutrient a deficiency, including those of vitamin A, iron, and iodine (Mamiro *et al.,* 2005; Lukmanji *et al.,* 2008). It was also found that, many Tanzanians appear to be deficient in energy and unable to sustain their expected level of physical activity (Lukmanji *et al.,* 2008). At the same time, over nutrition-related diseases such as obesity, diabetes, and hypertension are rapidly increasing among the adult population (Lukmanji *et al.,* 2008), most conspicuously in urban centers, but also in rural areas (Lukmanji *et al.,* 2008). Information of the concentrations of nutrients in indigenous foods forms important components of quantitative studies in human nutrition and nutrition intervention programmes (Lukmanji *et al.,* 2008).

In the present study the nutritional improvements of maize flour after blending with pumpkinseed and soybean seed flours at different ratios were evaluated, both in the uncooked composite flour and in the ready- to- eat stiff porridge(*ugali*) prepared using the various formulations

3.2 Materials and Methods

3.2.1 Samples

White maize, pumpkin- soy bean seeds were purchased at Chief Kingalu Market in Morogoro region, Tanzania.

3.2.2 Sample preparation

3.2.3 White maize grains

Maize was sorted to remove foreign material including stones and broken grains. The sorted grains were thoroughly cleaned using water to remove dust and mud. Cleaned white maize grains were then dried by sun drying.

3.2.4 Soy beans

Soy bean was sorted to remove damaged grain or infested by pests and to remove other extraneous matter. Soy bean seeds were boiled for 45 minutes, after cooling were dehulled then sun drying.

3.2.5 Pumpkin seeds

Pumpkin seeds were sorted to remove foreign material including stones and broken seeds. The sorted seeds were thoroughly cleaned using water, then sun drying 24 hours. The white maize grains, pumpkin and soy bean seeds were milled into fine flour (sieve size-1mm) using a commercial hammer mill (Mzinga corporation, Morogoro, Tanzania). The flour sample was taken and stored in polyethylene packets to be used for analysis in the study.

3.3 Sample Formulation and Composition

Four formulations of white maize, pumpkin- soy bean seeds flours were developed using Nutrisurvey (2007) software, with at least half of the targeted amount of the nutrients of interest in the study were taken. Nutri-survey software is an important analytical tool that helps in determining the potential of different foods to meet daily nutrient requirements of various target groups (Amegovu *et al.,* 2014; Onyango *et al.,* 2020).

Table 3.1: Formulations for the Flour Making (% W/W)

Sample	F1%	F2%	F3%	F4%	Control%
Maize	70	65	60	55	100
Soy bean seed	10	20	30	40	0
Pumpkin seed	20	15	10	5	0
Total	**100**	**100**	**100**	**100**	**100**

3.4 Proximate and Mineral Composition

The proximate composition including dry matter, crude protein, crude fibre, crude fat and ash of the sample formulation were determined according to official AOAC (1999). The results were presented as an average of duplicate determinations.

3.4.1 Crude fat

Total fat was determined by using Soxhlet ether extraction official method 945.87 (AOAC, 1999). The dry sample (5 g) was placed into the extraction thimble and assembled to the soxhtec apparatus. The petroleum ether 60 mL of was used for continuous reflux for 55 min in three phases, the boiling phase for 15 min, the fat extraction phase for 30 min and petroleum ether recovery phase for 10 min. Petroleum ether was then recovered by evaporation. Pre-weighed cups containing fat were dried in an oven at 105°C for 30 min to evaporate any remaining petroleum ether, cooled in a desiccators for 20 min and weighed.

Percentage fat was calculated by using the formula:

$$\%Crudefat = \frac{\text{Weight of crude (g)}}{\text{Weight of dry sample (g)}} x\ 100 \quad \text{.....(i)}$$

3.4.2 Ash content

Ash content was determined according to AOAC (1999), method 923.03. Five grams of dry sample was oven dried at 105°C for 24 h. The weight of crucible and dried sample were recorded. The dried samples in crucibles were incinerated in a muffle furnace at 550°C for 3 h, grey ash was obtained. Ash content was calculated as the difference between the weight of sample before and after incineration. Percentage ash was calculated from the relationship;

$$Ash(\%DM) = \frac{\text{Weight of ash(g)}}{\text{weight of dry sample(g)}} \text{x } 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(ii)}$$

