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Use of Cassava-Whole Chia Flour for Gluten-Free Cookie Production

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DECLARATION

I Edward Alan Mutaawe, declare that this dissertation is my original work and to the best of my knowledge it has never been submitted to any university or institution for any academic reward.

EDWARD ALAN MUTAAWE

Signature…………………………

Date…03/11/22………………….

This report has been submitted for examination with approval of my supervisor

DR. STELLAH BYAKIKA

Signed…………………………

Date…04/11/2022…………………...

DEDICATION

I dedicate my efforts and struggles of education life to my dear parents and family.

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First of all, I thank God who is the holder of my breaths, without his order nothing is possible.

Great thanks to my parents and siblings whose love and support has always been my energy to work hard and push through this project towards its success.

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TABLE OF CONTENTS

LIST OF TABLES

ABBREVIATIONS AND ACRONYMS

SFTNB	School of Food Technology, Nutrition and Bioengineering.
FTBIC	Food Technology Business Incubation Center
CD	Celiac Disease
EFSA	European Food Safety Authority
FAO	Food and Agriculture Organization
CTA	Technical Centre for Agricultural and Rural Cooperation
EC	European Commission
IFAD	International Fund for Agricultural Development
FW	Fresh Weight
DM	Dry Matter
NIDDK	National Institute of Diabetes and Digestive And Kidney Diseases
W/W	Weight by Weight
AR	Arrhenius
HMF	Hydroxymethylfurfural
HCL	Hydrochloric Acid
ANOVA	Analysis of Variance
LSD	Least Significant Difference

ABSTRACT

Previous studies have evidenced the importance of chia seeds flour in the enhancement of the nutritional value of various foods. However, no emphasis has been put on the supplementation of gluten-free cassava cookies with chia. Thus, its effects on the nutritional properties of cassava cookies were unknown. This research evaluated the effect of incorporating chia seeds flour on the protein content of cassava cookies and their sensory acceptability. Cassava flour was substituted with chia flour at levels of 5%, 10% and 15% chia seeds to form flour combinations for the production of the cookies. Addition of chia seed flour significantly ($P<0.05$) improved the protein content of the cookies. Cookies with 15% chia flour had 1.8times more protein than the control (0% chia). Acceptability scores reduced with increase in chia incorporation. Among the chia cookies, those with 5% chia flour were most accepted and were quite similar to the control. These were followed by cookies with 10% chia flour and lastly those with 15% chia flour. Although, incorporation of chia reduced the sensory acceptability of the cookies, all scores were above 6 'like slightly', implying they were accepted. Cassava cookies can, therefore, be supplemented with chia seed flour up to 15% without rendering them unacceptable to the consumer whilst improving their protein content.

Key words: cassava, cookies, gluten, chia

1 INTRODUCTION

1.1 Background

The increasing number of celiac and gluten-intolerant consumers has resulted into increasing growth of market for gluten-free products. Celiac disease (CD) is one the various medical conditions caused by intolerance to gluten (Chakrabarti et al., 2017). It is an autoimmune disorder in which the immune system responds abnormally to gluten (Ostermann-Porcel et al., 2017). It is an inflammatory disease of the small intestine triggered by gluten proteins from wheat, barley and rye (de la Barca et al., 2010).

One of the replacements of wheat flour and other common grains containing gluten is cassava flour (Chakrabarti et al., 2017). Considered as the "food of the poor", cassava is a multipurpose crop that responds to the priorities of developing countries and to trends in the global economy (FAO, 2013). Cassava as a crop has advantages including flexibility in planting and harvesting time, drought tolerance and its ability to grow and produce in low nutrient soils (Fregene et al., 2000). Cassava is known to be a good and cheap source of carbohydrates (65-70% dry matter starch) (Chakr (Dhankhar, 2013)abarti et al., 2017). Cassava flour has some functional properties due to which it can be used in bakery products.

The increasing urbanization has resulted into a rise in consumption of bakery products (Akubor, 2003). Cookies constitute a major portion of bakery products, they are feasible fiber carriers because of their longer shelf-life. They are relished as snack food product by children and adults due to variety in taste, crispiness and digestibility (Bamgbose A, 2015).

This project was conducted to produce Cassava Cookies. However, cassava flour alone provides little or no protein therefore requires enriching (CTA, 1995). Chia (Salvia hispanica) is an excellent source of protein. The protein content of chia ranges between 20-22% (EC, 2009) . Chia protein has a good balance of essential amino acids (Sandoval-Oliveros & Paredes-López, 2013), required for proper growth and maintenance of body. Incorporating whole chia flour in the production process will improve the nutritional quality it may also improve the flavor and texture of the cookies.

1.2 Problem statement

Proper nutrition is key to a healthy lifestyle for all individuals. The current essential therapy of celiac disease is a strict adherence to a gluten-free diet, which means a permanent withdrawal of gluten from daily food. For this reason, the market demand for gluten-free products has increased tremendously (Dhankhar, 2013). Previously, research has mostly been focused on gluten-free bread (Xu et al., 2020). In the same manner, gluten-free cookies have also been studied; using cereals (rice, maize, sorghum, millet), legumes, pseudocereals, their blends (Xu et al., 2020) and tubers like cassava (Chakrabarti et al., 2017).

