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Nutritional and Antinutritional Evaluation of Gluten-Free Pasta from Cocoyam Starch and Lima Bean Flour

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ABSTRACT

Pasta is a wheat-based food product which has gained universal popularity in recent years due to its versatility, low cost and convenience; however, it has low nutritional quality. It is traditionally produced from wheat that is largely imported in Nigeria, placing a heavy demand on the dwindling financial resources of the nation. Wheat also contains gluten, which triggers a multi-system autoimmune disorder called celiac disease in genetically predisposed individuals. Treatment for celiac disease is complete exclusion of dietary gluten. Efforts are currently being made to develop gluten-free pasta from indigenous crops in order to reduce heavy dependence on imported wheat and provide alternatives to consumers affected by celiac disease. Cocoyam and lima bean are gluten-free, underutilized, indigenous tropical crops with a high nutritional profile that can be used to replace wheat flour in pasta production. This study, therefore, aimed at developing a high-nutritional gluten-free pasta from lima bean flour and cocoyam starch. Composite blends of pregelatinized cocoyam starch (PCS) and germinated lima bean flour GLBF) were formulated in the following ratios: 100:0, 87.5:12.5, 75:25, 62.5:37.5 and 50:50. Xanthan gum was added to the composite flour at 2.5% while 100% wheat flour was used as the control and these were used to produce pasta. The protein, moisture and carbohydrate contents of the gluten-free pasta ranged from 6.54 -18.82%, 9.06-10.56% and 59.29-74.30% respectively. Mineral content of gluten-free pasta ranged from 61.54-108.20 mg/100 g for calcium and 0.94-6.09 mg/100 g for iron. Lysine values increased from 2.63 (100% Wheat Flour Pasta) to 3.85 g/100 g protein (50% PCS, 50% GLBF) while total amino acids ranged from 53.66-83.11 g/100 g protein. The antinutritional factors cyanide, oxalate and phytic acid ranged from 0.191-1.199 mg/100 g, 10.251-85.064 mg/100 g and 0.01-1.66%, respectively. This study has shown the potential of developing a high-nutritional gluten-free pasta from the blends of pregelatinized cocoyam starch and germinated lima bean flour using xanthan gum as a binder.

INTRODUCTION

Pasta is a popular and widely consumed food all over the world (IPO, 2021). It is traditionally produced from durum wheat semolina or soft wheat flour and water, kneaded until the final texture is obtained (Arcangelis *et al.*, 2020). In recent years, a lot of research efforts have been targeted at improving the nutritional composition of pasta by using unconventional and indigenous crops rather than the conventional wheat, which is imported (Bresciani *et al.*, 2022). Non-wheat flours are gaining ground as a substitute for wheat flour in pasta production as a means of increasing local indigenous crop utilization and reducing wheat importation (Makhuvha *et al.*, 2024). Cocoyam is an important underutilized indigenous tropical crop commonly grown by low-income earners in Nigeria (Amanyunose *et al.*, 2021). It is mainly a starchy food, even though there are reports that it has nutritional advantages over other roots and tubers because of its greater protein content and amino acid (Boakye *et al.*, 2018).

Cocoyam starch, which is the major nutritional component of cocoyam, can be extracted and used for varying food formulations, thus extending the utilization of cocoyam (Okunade and Arinola, 2021). Cocoyam starch, unlike cassava and corn starches, has not found wide application in food and nonfood industries (Arinola, 2019).

In its native form, starch is unusable for many applications. The undesirable properties of native starch can be reduced or eliminated through modifications; by reorganizing the structural arrangement of the starch granules, resulting in enhanced physicochemical properties (Ihemeje, 2024). Lima beans are leguminous plants with high protein and dietary fiber; it has good potential as a cheap and alternative source of protein (Seidu *et al*., 2018). Due to their high nutritional profile, lima beans can help in combating protein malnutrition commonly found in developing countries and among vulnerable groups who cannot afford animal protein (Owolabi *et al.* 2020). Lima beans contain some antinutrients, which can be completely removed or reduced to a minimal level by common processing and household cooking methods. Some researchers have attempted to use various indigenous crops such as millet, sorghum, pearl millet, prosso millet, rice flour and banana starch and the like in the production of gluten-free pasta (Palavecino *et al*., 2017; Fradinho *et al*., 2020).

There is, however, limited information available on the use of pregelatinized cocoyam starch fortified with germinated lima bean flour in the production of gluten-free pasta. This research was carried out to evaluate the proximate, mineral, antinutritional components and amino acid profile of gluten-free pasta made from pregelatinized cocoyam starch, germinated lima bean flour, using Xanthan gum as a binder.