3.4.3 Crude protein

Crude protein content of the samples was determined using the micro-Kjeldahl method 920.87 (AOAC, 1999). The dried sample 0.5 g were weighed and transferred into digestion tubes; 0.6 g of catalyst (mixture of 10 g K2SO4, 0.5 g CuSO4), 6 mL of concentrated H2SO4 were added to each tube. Samples were digested using Tecator digestion system 40 (Model 1016 digester, Sweden) for 3 hr to obtain a clear greenish solution. The digest was cooled and mounted in the distillation unit (Foss Tecator, Model 2200 Kjeltec auto distilling unit, Sweden). The distilled water, 70 mL was added to the digest followed by 70 mL of 40% NaOH and steam distilled for 4 min. The distillate, 50 mL was collected in conical Erlenmeyer flask containing 25 mL of 4% boric acid. The distillate was thereafter titrated with 0.105 g/100 mL hydrochloric acid. The blank volume was carried out and 0.04 mL obtained.

$$\%\text{Nitrogen} = \frac{14.01\text{x(titre}-\text{blank)mL xconcentration of acid in n/mol}}{\text{weight of sample(g)x10}} x100 \ldots\ldots\ldots\ldots\ldots\ldots \text{(iii)}$$

% CP=%$Nitrogenx$ 6.25……………………………………..…………………………..(vi)

3.4.4 Crude fibre

Crude fibre was determined by using AOAC (1999) official method 920.86. Ankomfibre analyzer (Model ANKOM 220, USA) was used for the determination of crude fibre. The sample of 1.0 g was digested in the fibre analyzer by dilute sulphuric acid (0.125 M H2SO4) for 30 min and washed with hot water. The residues were then digested by dilute alkali (0.125 M KOH) for 30 min and washed by hot water. Digested residues were dried in the oven 105°C for 5 h, cooled and weighed. The residues were then placed in muffle furnace and incinerated at 550°C for 2 h, cooled and weighed again. Total fibre content was taken as the difference between the residues before and after Incineration %

$$\%\text{C.F}= \frac{\text{Weight of sample residues before incinereation}-\text{Weight after)g}}{\text{Wheight of dry sample taken for determination(g)}} \text{x } 100 \ldots\ldots\ldots\ldots\ldots \text{(v)}$$

3.4.5 Moisture content determination

Moisture content determination was done using the oven drying method as per AOAC method 920.151 (AOAC, 1990). Five grams of a food sample was weighed into a previously dried and weighed glass crucible. The crucible and its content were placed in a thermostatically controlled oven at 105ºC for 24 h. The contents were cooled in desiccators and then weighed. The loss in weight was recorded as moisture content and it was expressed as a percentage of the total weight of sample used; using the formula:

% moisture = (W2- W3) x100 …………………………………………...…………………… 1
(W2 –W1)

Whereby: w1= weight of empty crucible (g)
W2=weight of container + sample before drying (g)
W3= weight of container + sample after drying (g)

3.4.6 Mineral composition

Mineral composition; iron, zinc, was determined by atomic absorption spectrophotometer (UNICAM, Cambridge, United Kingdom) using procedure of method number 968.08 as described by AOAC 1999. From each sample 5 g was measured in a pre-dried and weighed

crucibles then incinerated at 550oC overnight to ash. The ash was dissolved in 6 N HCl and left for 12 h to allow extraction of Minerals.

3.4.7 Vitamin Determination

3.4.7.1 Vitamin C

Vitamin C content was determined using 2,6-dichorophenol indophenols method as per AOAC Method 967.21 (2005); This was done as described by N.B Bineesh *et al.* (2005) whereby 2g of homogenized sample were ground using mortar and pestle 25ml of 10% trichloro acetic acid (TCA) added and extracted. The extracted sample were then transferred into 50ml volumetric flask and diluted to the mark with TCA solution. The diluted sample is then filtered using No. 1 Whatman filter papers. Standard vitamin C solution with concentration 0, 5, 10, 15, 20 and 25mg/100ml prepared by dissolving 50mg ascorbic acid powder into 100ml distilled water to obtain 50mg/100ml. This solution is then diluted by taking 0, 10, 20, 30, 40, and 50ml of stock solution containing 50mg/100ml vitamin C.