Cassava cookies present an excellent solution towards the treatment of celiac disease. Cassava however, has an extremely low protein content (1-3%) (Salcedo et al., 2010) which limits its nutritional potential in terms of protein provision. It is therefore, important that it is enriched with a protein source. Chia flour can be used to improve the protein content of cassava cookies by replacing part of the cassava flour with whole chia flour.

The purpose of this project was to enhance the nutritional quality of gluten-free cassava cookies in terms of protein. This would improve the nutritional diversity for celiac patients and gluten intolerant individuals. This study would also contribute towards the increasing market and demand for gluten-free foodstuffs.

1.3 General objective

To improve the nutritional quality of gluten-free cassava cookies by enriching them with whole chia flour.

1.4 Specific objectives

1. To improve the protein content of the cassava cookies using whole chia flour.
2. To determine the sensory acceptability of the cassava-chia cookies.

1.5 Hypotheses.

1. Substitution of cassava flour with chia flour increases the protein content of the cassava cookies.
2. Addition of whole chia flour to the cassava cookie formula improves the sensory acceptability of the cassava cookies.

2 LITERATURE REVIEW

2.1 Cassava

Cassava (Manihot esculanta crantz) is a perennial subtropical crop important for its underground starchy tubers (Grace, 1997). It is a starchy root crop grown mostly in the hotter low land tropics and is an important energy source (Kaur et al., 2016). It is a staple food for more than 500million people in Africa, Latin America and Asia (Alicai et al., 2007). Cassava is important in food security and nutrition as it is a source of income for producers, processors and trades contributing substantially to poverty alleviation (FAO and IFAD, 2001). It has features such as great potential for the production of starch, tolerance to drought, its adaptation to difficult ecosystems such as acid soils of low fertility and its good flexibility on planting and harvesting adapting to different growing conditions (Aristizábal et al., 2017).

Cassava root is an energy-dense food. The root possesses an energy reserve with high carbohydrate content ranging from 32% to 35% on a fresh weight (FW) basis, and from 80% to 90% on a dry matter (DM) basis (Montagnac et al., 2009). About 80% of the carbohydrates produced in cassava root is starch (Gil JL, 2002); 83% is in the form of amylopectin and 17% is amylose (Rawel HM, 2003). Roots contain small quantities of sucrose, glucose, fructose, and maltose (Tewe OO, 2004). The fiber content in cassava roots depends on the variety and the age of the root. It does not usually exceed 1.5% in fresh root and 4% in root flour (Gil JL, 2002). The lipid content in cassava roots ranges from 0.1% to 0.3% on a FW basis (Montagnac et al., 2009). The glycolipids are mainly galactose-diglyceride (Gil JL, 2002). The predominant fatty acids are palmitate and oleate (Hudson & Ogunsua, 1974). The protein content is low at 1% to 3% on a DM basis (Buitrago 1990) and between 0.4 and 1.5 g/100 g FW (Bradbury J.H. & Holloway W.D., 1998). The content of some essential amino acids, such as methionine, cysteine, and tryptophan, is very low. However, the roots have high amounts of arginine, glutamic acid, and aspartic acid (Gil JL, 2002). About half of the protein in the roots is whole protein and the other half is free amino acids (predominantly glutamic and aspartic acids) and non-protein components such as nitrite, nitrate, and cyanogenic compounds (Montagnac et al., 2009). Cyanogenic compounds are present and predominate in bitter varieties (Montagnac et al., 2009).

Cassava contains potassium, iron, calcium, vitamin A, folic acid, sodium, vitamin C, vitamin B-6 and protein (Montagnac et al., 2009). The cassava root contains significant amounts of iron, phosphorus and calcium and is relatively rich in vitamin C (*ENIDOK.Pdf*, n.d.). The calcium content is relatively high compared to that of other staple crops and ranges between 15 and 35 mg/100 g edible portion. The vitamin C (ascorbic acid) content is also high and between 15 to 45 mg/100 g edible portions (Okigbo, 2018). Cassava roots contain low amounts of the B vitamins, that is, thiamin, riboflavin, and niacin and part of these nutrients is lost during processing (Montagnac et al., 2009).

2.2 Cookies

Cookies are significant bakery products in the world. These are an important food product used as snacks by children and adults (Bamgbose A, 2015). Cookies hold an important position in snack foods due to variety in taste, crispiness and digestibility. The difference between cookies and other baked products like bread and cakes is their low moisture content that ensures they are free from microbial spoilage and long shelf life (Dhankhar, 2013). The principal ingredients during the manufacture of cookies are flour, fat, sugar and water (Panghal et al., 2011). Cookies are characterized by a formula high in sugar and shortening (Xu et al., 2020). They are usually referred to as baked products made from soft wheat flour with low final water content (Delcour & Hoseney, 1986)

2.2.1 Gluten-free cookies

Gluten network development in cookies is relatively limited and dependent on the type of desired final product (Xu et al., 2020). Cookies with hard texture requires some gluten network development (Di Cairano et al., 2018), whereas cookies from short dough with high proportion of fat and sugar rely on the starch gelatinization rather than gluten network development (Dapčević Hadnadev et al., 2013). A high amount of gluten protein in the short dough reduces cookie spread

(HadiNezhad & Butler, 2009). Using gluten-free flours therefore, assures the possibility of producing gluten-free cookies with desired texture and spread (Xu et al., 2020).