METHODOLOGY

Materials

Cocoyam (*Xanthosoma* sp) and lima bean (*Phaseolus lunatus*) used for this study were obtained from the International Institute of Tropical Agriculture, Moniya, Ibadan (IITA). Other materials and equipment used were obtained from the Department of Food Science, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.

Cocoyam starch extraction

Starch was extracted from cocoyam as described by Arawande and Ashogbon (2019). Cocoyam corms were washed, peeled, cut into small slices and ground in a milling machine and the resulting slurry was mixed with distilled water (1:4). The mixture was then sieved through a muslin bag and the starch suspension was left overnight at refrigerated temperature (4 °C) to settle. The supernatant was decanted and the white starch sediment was washed 3 to 4 times by re-suspending in distilled water. It was allowed to settle and decanted when the supernatant became transparent. The isolated starch was dried in an oven at 40 °C and milled using a conventional blender. It was sieved and packaged in ziplock bags and stored at ambient temperature (26 ± 2 °C) until required for use.

Pregelatinization of cocoyam starch

This was carried out according to the method described by Okunade and Arinola (2021). A known weight (100 g) of starch sample was mixed with 150 ml of distilled water and heated in a water bath at 80 °C for 15 min with slow intermittent manual mixing using a stirring rod. A thin film of the pregelatinized starch was spread on a stainless steel tray in the oven at 40 °C for 24 hr to dry. This was cooled, milled, sieved and packaged in ziplock bags and stored at ambient temperature (26 ± 2 °C) until required for use.

Processing of lima beans into flour

Lima bean flour was processed from wholesome lima beans using the method described by Farinde *et al.* (2018). Lima bean was thoroughly screened to remove husks, stones and dirt, and then washed in 5% (w/v) sodium chloride solution and soaked for 12 hr at room temperature. The water was drained and the beans allowed to germinate for 24 h, dried at 60 ℃, milled, sieved, packaged and stored until required.

Formulation of composite blends

Composite blends of pregelatinized cocoyam starch (PCS) and germinated lima bean flour (GLBF) were formulated in the following ratios: 100:0, 87.5:12.5, 75:25, 62.5:37.5 and 50:50. Xanthan gum was added to the composite flour at 2.5% while 100% wheat flour was used as the control. The composite blends were thoroughly mixed using a Kenwood blender until a homogenous blend was obtained.

Pasta production

Pasta dough was formulated using a method described by Bolarinwa and Oyesiji (2021). Composite flour (100 g) composed of pregelatinized cocoyam starch, lima bean flour and xanthan gum was mixed with water to form a uniform dough. The dough was kneaded and extruded using a pasta extruder. Control pasta was prepared using 100% wheat flour (WF) with no Xanthan. The pasta samples were dried at 60 ℃ for 3 hr, cooled and packaged in ziplock bags.

Proximate analysis

Composite blends and pasta samples were analyzed for proximate composition (AOAC, 2010)

Mineral analysis

The analysis for essential mineral elements (calcium, sodium, magnesium, zinc and iron) was carried out by Atomic Absorption Spectrophotometer (AOAC, 2010)

Amino acid profile determination

Amino acid profile was determined according to the method of Obreshkova *et al*. (2012).

Determination of anti-nutritional factors

Tannin was determined using the method of AOAC (2005), and the cyanide content of the samples was determined using the method described by Bradbury *et al*. (1991). Phytate content was evaluated using the process described by Haugh and Lantzsch (1992), while oxalate content was analyzed as described by N'zi *et al*. (2021).

Statistical analysis

Analysis was carried out in triplicate. The means and standard deviations of the analyses were thus calculated and the results were subjected to analysis of variance using Statistical Package for Social Science (SPSS), while means were separated using Duncan Multiple Range Test (DMRT) at $p < 0.05$ (Arise *et al*., 2022).

RESULTS AND DISCUSSION

Proximate Composition of the Composite Blends from Pregelatinized Cocoyam Starch (PCS) and Germinated Lima Bean Flour (GLBF).

Proximate composition of the blends from pregelatinized cocoyam starch and germinated lima bean flour is presented in Table 1. The moisture content of the blends ranged between 7.36 and 9.81%. The moisture values were significantly different ($p<0.05$) except for samples D (75% PCS & 25% GLBF) and E (62.5% PCS & 37.5% GLBF). The highest moisture content (9.81%) observed was for sample A (100%WF). The moisture content of 100% wheat flour obtained in this work is similar to the value reported by Radhika *et al*. (2019) for wheat flour, while the composite blends were lower than the range of 11.28 - 15.00% reported by Vellore *et al*. (2023) for sorghum and pearl millet flour blends. Moisture content of flour is important as it predicts the stability of the flour during storage

(Bolarinwa and Oyesiji, 2021). Moisture content obtained for all the samples was below the recommended moisture level (<13), indicating the shelf stability of the samples. The protein content of the blend ranged between 2.82 and 15.02%. The values were observed to be significantly different ($p<0.05$), with sample B (100% PCS) having the lowest value and sample F (50% PCS & 50% GLBF) having the highest value. Protein content increased with an increase in germinated lima bean flour. Tharise *et al*. (2014) reported a similar increase in protein content of flour blend from potato starch, cassava flour and soybean flour as an alternative to wheat flour.

