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The Effect of Fermentation and Extrusion on the Anti-nutritional Composition and Digestibility of Millet and Soybean Flour Blends

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Authors' contributions

This work was carried out in collaboration between all authors. Author JUO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors II and AOO managed the analyses of the study. Author JUO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To investigate the effect of fermentation and extrusion on the anti-nutritional properties, starch and protein digestibility of millet and soybean flour.
Methodology: Four combinations (100, 90:10, 80:20, and 70:30) of millet and defatted soybean flour blends were separated into two batches. A batch of the combination was extruded in a laboratory single screw extruder. The other batch was separately hydrated, preconditioned and extruded. The raw flour blends and extrudates were subjected to antinutritional analysis, starch and protein digestibility, using standard methods.
Results: Tannin, saponin, trypsin inhibitor, and phytate contents significantly changed in all the extrudates. However, the fermented-extruded (FE) blends recorded highest reduction in all antinutrients. Saponin contents observed a change from 11.65 -13.08% (raw flour blends), to 4.98-6.54% (extruded (E) blends), and 2.13-3.19% (FE blends). Tannin contents changed from 0.1235-0.1412 mg/g (raw flour blends) to 0.0988-0.1083 mg/g (E blends) and 0.0838-0.0962 mg/g

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(FE blends). Trypsin inhibitors changed from 50.13-56.02% (raw flour blends) to 34.10-37.76% (E blends) and 32.98-41.89% (FE blends). Phytic acid contents also changed from 9.311-10.600 mg/g (raw flour blends) to 6.810-7.416 mg/g (E blends) and 4.944-6.361 mg/g (FE blends). *In vitro* starch digestibility was significantly higher ($P<0.05$) from 0.2615-0.3509 mg/g (raw flour blends) to 0.3246-0.4204 mg/g (E blends), and 0.3877-0.4699 mg/g (FE blends). As compared to the raw flour blends, protein digestibility significantly changed from 0.0298-0.0328 mg/g (raw flour blends) to 0.0817-0.0665 mg/g (E blends) and 0.0931-0.0753 mg/g (FE blends).
Conclusion: From the results of this research, it is evident that fermentation and extrusion will produce acceptable products and increase the nutritional and sensory attributes of the product.

Keywords: Fermentation; extrudates; digestibility; millet; defatted soybean.

1. INTRODUCTION

Pearl millet (*Pennisetum glaucum*) ranks as the World′s 4th most important tropical food cereal, with 26 million ha (64million acres). It has been grown in Africa and Indian sub-continent since pre-historic times [1]. From a nutritional point of view point, pearl millet is an attractive food grain. Several studies indicate that metabolizable energy of pearl millet for non-ruminant animals is approximately equal to that of maize [2,3,4]. When compared to maize on a weight basis, pearl millet is about 60% higher in crude protein, 40% richer in lysine and methionine, and 30% richer in threonine [5].

Soybean (*Glycine max*) is a legume native to East Africa, widely grown for its edible bean which has numerous uses. The plant is classified as an oil seed, rather than a pulse by the United Nation Food and Agricultural organisation (FAO) [6]. Soybean belongs to the family Leguminosae. Soyabean is a very rich source of essential nutrients, and one of the most versatile food stuff. It possesses good quality protein which is comparable to other protein foods and is suitable for all ages, from infants to the elderly. Soybean protein contains all the essential amino acids, except methionine which is relatively low. However, it is a good source of lysine [7]. Soyabean is a good source of energy.

Fermentation is one of the oldest and most economical methods of processing and preserving foods. Whole or ground seeds, either raw or cooked can act as substrate for fermentation. The fermented legumes and cereals are popular due to improved sensory characteristics, protein quality, starch disgestibility and contents of some minerals and vitamins, as well as partial or complete elimination of antinutritional factors. The fermented legumes and cereals are popular due to improved sensory characteristics, protein quality, starch disgestibility and contents of some minerals and vitamins, as well as partial or complete elimination of antinutritional factors.

Extrusion cooking is a high-temperature, short-time process in which moistened, expansive, starchy and/or proteinacious food materials are plasticised and cooked in a tube by a combination of moisture, pressure, temperature and mechanical shear, resulting in molecular transformation and chemical reactions [8,9]. Extrusion cooking is preferable to other food processing techniques in terms of continuous process with high productivity and significant nutrient retention, owning to the high-temperature and short-time required.