Past studies on gluten-free cookies have used flours from cereals (such as rice, maize, sorghum, millet), legumes, pseudocereals, and their blends (Xu et al., 2020). Rice flour has most commonly been used among gluten-free flours, often combined with other flours, such as maize starch and pea protein (Mancebo et al., 2016). Cookies are also developed using oat (Duta & Culetu, 2015), millet flour, and chia seed (Brites et al., 2019).

2.3 Role of gluten in baking

Gluten is a complex mixture of storage proteins of wheat, a staple food for most populations in the world, and other cereals (rye and barley) (Rahim, n.d.). It is known that, the bread making quality of wheat flour depends on both the quantity and quality of its gluten proteins (Rahim, n.d.). The gluten proteins contribute 80–85% of the total wheat protein and are the major storage proteins of wheat (Rahim, n.d.). These proteins belong to the prolamin class of seed storage proteins (Shewry & Halford, 2002). Two functionally distinct groups of gluten proteins can be distinguished: monomeric gliadins and polymeric glutenins (Lindsay & Skerritt, 1999). The gliadin fraction contributes to the viscous properties and dough extensibility of wheat dough (Don et al., 2006); while, the glutenin fraction improves the elasticity and strength of the dough (MacRitchie, 1979). The relative proportions of gliadin and glutenin found in dough affect the physical properties of dough, with higher relative proportions of glutenin imparting greater dough strength (MacRitchie, 1987).

2.3.1 Gluten and health

Consumption of gluten has resulted into the rising frequency of three pathological conditions: 1) food allergy, which affects 0.2-0.5% of the population but has major clinical implications 2) gluten sensitivity, a recently discovered condition due to gluten intolerance and 3) celiac disease (Mancebo et al., 2015). Celiac disease (CD) is a permanent inflammatory disease of the small intestine triggered by the ingestion of gluten containing cereals (Chakrabarti et al., 2017). The prevalence of CD is 1%-2% in any population around the world (1). It is an autoimmune-mediated disorder that affects the gastrointestinal tract. Symptoms of celiac patients include diarrhea, malnutrition, anemia, abdominal crumpling, pain and fatigue (Faulkner-Hogg et al., 2009).

The only CD treatment is a gluten-free diet (NIDDK, 2008). Complete avoidance of gluten allows the intestine to heal, and the nutritional deficiencies and other symptoms to resolve (po on am, 2013). Following a strict gluten-free diet also reduces the risk of developing most of the long-term complications related to untreated CD.

2.3.2 Gluten in foods

According to the European Commission foods for people intolerant to gluten should contain a level of gluten less than 100 mg/kg (100 ppm) as sold to the final consumer (EC No 41/2009). It also regulates that gluten content less than 100 mg/kg can be labelled as “very low gluten” and gluten content less than 20 mg/kg as “gluten-free”, due to the fact gluten intolerance varies among individuals (McCabe, 2010). Similarly, the U.S. Food and Drug Administration defines gluten-free food as the food that is either completely gluten-free or does not contain any of these ingredients: 1) gluten-containing grain (e.g., wheat); 2) derived from gluten-containing grain that has not been processed to remove gluten (e.g., wheat flour); and 3) derived from a gluten containing grain that has been processed to remove gluten (e.g., wheat starch), if any use of these ingredients contains the presence of 20 ppm or more gluten in food. In summary, gluten-free foods should not contain gluten, or the presence of gluten should be lower than 20 ppm (Xu et al., 2020).

2.4 Chia

Chia (Salvia hispanica) belongs to the Salvia category of the Labiatae family (Zettel & Hitzmann, 2018). Salvia hispanica is a subtropical annual herbaceous plant which requires less water compared to other crops (Zettel & Hitzmann, 2018). The plant may reach 1m in height (Kulczyński et al., 2019). Its serrated leaves, arranged opposite are 4-8cm in length and 3-5cm in width (FAO, 2013). It bears white or blue bisexual flowers of 3-4mm in size, growing in whorls at shoot tips (Kulczyński et al., 2019). Chia forms round fruits, containing many tiny, oval seeds of 2mm length and 1mm width (Kulczyński et al., 2019). The surface of the seed is smooth, shiny, ranging in color from white, Green to brown with irregularly arranged black spots (FAO, 2013; Mohd Ali et al., 2012).

Salvia genus is commonly applied in flavoring and folk medicines worldwide (Lu & Foo, 2002). Chia has been used for medicinal purposes for thousands of years (FAO, 2013; P., 2003). Chia seeds have earlier been used as whole seeds, seed flour, seed mucilage and seed oil (Zettel & Hitzmann, 2018). They are consumed as ingredients or additives to many foods: diary, baked products, fruit smoothies, salads and others (Inglett et al., 2014; Luna Pizarro et al., 2013; Steffolani et al., 2015). They are also used as thickeners in soups and sauces (Kulczyński et al., 2019).