The fat content ranged between 2.10 and 2.92% with sample A (100%WF) having the lowest. The fat content observed for sample B (100% PCS) is higher than 0.08% reported by Ashogbon (2017) for 100% cocoyam starch. There was an increase in the fat content of the blend with increasing addition of lima bean flour. The fat content values reported in this work are lower than the values reported by Awolu *et al*. (2017) for composite flour made from maize flour, soyflour and tigernut flour. High fat content is undesirable in pasta products to prevent oxidative rancidity that can negatively impact their shelf life. The ash content of the blend ranged from 1.56 to 3.94%. The values were significantly different ($p<0.05$) from the control. Sample A (100% WF) had the lowest ash content, while sample F (50% PCS & 50% GLBF) had the highest value. The ash content of the blend samples increased with increasing addition of germinated lima bean flour. Tharise *et al*. (2014) reported lower ash content values in the range 0.75-1.12% for composite flour from potato starch, rice flour, cassava flour and soybean flour.

Table 1: Proximate Composition (%) of Composite Blends from Pregelatinized Cocoyam Starch and Germinated Lima Bean Flour

Sample	Moisture	Fat	Protein	Fiber	Ash	Carbohydrate
A	9.81 ± 0.02^{e}	1.38 ± 0.10^{a}	11.23 ± 0.25^{d}	0.67 ± 0.00^{b}	1.66 ± 0.03^{a}	75.25 ± 0.17^{d}
B	9.07 ± 0.02^{d}	2.10 ± 0.10^{b}	2.82 ± 0.25^{a}	1.10 ± 0.00^{a}	1.56 ± 0.03^{a}	83.35 ± 0.17^{e}
C	8.75 ± 0.06^{c}	2.42 ± 0.06^{b}	9.10 ± 0.00^{b}	3.19 ± 0.00^{c}	2.98 ± 0.01^{b}	73.56 ± 0.01^{c}
D	8.16 ± 0.02^{b}	2.61 ± 0.15^{b}	10.12 ± 0.32^{c}	3.22 ± 0.00^{c}	3.17 ± 0.02^{c}	72.72 ± 0.14^{c}
E	8.16 ± 0.03^{b}	2.87 ± 0.06^{c}	12.00 ± 0.22^{e}	3.79 ± 0.01^{d}	3.61 ± 0.01^{d}	69.57 ± 0.25^{b}
F	7.36 ± 0.03^{a}	2.92 ± 0.12^{c}	15.02 ± 0.13^{f}	3.88 ± 0.00^{d}	3.94 ± 0.01^{d}	66.88 ± 0.11^{a}

Mean values with different superscripts within the same column are significantly different at 5% level.
Keys A = 100% Wheat Flour,
B = 100% Pregelatinized Cocoyam Starch
C = 87.5% Pregelatinized Cocoyam Starch and 12.5% Germinated Lima Bean Flour
D = 75% Pregelatinized Cocoyam Starch and 25% Germinated Lima Bean Flour
E = 62.5% Pregelatinized Cocoyam Starch and 37.5% Germinated Lima Bean Flour
F = 50% Pregelatinized Cocoyam Starch and 50% Germinated Lima Bean Flour

They also reported an increase in ash content as levels of cassava flour and soybean flour increased. Crude fiber content of the blend ranged from 0.67 to 3.88%. The values were significantly different ($p<0.05$) from one another. Sample A (100%WF) had the lowest value, while sample F (50% PCS &

50% GLBF) had the highest value. Awolu *et al.* (2017) observed the same increase in crude fiber content of composite flour from toasted maize, soybean and tigernut flour. The crude fiber value of 100% WF obtained in this work is lower than 2.5% obtained by Vellore *et al.* (2023) and higher than 0.54% reported by Bala *et al.* (2015), both for wheat flour. The carbohydrate content of the pregelatinized cocoyam starch and germinated lima bean flour blend ranged from 66.88 – 83.95%. The values were significantly different (p<0.05), with sample B (100% PCS) having the highest value and Sample F (50% PCS & 50% GLBF) having the lowest carbohydrate content. The carbohydrate content decreased with a decrease in levels of PCS and an increase in levels of GLBF. James *et al.* (2017) reported a similar carbohydrate value for 100% wheat flour, while Vellore *et al.* (2023) reported a much lower value.