Sample 1.0ml of clear filtrate and 1.0ml of diluted standard were taken into respectively 15ml test tube. 2ml of pH 4.0 Acetate buffer (prepared by mixing 50% sodium acetate and acetic acid in 1:1 ration) added followed by the addition of 1.5ml of 2,6 dichlorophenol indophenols (prepared by taking 0.08g 2,6-dichlorophenol-indophenol and dissolved to 100ml using distilled water). To the mixture, 7.5ml of xylene added. Absorbance of the developed color is then read at 520nm using UV/Vis spectrophotometer (Double beam UV-3000 model X-ma3000 spectrophotometer Human Corporation, England). Vitamin C Concentration in the samples calculated using the following formula obtained from the standard plot

$$\text{Vitamin C content in the samples (mg/100g)} = \frac{(Y-0.052) \times V1}{0.027 \times Sw}$$

Where;

V_1 = Total extraction volume, ml

Y = Corrected sample absorbance (Sample absorbance – blank absorbance)

0.052 = Y-intercept from the standard plot

0.027 = Slope of the standard plot

Sw = Amount of sample taken for analysis

3.4.7.2 Vitamin A (beta- carotene) determination

Beta- Carotene was determined using standard AOAC Method 2005.07 (AOAC, 2005). Where 2.0g homogenized sample taken into mortar and pestle and was extracted 4 times uses 50mls proportions of cold acetone. The 4 portions of extracts were transferred into the separating funnel contained petroleum ether (40-60^{o}C Bp), followed by a thorough washing with about 300mls of distilled water until the extracts were acetone free. During the washing process, the distilled water was added by wall of the glass separating funnel to avoid formation of emulations (water stones) in the carotenoid extracts. The washed samples were then passed through anhydrous sodium sulphate to make it free from any trace of water. The dried carotene extracts was then collected into a clean and dry volumetric flask. The extract was then read under UV-Visible Spectrophotometer at 450nm (Double beam UV-3000 model X-ma3000 spectrophotometer Human Corporation, England).

Beta carotene standard solution with the concentration of 118µg/ml was prepared by taking 0.0118g of β-carotene standard powder obtained from Sigma-Adrich into 100ml volumetric flask. 10ml petroleum ether added and swirled to dissolve and finely petroleum ether added until the volume made to 100ml mark of the volumetric flask. Serial dilution of 0µg/ml, 0.1µg/ml, 0.2µg/ml, 0.4µg/ml and 0.6µg/ml were prepared by taking 0ml, 0.2ml, 0.4ml, 0.8mland 1.0ml of the stock standard solution (118µg/ml) into 25ml volumetric flask. Petroleum ether added to complete volumes (25ml). Absorbencies of the diluted standards read and standard calibration plot constructed the linear regression equation obtained which will be used to calculate the Beta carotene content of the sample as according to Rasaki (2009).

3.4.7.3 Vitamin B9 determination

Vitamin B9 was determined by spectrophotometer as per AOAC method 942.23 (AOAC, 2005). Homogenized sample (2 g) was accurately weighed into erlenmeyer flask and 20 ml of distilled water were added. The contents were placed in a boiling water bath for 20 minutes and cooled. The contents were then transferred to 100 ml volumetric flask and diluted to 100 ml mark with distilled water then filtered using Whatman No 42 filter paper. Standard stock solutions (1 mg/ml) of thiamine HCl (vitamin B1) were prepared by dissolving 0.1 g thiamine HCl in deionized water and diluting to the mark in a100 ml volumetric flask. Working solutions (0 – 1 mg/ml) were prepared by diluting the standard solution serially in deionized water. 1 ml of sample extract and diluted standard were taken into 100ml volumetric flask, 0.19 ml of 0.1M Fe(NO3)3.9H2O added and shaken. 0.6 ml of 0.1M K3[Fe(CN)6] and diluted to mark with distilled water and left to stand for 20 minutes in the water bath at 40ºC and absorbance measured at 747 nm using UV-visible spectrophotometer (X-ma Spectrophotometer, Human Corporation, UK). Thiamine content in the samples was then calculated using the linear regression equation of the standard plot.

3.5 Anti- Nutritional factors

3.5.1 Determination of phytate (Phytic acid)

Phytic acid was determined by the procedure described by Lucas and Markakas (1975) with some modification where by 2.0g of the sample was weighed into a 250 ml conical flask. One hundred mL 2% concentrated HCl was used to dissolve sample for 3 hours and then filtered with Whatman No. filter paper. About 50 ml of the filtrate and 10ml of distilled water were added in each case to give proper acidity. Ten mL 0.3% ammonium thiocyanate solution was added in the solution as indicated and titrated with standard Iron II Chloride solution containing 0.00195 g Iron/mL, end point observed to be yellow which persisted for 5 minutes. The percentage of phytic acid was calculated thus:

% Phytic acid= y×1.19×100

Where, y= titre value ×0.00195g

3.5.2 Determination of tannin

Tannin was evaluated by a procedure described by Pearson (1991). A measured weight of each sample (1.0g) was dispersed in 10ml distilled water for 30 minutes at room temperature being shaken after every 5 minutes. At the end of 30 minutes, it was centrifuged and the extract gotten. The supernatant 2.5ml (extracts) was disposed into a 50ml volumetric flask. Similarly, 2.5ml of standard tannic acid solution was disposed into separate 50ml flask. A 1.0ml Folin-Denis reagent was measured into each flask followed

by 2.5ml of saturated Na_2CO_3 solution. The mixture was diluted to mark in the flask (50ml) and incubated for 90 minutes at room temperature. The absorbance were measured at 250nm in a Genway model 6000i electronic spectrophotometer (Bibby Scientific Ltd, Beacon Road Stone Staffordshire ST15 0SA, United Kingdom)

3.6 Results and Discussion

The findings from this study are presented below. Table 1 presents the proximate composition of the blended flours.

Table 3.1: Proximate composition of maize-soybean-pumpkin seeds composite flour.

Sample	Protein (g/100g)	Fat (g/100g)	Fibre (g/100g)	Moisture (g/100g)	Ash (g/100g)
F1	13.095 ± 0.35^{a}	10.65 ± 0.42^{a}	4.68 ± 0.007^{b}	10.29 ± 0.03^{ab}	2.79 ± 0.007^{a}
F2	13.85 ± 0.00^{b}	13.27 ± 0.11^{c}	5.95 ± 0.02^{c}	9.84 ± 0.007^{ac}	2.62 ± 0.007^{b}
F3	24.04 ± 0.06^{c}	32.26 ± 0.22^{b}	13.45 ± 0.014^{d}	6.82 ± 0.014^{ad}	3.11 ± 0.007^{c}
F4	24.09 ± 0.02^{c}	31.79 ± 1.74^{b}	12.31 ± 0.007^{e}	7.14 ± 0.007^{af}	3.44 ± 0.014^{d}
Control	8.1 ± 0.01^{c}	3.6 ± 0.02^{c}	7.3 ± 0.001^{b}	8.1 ± 0.002^{af}	1.09 ± 0.021^{c}

The Values are expressed as **mean± standard deviation** at two replications (**n=2**). Mean values with different superscript letters along the same column are significantly different at **p≤0.05.**

3.6.1 Crude fat

Crude fat ranged from 3.6%in control sample to F3 32.26%, the increase in fat content was observed between formulation F1 and formulation F4 this can be due to variation in the amount of soy bean and pumpkin seeds. The combination of pumpkin and soy bean seeds flour in F3 results into high fat contents. It was observed a significant difference at p≤0.05 in fat content between the milled sample formulations F1, F2, F3, F4 and control of maize, pumpkin- soy bean seed flour. This was reported by Byamukama (2019). It was observed that as amount of soybean flour and maize germ increased in bread samples, the fat content of bread increased because of the high amount of fat in soybean and maize germ.

3.6.2 Ash

The ash content indicates the inorganic minerals present in the sample; the high level of ash indicated the presence of high levels of minerals. From the study the range of ash was from 1.09% in control sample formulation to 3.44% in F4 formulation. There was a significant difference ($p \leq 0.05$) in minerals between the milled flour samples for all formulations. Similar increase in ash and inorganic nutrients in soybean flour supplementation in composite bread have been reported in previous studies (Mashayekh *et al*., 2008; Sanful and Darko, 2010; Byamukama, 2019). Soybean and maize bran contribute towards high ash contents in the blend because of their high mineral contents. The level of ash in food is an important nutritional indicator for mineral density and quality parameter for contamination, especially with foreign matter (Fennema, 1996). Ndibalema (2011) found that as sardines and soybeans were added to the plain sorghum, the ash content increased significantly.