The chemical composition of chia seeds has been analyzed in many studies. Chia seeds have got high nutritive value particularly due to their high contents of dietary fiber and fat. The European commission gives the approximate composition of chia as: 91-96% dry matter, 20-22% protein, 30-35% fat, 25-41% carbohydrate, 18-30% crude fiber and 4-6% ash (EC, 2009).

2.4.1 Dietary fibre

Chia seeds contain approximately 30-34% dietary fiber of which the insoluble fraction (IDF) accounts for approximately 85-93% while soluble dietary fibre (SDF) is approximately 7-15% (Marineli et al., 2015; Reyes-Caudillo et al., 2008). Total dietary fiber includes polysaccharides, oligosaccharides, lignin and other associated substances (Zettel & Hitzmann, 2018). The dietary fiber content of chia seeds exceeds dried fruits, cereals or nuts.

Chia seeds form mucilage hydrated in water due to their composition; the mucilage could be used as industrial additive due to its outstanding physiochemical properties (Salgado-Cruz et am, 2013). Dietary fiber is most commonly incorporated in baked products to prolong freshness for their capacity to retain water (Zettel & Hitzmann, 2018). The suggested use of chia fiber as hydrocolloid is due to its high-water holding capacity of 15.41g/g of chia fiber (Alfredo et al., 2009).

2.4.2 Protein

Chia seeds are a good source of plant protein with approximately18-24% of their mass (Grancieri et al., 2019). The amino acid composition analysis confirmed the presence of 10 exogenous amino acids, with the greatest contents for arginine, leucine, phenylalanine, valine and lysine (Kulczyński et al., 2019). Chia proteins also have a significant amount of endogenous amino acids, especially glutamic and aspartic acids, alanine, serine, and glycine (BUSHWAY et al., 1981; Nitrayová et al., 2014). The protein has a good balance of essential amino acids (Sandoval-Oliveros & Paredes-López, 2013). Chia seeds ate gluten-free and therefore fit for consumption by celiac patients (Kulczyński et al., 2019).

2.4.3 Fatty acid composition

The fatty acid profile of chia seeds is characterized by high contents of polyunsaturated fatty acids, mainly alpha linoleic acid (ALA), which accounts for approximately 60% of all fatty acids. Linoleic, oleic and palmitic acids are found in lower amounts (Kulczyński et al., 2019). The ratio of omega-6 to omega-3 acids of 0.3:0.35 is advantageous (Ayerza (h), 1995; Ayerza h & Coates, 2011; Ciftci et al., 2012; Nitrayová et al., 2014; Peiretti & Gai, 2009). Chia oil rich in alpha linoleic acid might be an alternative omega-3 fatty acid source for vegetarians and people allergic to fish and fish products (Zettel & Hitzmann, 2018).

2.4.4 Chia in bakery products

Chia is used in gluten-free diet especial breads, to enhance the nutritional quality (Zettel & Hitzmann, 2018). Gluten-free bakery products have a low nutritional quality. They try to mimic gluten and contain more fat and salt but fewer minerals and vitamins than their equivalents with gluten (Pellegrini & Agostoni, 2015). Due to the mucilage release chia can also be regarded as technological improver for gluten-free breads. Combining chia with other nutritionally important materials can produce excellent applications (Costantini et al., 2014; Moreira et al., 2013). (Costantini et al., 2014) were substituting 10 % buckwheat flour with chia flour in gluten-free bread. They obtained no significant change in the specific volume, but an increase in moisture, fat, dietary fibre content, and a decrease in carbohydrate content. The amount of linoleic acid and linolenic acid increased. Corn tortillas with reduced glycemic index and significant higher levels of protein, lipids and total dietary fibre were produced with 15 % and 20 % addition of milled chia flour (Rendón-Villalobos et al., 2012). Chia flour was also used for rice and soy-based gluten-free breads (Huerta et al., 2016).

2.4.5 Regulations on use of chia

Chia is allowed in baked products up to 5 % as a novel ingredient (EC, 2009). Furthermore, the use of chia as novel food ingredient was extended to 10% (EC, 2013). The limitation on its use was due uncertainties towards its potential allergenicity (EC, 2013). According to the EC (2013), baked products, breakfast cereals and fruit, nut and seed mixes should not contain more than 10 % chia. The daily intake of chia should not exceed 15 g.

This toxigenicity is concerned with the formation of process contaminants such as acrylamide, hydroxymethylfurfural (HMF) or furfural during thermal treatment (Mesías et al., 2016). These contaminants are produced during a Maillard reaction initiated by carbohydrates or carbonyls generated from lipid oxidation (Zamora & Hidalgo, 2005). Asparagine and reducing sugars are the mains precursors for the generation of acrylamide (Mesías et al., 2016). The presence of acrylamide in food is a public health concern requiring continued efforts to reduce its exposure (E. Panel & Chain, 2015). HMF and furfural are formed as intermediate products of the Maillard reaction; HMF is also produced by caramelization of sugars at high temperature (Morales, n.d.). HMF is suspected to have potential genotoxic and mutagenic effects (Moura & Houten, 2010) while furfural may lead to hepatotoxicity (S. Panel et al., 2004).