Proximate Composition of Gluten-free Pasta Produced from Blends of Pregelatinized Cocoyam Starch, Germinated Lima Bean Flour and Xanthan Gum

The proximate composition of gluten-free pasta is presented in Table 2. The moisture content of gluten-free pasta ranged between 9.04 and 10.56% with sample D (75% PCS & 25%GLBF Pasta) having the highest value and sample E (62.5% PCS & 37.5% GLBF Pasta) having the lowest value. These values are similar to the range 10.44 – 11.22% obtained by Ikujenlola (2016) for sorghum pasta and higher than the range (0.57 – 2.89%) obtained by James *et al.* (2017). The low moisture content of these pasta samples will promote the keeping quality of the pasta, as the moisture content of pasta and similar products should be equal to or less than 12.5% after drying to avoid microbial contamination (Radhika *et al.*, 2019). The moisture content of a food is indicative of the dry matter in that food. Also, low residual moisture content in pasta is beneficial because microbial proliferation will be reduced, and storage life may be prolonged if stored appropriately under standard environmental conditions. The protein content of gluten-free pasta ranged between 6.54 and 18.82%. The protein content of the pasta samples was significantly different (p<0.05) from one another. The pasta from blends of PCS and GLBF had higher protein and the protein content increased as the percentage level of GLBF increased. James *et al.* (2016) reported an increase in protein content of pasta as levels of African breadfruit and soybean increased. A similar increase was also reported by Ikujenlola (2016) for gluten-free pasta produced from quality protein maize, sorghum and watermelon seed flour. Protein is important for good development and growth as it replaces worn-out tissues. Increased protein content of the gluten-free pasta suggests that they could be used as a solution to the increased rate of protein malnutrition in less developed countries. The fat content of gluten-free pasta ranged from 1.70-5.57%. The highest fat content was recorded for sample C (87.5% PCS & 12.5%GLBF Pasta), while sample A (100% WF Pasta) had the lowest fat content. The fat contents of the pasta samples were significantly different (p<0.05) and there was an increase in the fat content of pasta as levels of GLBF increased. This is in agreement with the work of Sule *et al.* (2019), who reported increased levels of fat (1.38-1.48%) in pasta as the levels of carrot powder increased. Bolarinwa and Oyesiji (2021) also reported fat content in the range of 3.51-5.77% for rice soy pasta. Thatsanasuwan *et al.* (2023) equally reported increased fat content for gluten-free pasta produced from jackfruit seeds. Fat is an important nutrient, containing fat-soluble vitamins (A, D, E and K). A high fat content in pasta and other dried food products is undesirable as it can cause off-flavor and rancidity, resulting in short shelf life (Ikujenlola, 2016).

Table 2: Proximate Composition (%) of Gluten-free Pasta Produced from Pregelatinized Cocoyam Starch, Germinated Lima Bean Flour and Xanthan Gum

Sample	Moisture	Fat	Protein	Fiber	Ash	Carbohydrate
A	10.29±0.05^{c}	1.70±0.06^{a}	10.15±0.05^{b}	2.20±0.02^{a}	1.51±0.02^{a}	74.15±0.04^{d}
B	9.92±0.02^{b}	4.20±0.00^{c}	6.54±0.02^{a}	2.48±0.02^{a}	2.56±0.03^{b}	74.30±0.04^{d}
C	10.37±0.03^{c}	5.57±0.06^{e}	10.82±0.02^{c}	2.41±0.02^{a}	2.88±0.02^{c}	67.95±0.14^{b}
D	10.56±0.04^{c}	4.91±0.02^{c}	11.32±0.05^{d}	5.98±0.03^{d}	2.22±0.00^{b}	65.01±0.07^{b}
E	9.06±0.02^{a}	2.96±0.05^{b}	12.53±0.12^{e}	4.10±0.01^{c}	2.31±0.01^{b}	71.04±1.48^{c}
F	10.53±0.03^{c}	5.42±0.02^{d}	18.82±0.00^{f}	3.48±0.02^{b}	2.46±0.03^{b}	59.29±0.05 ^{a}

Mean values with different superscripts within the same column are significantly different at 5% level.
Keys A = 100% Wheat Flour Pasta
B = 100% Pregelatinized Cocoyam Starch Pasta
C = 87.5% Pregelatinized Cocoyam Starch and 12.5% Germinated Lima bean Flour Pasta
D = 75% Pregelatinized Cocoyam Starch and 25% Germinated Lima bean Flour Pasta
E = 62.5% Pregelatinized Cocoyam Starch and 37.5% Germinated Lima bean Flour Pasta
F = 50% Pregelatinized Cocoyam Starch and 50% Germinated Lima Bean Flour Pasta