3.6.3 Crude protein

The range of protein observed were 8.1% for control sample to 24.09% for milled Maize, pumpkin-soy bean seeds flour respectively. This was due to the increase in the amount of soy bean seeds in formulation F4 of 40% compared to formulation F1 of 10%. This shows there were significant different in crude protein within the formulations sample. Dengegh *et al*. (2021) reported that the protein content tends to increase with increase in African Yam Bean (AYB) flour supplementation. This was expected due to the high amount of protein in African Yam Bean (AYB) that means there was significant difference in protein content of the composite flour blends, This variation was shown in Tanzania food composition table Lukumanji *et al.,* 2008) the amount of proteins in soy bean and pumpkin seeds differ, Soy have higher amount of proteins compared to pumpkin seed. A similar increase in the protein content of the composite flour bread with soybean flour addition was reported by (Byamukama, 2019). Protein is very important in general body growth and maintenance of body tissues; it assists the enzymes action in the body which catalyze different metabolic reactions (FAO/WHO/UNU, 1985).

3.6.4 Crude fibre

Crude fibre ranged from 7.3% in control sample to 13.45% in formulation F3. This was due to the amount of maize, soy bean and pumpkin seed in the milled flour both (Lukumanji *et al.,* 2008) and Usda (2020) reported the level of fibre in maize pumpkin and soy bean seeds both indicated that soy bean are rich in dietary fibre. This shows there was significant different in crude fiber between each formulated sample of maize, soy bean and pumpkin blended flour. This was reported by Byamukama (2019) that differences were observed because the crude fiber contents of the breads increased steadily with increasing content of maize bran and soybean flours. Fiber is important in prevention of constipation, overweight, cardiovascular diseases, diabetes and colon cancer however too much of it for older infants and young children increase dietary bulkiness, hence limiting adequate food intake by infants and young children (FAO, 1985).

3.6.5 Moisture content

There were significant differences ($p \leq 0.05$) in the moisture content of the four formulations of blended maize flour. The moisture ranged from 6.82% F3 to 10.29% F1sample formulation which indicate there was significant different in moisture contents within the mixed formulated samples at ($p \leq 0.05$). According to Dengegh *et al.* (2021) there were significant differences in moisture content of the samples. The result shows that African Yam Bean (AYB) flour increased the percent moisture in the samples.

This indicates that, the flour may spoil if not properly stored. Moisture has an implication in terms of the consistency/texture and microbiological quality of food. Generally, grain of higher moisture content is highly susceptible to deterioration. Furthermore, moisture content is highly dependent on the duration of the drying process thus an index of storage stability of the flour (Brewbaker, 2003).

3.6.6 Mineral composition

The mineral compositions of maize, pumpkin –soy bean seeds blended maize flours are shown in Table 3.4 below. The minerals content from the Table increases with increase in pumpkin and soy bean seeds addition. This simply implies that the seeds contain much mineral elements as compared to white maize flour (Lukumanji *et al.,* 2008). Quantitative

determination of mineral elements present in foods is significant because its concentration and type present must often be labeled on food packages. Mineral elements are needed for the proper functioning of the human system, health growth and development. The content of mineral elements in foods depends on the degree of the soils elements and its abundance, including the intensity of fertility (Angeline, 2015).

3.6.7 Iron

Iron performs a wide range of biological functions. Iron occupies a unique role in the metabolic process. The role of iron in the body is clearly associated with hemoglobin and the transfer of oxygen from lungs to the tissue cells. Iron deficiency is the most prevalent nutritional deficiency in humans. Iron is an essential element for human beings and animals and is an essential component of hemoglobin. Furthermore iron facilitates the oxidation of carbohydrates, protein and fat to control body weight, which is very important factor in diabetes. From the study the Iron content ranged from 3.5mg/100g in control sample (100:0:0) to 20.81mg/100g in F3 formulation (60:30:10), maize, soy bean –pumpkin seeds. This was due to the increase in the amount of soy bean flour, this was reported by (Byamukama, 2019) that Soybean contributed to high iron contents in the composite breads because soybean is high in mineral contents. This scenario is also reported by (Gargm *et al.*, 2016). From the study there was a significant different at ($p \leq 0.05$) in the level of iron between the four formulations samples. The study showed that it is possible to enhance the micronutrient contents of maize flour by blending with soybean and pumpkin seeds to suppress micro-nutrient deficiency disorders.

3.6.8 Zinc

The Zinc content ranged from 1.8mg/100g in control sample (100:0:0) to 3.43mg/100g in F3 formulation sample (60:30:10) maize: soy bean: pumpkin seeds. There was significant difference in zinc content among the milled flour sample formulations at ($p \leq 0.05$) and the control sample. There was an increase in the zinc content levels as the combination of pumpkin seeds soy bean seeds were increase in the formulation sample, it was also reported by (Marcel, 2022) since pumpkin seeds are rich in zinc content compared to soy bean seeds (Lukumanji *et al.,* 2008). Zinc is an important micronutrient it is used in the synthesis of enzymes, hormones, proteins and other materials that promote optimal physical and mental growth (King and Burgess, 1993).