(Mesías et al., 2016), investigated the effect of incorporating different amounts of chia flour in wheat-based biscuits on the formation of acrylamide, HMF and furfural. They found that the presence of chia has a strong influence on acrylamide, HMF and furfural formation in biscuits. Addition of 5% chia significantly increased acrylamide formation. When 10 % chia (acceptable level) was incorporated, levels of acrylamide even exceeded the indicative value established by the European commission (500µg/kg) (EC, 2011). Similar results were found to the HMF and furfural content in biscuits.

3 MATERIALS AND METHODS

3.1 Cassava flour

Packed pure refined cassava flour from a supermarket in Kampala. The cassava flour was stored at ambient temperature.

3.2 Whole Chia flour

Properly packaged good quality whole chia flour from SAGE Uganda, Kampala. The whole chia flour was stored in a plastic container at ambient temperature.

3.3 Cookie production

Cookies were made based on four formulations (Table 1). The procedure for making the cookies is summarized in Figure x.

Table 1: Composition of prepared flours

Formulations	Cassava	Whole Chia flour
0%	100	0
5%	95	5
10%	90	10
15%	85	15

Weighed All the Ingredients

↓

Beat butter for one minute

↓

Added sugar and beat till light and fluffy

↓

Added the eggs and beat well for 30 s

↓

Added Baking powder and mix well

↓

Mixed and kneaded well to make a non-sticky dough

↓

Roll the dough on a pastry board in uniform thickness

↓

Cut the dough using a Cookie Cutter

↓

Line cookies on an oven try by placing them on a layer butter

↓

Baked at 180°C for 20 min

↓

Removed tray and cooled for 30 min

↓

Packaged in polyethylene bags

Figure x: …………………..

3.4 Analyses

3.4.1 Crude Protein

The Kjeldahl method was used as described by Kirk and Sawyer (1991). It is assumed that all protein in the sample contains 14% nitrogen. About 0.5 g of sample was weighed out in triplicates into clearly labeled digesting Kjeldahl tubes. An aliquot of 10 mL of AR concentrated sulphuric acid and one spatula of mixed complex Kjeldahl catalyst was added to each tube. The samples in each tube were digested at 360°C on a digesting block to clear solution; this converted the nitrogen in the protein to ammonium sulphate. The digested samples are then cooled down and diluted to exactly 50 mL with distilled water. An aliquot of 5 mL of diluted samples were taken for distillation in Markham distillation apparatus to distill off ammonia which is trapped in calibrated 2% boric acid solution. Three drops of 1% phenolphthalein were added & about 5 mL of 40% sodium hydroxide to neutralized the acid and facilitate liberation of ammonia gas. The distillate was titrated against 0.05 M hydrochloric acid to determine the ammonia absorbed by boric acid. Protein content was calculated as;

$$\% \text{ Crude protein} = \frac{(V_2 - V_1)M_{HCL}}{W} X\ 14 X 6.25 X 100$$

Where,

V_2 = Volume (ml) of hydrochloric acid solution required for the test sample

V_1 = Volume of hydrochloric acid required for the blank test

M_{HCL} = Morality of Hydrochloric acid

W = Weight in grams of test sample

6.25 = Nitrogen conversion factor of protein

14 = Atomic mass of Nitrogen

3.5 Dietary Fiber

Dietary fiber of food matrix was determined using the method described by Kirk and Sawyer (1991). About one gram of the sample was weighed out in triplicate into a 600 ml conical flask, add 100 ml of acid detergent fiber and boiled for one hour in fiber analyzer (Labconco, Uk). The digest was filtered through glass sinter crucible connected to a vacuum pump (Charles Austen pumps ltd, UK) to collected the residues which are then taken to the oven for drying at 100°C for 45 min to drive off the moisture. Dietary fiber was calculated using equation below.

$$\% \text{ Dietary fibre} = \frac{w_{1-w_2}}{w_s} x\ 100$$

Where: W_2 = Weight of dry sinter-glass (g) and W_1= Weight of dry sinter-glass and sample (g) and W_s = weight of the sample.

3.6 Sensory evaluation

The organoleptic evaluation of the coded cookies was carried out by an untrained panel of 30 members in partitioned booths. Sensory attributes: appearance, taste, aroma, mouthfeel, and overall acceptability were evaluated under amber light while appearance shall be evaluated under bright illuminated light, using a nine-point hedonic scale as shown in the Table 3 below.

3.7 Statistical data analysis

Analyses were performed in duplicate. Means and standard deviations were calculated. Analysis of variance (ANOVA) was used to detect significant differences among treatments (4 treatments: control cookies, 5% chia, 10% chia and 15% chia). Mean comparisons were made using Least significant difference (LSD). A significance level of 0.05 was used. All statistical analyses. Data were analyzed using the R software (R 4.2.0).