The ash content of the pasta sample ranged between 1.51 and 2.88% and the values are significantly different ($p<0.05$) from sample A (100% WF Pasta), which also had the lowest ash content, while sample C (87.5% PCS & 12.5% GLBF Pasta) had the highest ash content. Radhika *et al*. (2019) reported a similar trend where the ash content of composite pasta was significantly increased compared to the control sample of 100% wheat flour. The ash content of a food is related to its mineral content (Edet *et al*., 2017). This could be a reflection of the high mineral content of cocoyam and lima bean. Crude fiber content for pasta ranged between 2.20 and 5.98% with sample A (100% WF Pasta) having the lowest value and sample D (75% PCS & 25% GLBF Pasta) having the highest value. The samples were significantly different from the control except for samples B (100% PCS Pasta) and C (87.5% PCS & 12.5% GLBF Pasta). Consumption of dietary fiber is important as it helps in lowering serum cholesterol level, reducing the onset of coronary heart disease, the development of colon cancer and hypertension. Dietary fiber promotes glucose tolerance and increases insulin sensitivity (Hassan and Umar, 2004). The carbohydrate content of gluten-free pasta ranged between 59.29 and 74.30% showing a decrease in carbohydrate content as levels of pregelatinized cocoyam starch decreased and levels of germinated lima bean flour increased. There was no significant difference ($p>0.05$) in carbohydrate content of samples A (100% WF Pasta) and B (100% PCS Pasta), while sample F (50% PCS & 50% GLBF Pasta) had the lowest carbohydrate content. James *et al*. (2016) reported similar carbohydrate content for pasta obtained from blends of wheat, bambara groundnut and cassava flour. Carbohydrate evaluation based on differences is highly affected by the protein, ash and fat content and an increase in the content of these nutrients can reduce or elevate total carbohydrates (Putri *et al*., 2023).

Mineral Composition of Gluten-free Pasta Produced from Pregelatinized Cocoyam Starch, Germinated Lima Bean Flour and Xanthan Gum

The mineral content of the gluten-free pasta is presented in Table 3. The calcium content of pasta samples ranged between 61.54 and 108.20 mg/100 g, with sample B (100% PCS Pasta) having the lowest value and sample F (50% PCS & 50% GLBF Pasta) having the highest value. The samples were all significantly different (p<0.05) from one another. Calcium content increased with the addition of germinated lima bean flour. Jalgaonkar *et al*. (2018) reported lower values (21.66 mg/100 g) for 100% wheat flour, while Ikujenlola (2016) observed a similar increase in calcium content as the incorporation of watermelon seed into quality protein maize increased. Calcium is important in the formation of strong, healthy bones and teeth. Calcium is also needed for normal cell regulation of the nerves, heart and proper functioning of the enzymes. Lack of calcium in the diet can result in rickets in children and osteoporosis in premenopausal and post-menopausal women (Ibitoye *et al*., 2013).

Sodium content of pasta ranged between 25.31 and 257.10 mg/100 g, with the samples significantly different (p<0.05) from each other. Sample A (100% WF Pasta) had the highest sodium content, while sample B (100% PCS Pasta) had the lowest sodium content. James *et al*. (2017) reported similar sodium content for 100% wheat flour pasta but far lower sodium values for blends of wheat, cassava and bambara groundnut pasta. The increase in sodium content with increased addition of lima bean flour observed in this research is corroborated by the findings of Yadaf *et al*. (2014), who also observed an increase in sodium content with the incorporation of selected vegetables in pasta.

Magnesium content of pasta ranged between 102.607 and 146.83 mg/100 g, with sample A (100% WF Pasta) having the highest magnesium content and sample B (100% PCS Pasta) having the lowest value. There was an increase in the magnesium content of pasta samples as levels of germinated lima bean flour increased. The composite pasta samples were also significantly different (p<0.05) from the 100%WF pasta. Sule *et al*., (2019) observed a different trend with a decrease in magnesium content of carrot-incorporated pasta as levels of carrot powder increased. The magnesium values observed in this work are higher than the values reported by James *et al*. (2017) who reported magnesium content in the range 10.0-14.0 mg/100 g. Magnesium is essential for over 300 enzymes in the body. It is needed in the formation of ATP, storing of carbohydrates, fats and proteins, in nerves and muscle activities. Humans have a unique ability to conserve magnesium. Its deficiency leads to growth abnormalities and defective central nervous systems (Ikujenlola, 2016).