3.7 Vitamin composition

3.7.1 Vitamin A

Vitamin A content ranged from 0.00 µg/100gin control sample to 78.82 µg/100g in F1. This indicates the significant difference at ($p \leq 0.05$) in vitamin A content between the control and formulated samples. There was an increase in vitamin A contents when pumpkin and soy bean seeds flour were added in the formulation samples. This was reported by (Ewulo *et al.,* 2017), substitution of soybean for maize in the formulated flour significantly improved Kokoro vitamin A. Similar finding was reported by (Marcel, 2017).The significant variation and increase in Vitamin A (beta carotene) between formulation and control groups was due to the addition of soybean, amaranth grains, pumpkin seeds and orange fleshed sweet potato (SAPO). The increase in vitamin A in food sample will help the consumer to stimulates the production and activity of white blood cells, takes part in remodeling bone, helps maintain healthy endothelial cells, and regulates cell growth and division such as needed for production.

3.7.2 Vitamin B9

Folic acid content ranged from 2.5µg/100g in control to 5.33µg/100gin F1. There was significant difference in vitamin A content between the formulated and control sample at ($p \leq 0.05$). The content of vitamin B9 was increased as the pumpkin and soy bean seed flour were added in each sample formulations. Folic acid contents help to form DNA and RNA, and are involved in protein metabolism. It is used to produce healthy red blood cells and critical during periods of rapid growth, such as during pregnancy and fetal development.

3.7.3 Vitamin C

The vitamin C content ranged from 0.00mg/100g in control sample to 12.23mg/100g in F3 formulated sample. There was significant difference in vitamin C content among formulated samples at $p \leq 0.05$. The difference was due to the amount of pumpkin and soy bean seeds added in each formulation samples. It indicates that pumpkin and soy bean seeds flour were the good source of vitamin C. Similar finding was reported by (Ewulo *et al.,* 2017) that the Substitution of soybean for maize in the formulated flour significantly improved Kokoro vitamin C. Similar finding was reported by (Marcel, 2017). The significant variation and increase in Vitamin C (ascorbic acid) between formulation and control groups was due to the addition of soybean, amaranth grains, pumpkin seeds and orange fleshed sweet potato (SAPO). The ascorbic acid acts as antioxidant and enhance the shelf life of fat containing food, and is therefore able to protect body against free radical induced degenerative diseases due to its antioxidant properties, the vitamin C content in foods also helps in absorption of iron from the food (Ewulo *et al.,* 2017).

The findings are summarized in Table 3.2 below.

Table 3.2: Micronutrient composition of maize-soybean-pumpkin seeds composite flours

Sample	Vitamin C (mg/100g)	Vitamin A (µg/g)	Folic acid (µg/100g)	Iron (mg/100g)	Zinc (mg/100g)
F1	2.24 ± 0.06^{e}	78.82 ± 0.13^{a}	5.33 ± 0.08^{d}	5.22 ± 0.15^{a}	2.13 ± 0.08^{e}
F2	3.79 ± 0.099^{f}	74.01 ± 0.25^{b}	5.29 ± 0.007^{d}	7.57 ± 0.05^{b}	2.37 ± 0.06^{e}
F3	12.23 ± 0.12^{g}	13.50 ± 0.08^{c}	4.42 ± 0.007^{e}	20.81 ± 0.31^{c}	3.43 ± 0.02^{k}
F4	10.74 ± 0.14^{h}	13.59 ± 0.085^{c}	4.45 ± 0.02^{e}	18.50 ± 0.014^{d}	3.25 ± 0.11^{k}
Control	0.0 ± 0.11^{g}	0.0 ± 0.000^{a}	2.5 ± 0.005^{e}	3.5 ± 0.02^{b}	1.8 ± 0.03^{a}

The Values are expressed as **mean± standard deviation** at two replications (**n=2**). Mean values with different superscript letters along the same column are significantly different at **$p \leq 0.05$.**

3.8 Phytate (Phytic Acid)

From the Table 3.3 below indicated that there were significant differences in phytate content between the sample formulations and control atp≤0.05 in both raw and cooked samples of stiff porridge. For the all raw sample and control sample the amount of phytic acid content was higher compared to cooked stiff porridge samples in all formulation. This was similar reported by (Jati, 2008) that was the phytic acid in the maize sample was higher compared to the cooked or boiled samples (Gitau, 2018) indicated the reduction of phytate on boiling of cowpeas, groundnuts, kidney beans. This was due to the fact that

boiling and cooking is the one of the methods used in reducing phytate contents in food samples (Samtiya *et al.,* 2020).