4 RESULTS AND DISCUSSION

4.1 Effect of chia on the protein content of the cookies

The protein content of the cookies (Table 4) ranged from 2.5±0.2g/100g to 4.4±0.3 g/100g. A protein content of 2.5g/100g (all data presented on wet basis) was obtained for cookies made with cassava flour (control). The protein content increased significantly among chia-supplement cookies (table 4). The differences were statistically significant ($p<0.05$).

Table 4: Protein composition of cookies

Cookie	Protein (g/100g)
Control	2.5±0.2[a]
5% chia flour	3.1±0.0[a]
10% chia flour	3.2±0.1[a]
15% chia flour	4.4±0.3[b]

Several studies reported increase in protein content of foods incorporated with chia. According to (Mesías et al., 2016), the protein content of wheat-based biscuits supplemented with different amounts of chia four; 5%, 10% and 20% ranged from 8.8g/100g (control) to 11.7g/100g (20%). Barrientos, 2012) reported that, the protein content of sugar-snap cookies from wheat flour supplemented with chia seeds flour at 10% and 20% chia increased from 7.55g/100g (control) to 10.44g/100g (20% chia). The differences were due to the high content of protein of chia seeds (Peiretti & Gai, 2009).

4.2 Effect of chia on sensory acceptability of the cassava cookies

Generally, sensory acceptability of the cookies reduced with increase in flour percentage of whole chia flour. All mean scores for all the attributes for all cookie samples were above 6 (Table 4). There were no statistical differences ($p>0.05$) between scores for taste, aroma, mouthfeel and overall acceptability of cookies from all samples. Appearance scores of the cookies reduced ($p<0.05$) with increase in percentage of chia in the cookies. The appearance for the control was not different from the 5% chia cookies. There was a statistical difference between the appearance for the control with the 10% and 15% chia cookies.

Table 6: Sensory acceptability scores

Attribute	Control	5% chia	10% chia	15% chia
Appearance	7.6±1.2[a]	6.8±1.3[ab]	6.3±1.4[b]	6.1±1.9[b]
Taste	7.0±1.5[a]	7.1±1.4[a]	6.5±2.0[a]	6.3±2.0[a]
Aroma	6.9±1.4[a]	6.7±1.2[a]	6.3±1.6[a]	6.2±1.9[a]
Mouthfeel	6.8±1.2[a]	6.8±1.2[a]	6.6±1.9[a]	6.4±2.1[a]
Overall acceptability	7.3±1.2[a]	7.2±1.2[a]	6.6±1.5[a]	6.4±1.9[a]

(Barrientos et al., 2012), studied the effect of adding chia seeds to sugar-snap cookies on their sensory preference. They found that, as chia supplementation increased, the liking score decreased. The reduction in cookies preference for the samples with chia seeds could be explained by the changes in color; the cookies become darker with addition of chia (Barrientos et al., 2012).

The sensory evaluation of cookies supplemented with chia showed that as the supplementation level increased, there is a decrease in the preference for the product. However, even at the highest supplementation level (15%) the average acceptability rating was above 6.0 (‘like slightly’ category). This fact shows that cassava-chia cookies are not rejected by a panel of consumers.

5 CONCLUSIONS AND RECOMMENDATIONS

Substitution of cassava with whole chia flour reduces the sensory acceptability of cassava cookies. The reduction is however not significant. Up to 15% whole chia flour could be used to produce cassava-chia cookies that are acceptable to the consumer. Addition of whole chia flour significantly improves the protein content of the cookies. Therefore, whole chia flour can be used to produce cassava-chia cookies with improved protein content and acceptable sensory attributes

Further research should focus on determining the effect of whole chia flour on other nutritional components of cassava cookies such as carbohydrates, dietary fiber, fatty acid composition, antioxidants, total sugars and ash. The effect of addition of chia on the physical properties on cassava cookies could also be studied. How chia flour affects the processing parameters of cassava cookies such as dough spreadability, dough volume and others is another area of potential research.