The zinc and iron content of composite pasta ranged between 1.30 and 3.75 mg/100 g and 0.94-6.09 mg/100 g. The zinc and iron content were all significantly different (p<0.05) and the values improved with the addition of lima bean flour. Sample B (100% PCS Pasta) recorded the lowest zinc and iron content, while sample F (50% PCS & 50% GLBF Pasta) recorded the highest value. This shows improved zinc and iron content compared to that of pasta from 100% wheat flour. Jalgaonkar *et al*. (2018) reported a similar range for zinc content (2.93-4.88 mg/100 g) of composite pasta. The iron content reported for 100% wheat flour pasta in this work is higher than 1.30 mg/100 g reported by James *et al*. (2017) for 100% wheat flour pasta. Iron is essential for blood formation and it helps in transporting oxygen throughout the body (Ikujenlola, 2016).

EDXRF Study: Chemical Composition and Toxic Element Adsorption Potential

EDXRF results, as shown in Table 3 and Figure 4, reveal a chemically complex adsorbent with strong potential for TE adsorption and catalytic oxidation, consistent with recent advancements in adsorption science.

The EDXRF analysis revealed that complex oxides exhibited adsorptive capabilities for heavy metals (in Table 3). Calcium Oxide (CaO, 5.334%), Silica (SiO_2, 4.836%), Cerium Oxide (CeO_2, 5.009%), Phosphorus Pentoxide (P_2O_5, 1.902%), and Sulfur Trioxide (SO_3, 2.2966%) were found. Wang *et al.* (2020) reported that CaO provides alkaline sites that enhance cation exchange for metals like Pb^{2+}, Cd^{2+}, and Cu^{2+}. Ca^{2+} from CaO exchanges with heavy metals (M^{2+} = Pb^{2+}, Cd^{2+}, Cu^{2+}) on the adsorbent surface:

Table 3: Mineral Composition (mg/100 g) of Gluten-free Pasta Produced from Pregelatinized Cocoyam Starch and Germinated Lima Bean Flour and Xanthan Gum

Sample	Calcium	Sodium	Magnesium	Zinc	Iron
A	101.02±0.02^{d}	257.10±0.01^{f}	146.83±0.03^{f}	2.17±0.04^{b}	3.21±0.03^{b}
B	61.54±0.03^{a}	25.31±0.00^{a}	102.607±0.01^{a}	1.31±0.00^{a}	0.94±0.02^{a}
C	97.78±0.09^{b}	146.38±0.04^{b}	104.078±0.01^{b}	2.30±0.06^{c}	4.29±0.01^{c}
D	99.66±0.55^{c}	163.45±0.36^{c}	114.187±0.02^{c}	2.77±0.01^{c}	4.54±0.06^{c}
E	104.62±0.01^{e}	167.57±0.09^{d}	120.432±0.00^{d}	3.54±0.03^{d}	5.02±0.04^{d}
F	108.20±0.04^{f}	175.21±0.02^{e}	121.619±0.03^{e}	3.75±0.03^{d}	6.09±0.01^{e}

Mean values with different superscripts within the same column are significantly different at 5% level.

Keys A = 100% Wheat Flour Pasta
B = 100% Pregelatinized Cocoyam Starch Pasta
C = 87.5% Pregelatinized Cocoyam Starch and 12.5% Germinated Lima bean Flour Pasta
D = 75% Pregelatinized Cocoyam Starch and 25% Germinated Lima bean Flour Pasta
E = 62.5% Pregelatinized Cocoyam Starch and 37.5% Germinated Lima bean Flour Pasta
F = 50% Pregelatinized Cocoyam Starch and 50% Germinated Lima Bean Flour Pasta

Amino Acid Composition of Gluten-free Pasta Produced from Pregelatinized Cocoyam Starch, Germinated Lima Bean Flour and Xanthan Gum

The amino acid content of pasta made from pregelatinized cocoyam starch and germinated lima bean flour is presented in Table 4. The total amino acid composition of gluten-free pasta samples ranged between 53.66 and 83.11 g/100 g protein, with sample B (100% PCS Pasta) having the lowest amino acid values and sample F (50% PCS & 50% GLBF Pasta) having the highest values. Pasta samples containing germinated lima bean flour showed an increase in lysine content compared to the 100% wheat flour pasta. This is similar to the findings of Filip and Vidrih (2015), in the production of pasta using plant protein extract and spinach powder and Gopalakrishnan *et al*. (2011) in the production of pasta using sweet potato flour and defatted soy flour. The lysine content of pasta with added germinated lima bean flour showed an improvement compared to the 100% WF pasta; this is similar to the report of Filip and Vidrih (2015). Lysine is one of the essential amino acids that is necessary for cell growth and repair (Matthews, 2020). The lysine content of 100% WF pasta (2.63 g/100 g protein) obtained in this work is lower than that obtained. (3.1 g/100 g protein) for 100% WF pasta by Veena *et al*. (2022), while the tryptophan levels were higher.

Table 4: Amino Acid Composition (g/100 g protein) of Gluten-free Pasta Produced from Pregelatinized Cocoyam Starch and Germinated Lima Bean Flour.