3.8.1 Tannin

From the Table 3.3 below indicated that there were significant differences in phytate content between the sample formulations and control atp≤0.05 in both raw and cooked samples of stiff porridge. For the all raw sample and control sample the amount of tannin acid content was higher compared to cooked stiff porridge samples in all formulation. This was similar reported by (Bello *et al.,* 2020) that the lower tannin content in the cookies (made from composition of wheat, unripe plantain and fluted pumpkin seed composite flour) made it safe for consumption. This was due to the fact that boiling and cooking is the one of the methods used in reducing tannins contents in food samples (Samtiya *et al.,* 2020). Tannins known to bind protein including digestive enzymes leading to poor protein digestibility (Bello *et al.,* 2020).

Table 3.3: Antinutritional factors composition of white maize-soybean-pumpkin seeds composite flour

	Phytate (g/100g)		Tannins (g/100g)	
Sample	**Before**	**After cooking**	**Before**	**After cooking**
1	1.18±0.014^{a}	1.01±0.011^{b}	2.51±0.028^{a}	1.20±0.029^{c}
2	1.12±0.021^{b}	0.54±0.014^{a}	2.22±0.049^{c}	0.86±0.024^{b}
3	0.53±0.0000^{c}	0.245±0.012^{a}	1.86±0.014^{b}	0.65±0.061^{c}
4	0.58±0.007^{c}	0.25±0.018^{b}	2.345±0.021^{c}	1.016±0.212^{b}
Control	0.34±0.018^{a}	0.12±0.023^{b}	1.05±0.001^{c}	0.15±0.034^{a}

The Values are expressed as **mean± standard deviation** at two replications (**n=2**). Mean values with different superscript letters along the same column are significantly different at **p≤0.05.**

3.9 Effect of cooking on the Composition of vitamin A and B9

3.9.1 Vitamin A

There was significant difference in vitamin A content between raw and cooked sample of composite flour stiff porridge at p≤0.05 between the control and formulated sample formulation as shown in Table 3.7 below. There were slightly decrease in vitamin A among formulations, this decrease can be due to increase in temperature during cooking, by degradation, this can occur by destruction or by other chemical changes such as oxidation, and secondly by leaching into cooking medium (Mosha *et al.,* 1995; Mosha *et al.,* 1997; Gibson *et al.,* 2006). The percentage losses partly depend on the cooking temperature and method of cooking. Therefore the slightly decrease does not affect the content of vitamin A regardless in a control sample was zero, so it was still available upon cooking.

3.9.2 Vitamin B9

Folic acid is a water soluble vitamin, they are not stored in appreciable amounts and therefore, provision of these vitamins is essential in the diets. From the Table 3.7 below shows the significant difference in vitamin B9 between control and formulated samples, the decrease in vitamin B9 in cooked samples was due to degradation, this can occur by destruction or by other chemical changes such as oxidation, and secondly by leaching into

cooking medium. This finding is similar to that reported by (Kailembo, 2011; Mosha *et al.,* 1995; Mosha *et al.,* 1997; Gibson *et al.,* 2006) on the decrease in vitamin B9 in vegetables during boiling and cooking. But it still available in large amount compared to the control sample which was very minimal.

Table 3.7: Effect of cooking on the Composition of vitamin A and B9

Sample	Vitamin A(µg/g)		Folic acid(µg/100g)	
	Raw	Cooked	Raw	Cooked
F1	78.82±0.134^{a}	78.76±0.39^{ab}	5.36±0.035^{ac}	4.79±0.04^{b}
F2	73.99±0.276^{b}	73.33±0.76^{ac}	5.25±0.057^{ad}	4.21±0.021^{c}
F3	13.38±0.092^{c}	12.50±0.46^{ad}	4.57±0.21^{e}	4.05±0.021^{d}
F4	13.52±0.014^{d}	13.36±0.12^{ef}	4.49±0.05^{af}	3.86±0.014^{e}
Control	0	0	2.5±0.31^{e}	2.4±0.03^{e}