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7 APPENDICES

7.1 Appendix I: Samples of the cookies from the control and the three treatments

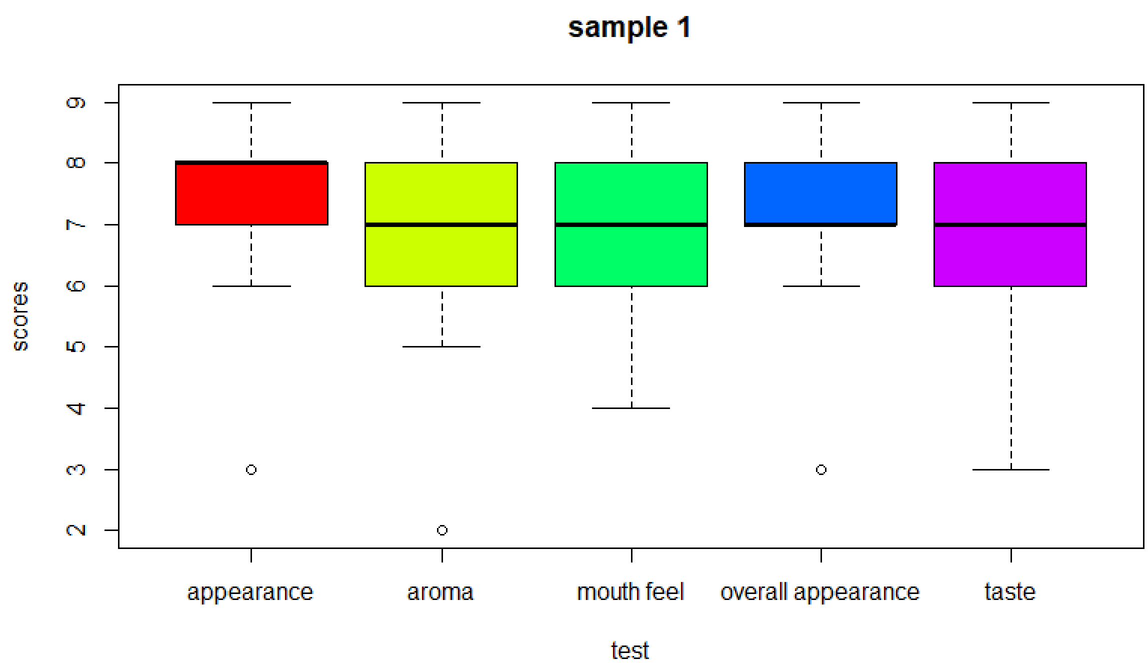
7.2 Appendix 2: Box plots showing distribution of sensory evaluation scores for the control cookies, 5% chia cookies, 10% chia cookies and 15% chia cookies

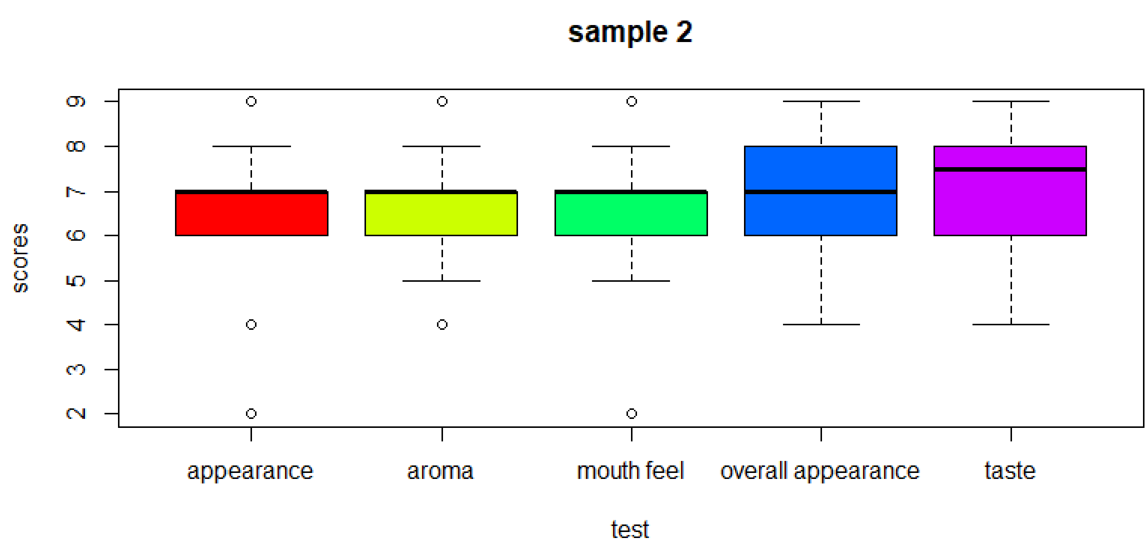
Sample 1= control.

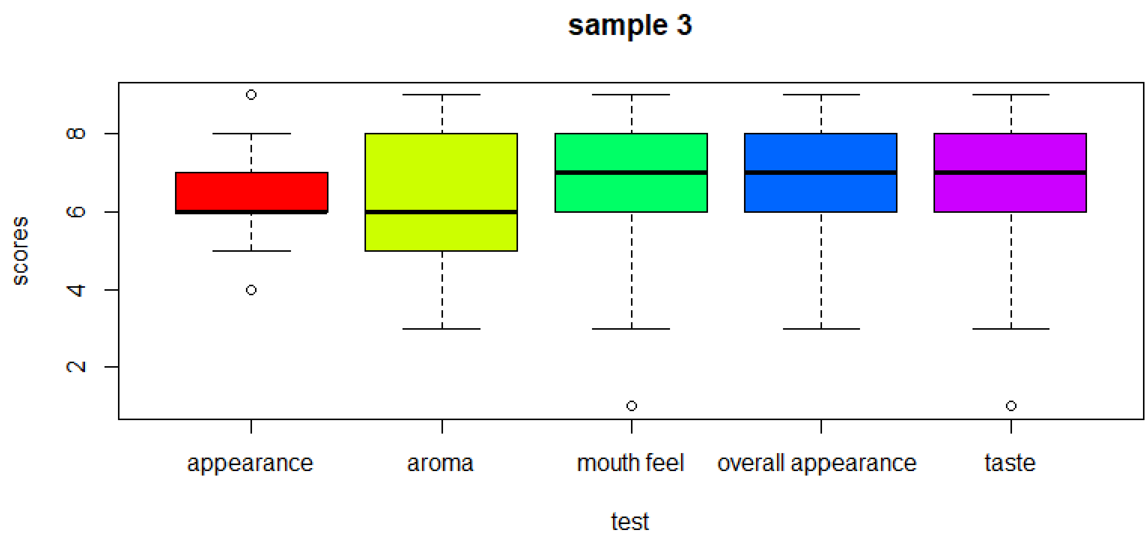
Sample 2= 5% chia.