Amino Acid	A	B	C	D	E	F
Leucine	7.62	5.02	5.51	6.91	7.44	7.77
Lysine	2.63	2.57	2.94	3.08	3.74	3.85
Isoleucine	4.03	2.42	2.81	3.11	3.70	3.83
Phenylalanine	4.52	3.10	3.01	3.28	3.99	4.26
Tryptophan	1.29	0.74	0.87	1.05	1.26	1.28
Valine	4.27	2.81	3.10	3.07	3.97	4.15
Methionine	1.76	0.80	0.91	1.07	1.55	1.55
Threonine	3.41	2.30	2.33	2.72	3.25	3.61
Total Essential	29.53	19.76	21.48	24.29	28.90	30.30
Non-Essential						
Proline	3.45	2.54	2.44	2.94	3.25	3.45
Arginine	5.85	3.87	4.04	4.39	5.59	5.64
Tyrosine	3.61	2.75	2.58	2.75	3.27	3.40
Histidine	2.81	2.08	2.27	2.62	3.03	3.68
Cystine	1.82	1.21	1.39	1.76	2.06	2.82
Alanine	4.25	3.00	3.30	3.60	4.17	4.55
Glutamic acid	11.28	8.17	9.23	9.61	11.58	12.12
Glycine	3.97	2.61	3.02	3.30	4.37	4.61
Serine	4.16	2.62	3.00	3.24	3.81	4.32
Aspartic acid	8.38	5.05	5.46	7.01	8.03	8.22
Total Non-Essential	49.58	33.9	36.73	41.22	49.16	52.81
Total Amino Acid	79.11	53.66	58.21	65.51	78.06	83.11

Keys A = 100% Wheat Flour Pasta
B = 100% Pregelatinized Cocoyam Starch Pasta
C = 87.5% Pregelatinized Cocoyam Starch and 12.5% Germinated Lima bean Flour Pasta
D = 75% Pregelatinized Cocoyam Starch and 25% Germinated Lima bean Flour Pasta
E = 62.5% Pregelatinized Cocoyam Starch and 37.5% Germinated Lima bean Flour Pasta
F = 50% Pregelatinized Cocoyam Starch and 50% Germinated Lima Bean Flour Pasta

Recommended dietary intake for tryptophan is 1.1 g/day (Cavada *et al*., 2011), indicating that pasta samples with ≥ 25% germinated lima bean flour will be suitable to fulfill this minimum requirement. The biological value of proteins is determined by their essential amino acid, which are dependent on varying raw materials and their formulations (Messia *et al*., 2023). Pasta samples obtained from 50%GCS and 50%GLBF had higher total amino acid composition (83.11 g/100 g protein) than the 100%WF pasta (79.11 g/ 100 g protein), with higher levels of leucine, lysine, threonine (essential amino acid), histidine, cystine, alanine, glutamic acid, glycine and serine (non-essential amino acid). The essential amino acids are important from a nutritional point of view since the body cannot synthesize them and should therefore be supplemented in the diet.

Anti-nutrient Factors of Gluten-free Pasta Produced from Pregelatinized Cocoyam Starch and Germinated Lima Bean Flour.

The antinutrient factors of gluten-free pasta produced from pregelatinized cocoyam starch and germinated lima bean flour are presented in Table 5. The cyanide content of gluten-free pasta ranged from 0.191-1.199 mg/100 g with the samples significantly different from sample A (100% WF Pasta) and sample B (100% PCS Pasta) All the pasta samples are safe for human consumption as they were very low in cyanide content and do not exceed the WHO safe limit of 10 mg/kg (Puspitojati *et al*., 2019). Germination of lima bean flour drastically reduced the cyanide content of gluten-free pasta, coupled with drying of pasta samples after extrusion. This is similar to the observation of Adebowale *et al*. (2016), who reported a decrease in cyanide content after initial processing of raw materials followed by extrusion. Processing has been proven to reduce the cyanide content of food, as evident in cassava processing to various products that are safe to consume. High cyanide content of food leads to toxicity, causing paralysis and death (Onyenweaku, 2021).