3.10 Conclusion

Soybean, pumpkin seeds and maize are locally available and affordable raw materials that can be incorporated in stiff porridge (*Ugali*) making to improve its nutritional quality. From the study, it was observed that there was increase in nutrient content of the composite stiff porridge formulated, particularly samples F3and F4 that had significantly the highest protein (24.04%), fibre (13.45%), fat (32.26%), iron (20.81 mg/100g) and zinc (3.43mg/100g). Formulation F4protein (24.09%), fibre (12.31%), fat (31.79%), ash (3.44%), iron (18.50 mg/100g) and zinc (3.25mg/100g).The enhancement of the nutritional composition of stiff porridge with addition of soybean flour, maize and pumpkin seeds flour could help to alleviate the problems of micronutrient malnutrition and protein- energy malnutrition in children and adults which affects the quality of life.

3.11 Recommendations

Production of maize, pumpkin and soy bean seeds should be encouraged by Tanzania government because of its nutritional values and income generation potential. The blending of maize, pumpkin and soybean seeds to composite flour should be promoted within the communities by local leaders and village health teams to eliminate the case of malnutrition.

3.12 Acknowledgement

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CHAPTER FOUR

4.0 CONCLUSIONS AND RECOMMENDATION

4.1 Conclusions

The study showed the possibility of combination of white maize, pumpkin and soy bean seeds flours in different ratios so as to improve the nutrients composition and therefore increase the utilization of pumpkin-soy bean seeds blended maize flour.

Nutritionally, cereals perform better when combined with legumes such that the formulations in which pumpkin and soy bean seeds were added had better nutritional composition than control plain white maize flour. The combinations made at ratios (w/w) 65:15:20; 60:10:30; and 55:5:40 maize –pumpkin and soy bean seeds, had the best nutritional quality based on studied nutrients.

Maize blended flour with pumpkin and soy bean seeds improved the minerals and vitamins content such as iron, zinc, vitamin A, folic acid, and vitamin C which are important in the diet.

From sensory evaluation test indicated that there were no significant difference among the attributes in sample formulations, since the formulations are practical the panelist members accepted the stiff porridge samples (*ugali*).

This study confirms that the approach of blending maize, pumpkin and soy bean seeds to composite flour can provide a sustainable alternative to current chemical fortification approaches if indigenous food crops of high nutrient content are incorporated in common staple diets. Strategies such as nutrition education can be explored in order to enhance acceptability.

The finding from this study will contribute to food science and nutrition research and practice specifically blending of maize with pumpkin and soy bean seeds in addressing household nutrition and malnutrition in developing countries.

Therefore according to table 6 above USDA dietary guideline formulation 1 and 2 are suitable for age 1-3years, 3 and 4 for 4-13years. 14-30+years that provide at least half of recommended daily allowances (RDA).

4.2 Recommendations

This work recommends the production, promotion and utilization soybean flour, maize and pumpkin seeds flour in stiff porridge making in Tanzania. However, further research work should be focused on how to improve sensory quality to enhance overall acceptability of the final composite stiff porridge

Recommendation for policy: Policies on blending of commonly consumed staples in Tanzania with pumpkin and soy beans to improve the nutritional adequacy of the diets should be made.

Recommendation for practice: As one of the ways of reducing the macro and micronutrient malnutrition in Tanzania awareness of the nutritional benefits of white maize, soy bean and pumpkin seeds should be raised to increase its adoption and therefore consumption in the households. This can be done though nutrition education programs.

APPENDICES

Appendix 1: Questionnaires for sensory evaluation of the Developed Products.

SOKOINE UNIVERSITY OF AGRICULTURE

SCHOOL OF AGRICULTURE

DEPARTMENT OF FOOD SCIENCE AND BIOPROCESSING

P.O. Box 3006, MOROGORO – TANZANIA

Name:……..…….. (Optional) Date:…….

Age: …….. Sex: …….. Panelist No.: ……..

CONSUMER ACCEPTANCE TEST

In front of you are FOUR *UGALI* samples: starting with sample on your left, evaluate each **attribute** and indicate the degree of liking by writing its correspondence number. Use the mouth cleanser (Drinking water) provided to rinse the mouth between samples. Take time to observe and taste the samples parameters before deciding the degree of liking.

Hedonic scale

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

Attributes	SAMPLES			
	347	603	195	481
Aroma				
Color				
Smell				
General appearance				
Texture (mouth feel)				
Taste				
Flavor				
Oiliness				
Springiness				
Hardness				
General acceptability				

Comments:

……

……

Are you the frequent user of the food product provided?. **Yes/ No (Circle your response)**