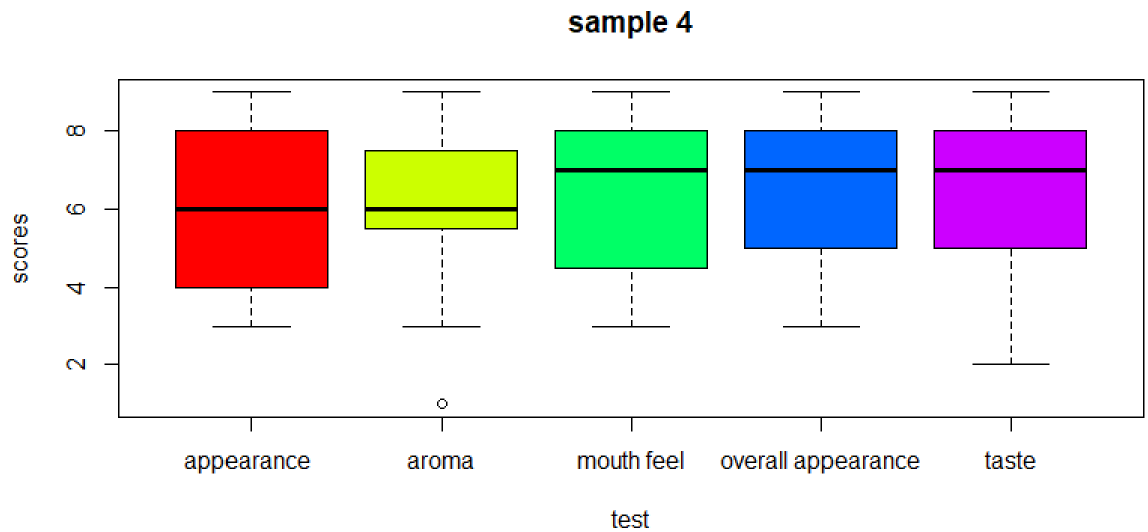
Sample 3= 10% chia.

Sample 4= 15% chia.






sample 3
scores
2
4
6
8
appearance
aroma
mouth feel
overall appearance
taste
test



7.3 Appendix III: ANOVA table for protein content

	DF	Sum square	Mean square	F-value	Pr>F
Test	3	3.903	1.3011	34.01	**0.00264**
Residuals	4	0.513	0.0383		

7.4 Appendix IV: ANOVA tables for sensory evaluation of cassava-chia cookies

Analysis of variance (appearance).

	DF	Sum square	Mean square	F-value	Pr>F
Test	3	41.23	13.74	6.162	**0.000635**
Residuals	116	258.73	2.23		

Analysis of variance (taste)

	DF	Sum square	Mean square	F-value	Pr>F
Test	3	13.9	4.633	1.524	**0.212**
Residuals	116	352.6	3.040		

Analysis of variance (aroma)

	DF	Sum square	Mean square	F-value	Pr>F
Test	3	9.97	3.322	1.406	**0.244**
Residuals	116	274.00	2.362		

Analysis of variance (mouthfeel)

	DF	Sum square	Mean square	F-value	Pr>F
Test	3	3.53	1.178	0.494	**0.687**
Residuals	116	276.53	2.382		

Analysis of variance (overall acceptability)

	DF	Sum square	Mean square	F-value	Pr>F
Test	3	16.67	5.556	2.694	**0.0493**
Residuals	116	239.20	2.062		

7.5 Appendix V: Sample questionnaire for sensory evaluation of the cookies

Date......................... Gender............................**.**

Questionnaire for sensory evaluation of cassava-chia cookies.

You are provided with four coded samples of cassava cookies. Assess them for appearance, taste, aroma, mouth feel and overall acceptability using the scale below. Write down the figure that corresponds with your response in the table below. Please rinse your mouth with water before and after tasting each sample. Please remember to write your comment(s) about each sample.

Scale.

Dislike......................................1

Dislike very much....................2

Dislike moderately..................3

Dislike slightly.........................4

Neither like nor dislike............5

Like slightly...................... 6

Like moderately..................... 7

Like very much.......................8

Like extremely........................9

Sample code	**Appearance**	**Taste**	**Aroma**	**Mouthfeel**	**Overall acceptability**	**Comments**
197						
710						
245						
513						

Other comments.

..............................,...

Thank you

7.6 Appendix VI: Box plots showing the range of protein content of the cassava-chia cookies.