Table 5: Anti-nutrient Factors of Gluten-free Pasta Produced from Pregelatinized Cocoyam Starch, Germinated Lima Bean Flour and Xanthan Gum

Sample	Cyanide (mg/100 g)	Oxalate (mg/100 g)	Phytic Acid (%)	Tannin (mg GAE/100 g)
A	0.191±0.002[a]	10.251±0.003[a]	0.01±0.06[a]	0.002±0.001[a]
B	0.465±0.062[a]	27.015±0.003[b]	0.21±0.10[b]	0.007±0.001[b]
C	1.176±0.000[b]	55.527±2.599[c]	1.66±0.01[c]	0.442±0.004[c]
D	1.105±0.031[b]	78.201±0.201[d]	1.51±0.19[c]	0.598±0.000[d]
E	1.199±0.031[b]	85.064±0.006[e]	1.44±0.95[c]	0.641±0.002[e]
F	1.034±0.001[b]	85.037±0.032[e]	1.32±0.00[c]	1.016±0.004[f]

Mean values with different superscripts within the same column are significantly different at 5% level.
Keys A = 100% Wheat Flour Pasta
B = 100% Pregelatinized Cocoyam Starch Pasta
C = 87.5% Pregelatinized Cocoyam Starch and 12.5% Germinated Lima bean Flour Pasta
D = 75% Pregelatinized Cocoyam Starch and 25% Germinated Lima bean Flour Pasta
E = 62.5% Pregelatinized Cocoyam Starch and 37.5% Germinated Lima bean Flour Pasta
F = 50% Pregelatinized Cocoyam Starch and 50% Germinated Lima Bean Flour Pasta

Oxalate content of gluten-free pasta ranged from 10.251-85.064 mg/100 g, with sample A (100% WF Pasta) having the lowest level and sample E having the highest content. The samples were significantly different ($p<0.05$) in terms of oxalate composition, showing an increase as levels of germinated lima bean flour increased. The range of oxalate content observed in this work is higher than that reported by

Liebman and Okombo (2009) and Veena *et al.* (2019), who reported a range between 12.7 and 15.6 mg/100 g for different brands of pasta and a range of 21.5-76.0 mg/100 g for vegetable pasta. Oxalates are known to form insoluble salts with minerals like zinc, calcium, magnesium and iron, thereby making such minerals unavailable for utilization in the body (Olaoye and Obidegwe, 2018). Oxalates, when present in large quantities in foods, chelate some metal ions and render them insoluble and hence, the metal ions cannot be absorbed in the intestine (Sanni *et al*., 2018). A diet high in oxalate increases the risk factors of renal calcium absorption and can lead to kidney stones (Gwer *et al*., 2020).

The phytic acid content of gluten-free pasta ranged from 0.01 - 1.66% with a significant difference ($p<0.05$) existing between the 100% WF samples and other samples. The 100% WF pasta had the lowest phytic acid content, while sample C (87.5% PCS & 12.5% GLBF Pasta) had the highest content. The level of phytic acid decreased as the level of lima bean flour increased. The trend observed is contrary to the report of Olopade and Ogundeji (2019) for acha/bambara pasta. Kamble *et al.* (2019) equally reported a minute increase in phytic acid content of multi-grain pasta as levels of sorghum and millet incorporation in pasta increased. The phytic acid content of 100% Wheat flour pasta obtained in this study is slightly higher than that obtained by Olopade and Ogundeji (2019) for 100% wheat flour pasta. The phytic acid content observed for all the samples is lower than the permissible level of 250-500 mg/100 g dry mass, showing that the gluten-free pasta is safe for consumption (Ndidi *et al*., 2014). Phytic acid (phytates), are compounds that occur naturally and can be found in several grains and seeds. Phytates prevent the absorption of minerals such as iron, zinc, and calcium and the hydrolysis of starch (Bethapudi *et al*., 2023). Phytic acid can also bind with protein to configure a protein–mineral compound which inhibits the enzymatic degradation of protein (Jalgaonkar *et al*., 2018).

Tannin content of gluten-free pasta ranged from 0.002 - 1.016 mgGAE/100g, with sample A (100% WF Pasta) having the lowest content and sample F (50% PCS & 50% GLBF Pasta) having the highest content. The samples are significantly different ($p<0.05$) from each other. The tannin content observed in this work is lower compared to the values observed by Kamble *et al*. (2018) for wheat and multi-grain pasta, but it's similar to the values observed by Olapade and Ogundeji (2019) for acha / bambara pasta. The tannin content observed for all the samples was lower than the permissible level of 20 mg/g dry mass, showing that the gluten-free pasta is safe for consumption (Ndidi *et al*., 2014). Tannins are soluble astringent, complex and phenolic compounds from plants that greatly influence the reduction of dietary protein digestibility (Olapade and Ogundeji, 2019). Tannins are known to have acerbic characteristics that can quicken the healing of wounds and prevent decay (Adebowale *et al*., 2017).

CONCLUSION

Gluten-free pasta had increased protein, ash and mineral content with an increase in the addition of cocoyam starch and lima bean flour, while a decrease in carbohydrate content was observed. Pasta samples containing germinated lima bean flour showed an increase in lysine content compared to the 100% wheat flour pasta. The study has established that gluten-free pasta with good nutritional attributes can be developed using underutilized locally available food crops.

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