Fermentation may improve the quality of legumes and cereals due to the removal of some antinutritional factors. In most cases, fermentation contributes to the masking of undesirable odours and flavours while impacting desirable flavour to the finished products. More importantly, fermentation is reported to enhance digestibility of starting materials by breaking down complex protein structures. Fermentation can also be a cheaper means of food preservation. Thus a combination of two or more methods of processing is required.

Extrusion cooking is preferable to other food processing techniques in terms of continuous process with high productivity and significant nutrient retention owing to high temperature and short time required. According to Pehanich [10], many consumers have taken gradually to extruded foods. Hence, there is the need to improve the nutritional quality of extruded foods by supplementing with proteinacious food plants. In addition, the extrusion process denatures undesirable enzymes inactivates some antinutritional factors (trypsin inhibitors, haemoglutinins, tannins and phytates) extrusions

also sterilizes the finished product, and retains natural colours and flavours of foods [11,12].

Determination of nutritional quality owing to undesirable antinutrients is a challenging problem in most traditional cooking methods. Antinutritional factors lower the nutritional value of foods by lowering the digestibility or bioavailability of nutrients [13]. Some of these substances may reduce the bioavailability of minerals either due to formation of extreme insoluble salts or very poorly dissociated chelates [14]. The removal of undesirable components is essential to improve the nutritional quality of legumes and cereals. In this way, they could effectively be utilized to their full potential as human food. It is widely accepted that simple and inexpensive traditional processing techniques are effective methods of achieving desirable changes in the composition of seeds.

In many instances, the use of only one method of processing may not impact the desired level of removal of anti-nutritional compounds, improvement of nutritional quality and digestibility as well as bioavailability of minerals. New food processing technologies such as extrusion cooking combined with fermentation can provide alternatives for improving the nutritional quality of the food.

The objective of this study was to determine the effect of the combination of both processing techniques on the starch/protein digestibility and some non-nutrient components of extruded food processed from millet and defatted soybean flour. The specific objective was therefore aimed at making extrusion and fermentation, a process that is more efficient in terms of digestibility and safety.

2. MATERIALS AND METHODS

2.1 Materials

Millet (*Pennisetum glaucum*) was purchased from Uchi market, Auchi, Edo state; while defatted soy bean flour (Variety TGX 1448-2E) was purchased from IITA (International Institute of Tropical Agriculture), Ibadan, Oyo state.

2.2 Processing of Millet Grains to Flour

Dried millet grains were sorted and cleaned to remove stones and other foreign materials. The sorted grains were washed and thoroughly sun dried for 24 hours. The clean dried millet grains were then fed into attrition mill (Model 200L090, E.H. Bentall, UK). The milled flour was sieved into fine flour.

2.3 Processing of Soybean to Defatted Flour

Soybean seeds were cleaned by sorting out dirts, leaves and stones. The clean soybean seeds were coarsely milled to separate the coat from the cotyledon. The dehulled seeds were milled to fine soybean flour using an attrition mill. The fine soybean flour was then deffated using cold extraction with n-hexane from a 21% fat content to 15.17%. The deffated flour was then air-dried and the clumps broken into fine flour, and then sieved through 60 µm mesh screen.

2.4 Formulation of Millet and Defatted Soybean Flour Blends

The flour samples from millet and defatted soybean were mixed at four (4) level combinations: 100; 90:10; 80:20 and 70:30.

The blends were separated into two batches from each combination. The two batches of the flour blends were stored in a clean sterile polythene bags, tightened at tips, and kept in appropriately labelled plastic bowls with cover lids. A batch of the flour blends were separately hydrated and preconditioned by adding the appropriate amount of water, and manually mixed in a sterile wide bowl, to ensure even moisture distribution.

The second batch of the flour blends were separately fermented (75 ml of water to 100 g of the flour blend) for 72 hours using solid state fermentation method of AOAC [15].

2.5 Extrusion Process

Extrusion of the two batches of flour blends was carried out in a Brabender 20DN single-screw laboratory extruder (Brabender OHG, Duisburg, Germany) having a uniformly tapered screw with a nominal compression ratio of 2:1, diameter 19/mm, length to diameter ratio 20:1, die diameter 3mm, screw speed at feed inlet was kept constant at 30/rpm and a temperature profile of 100°C. Electrical heating was applied to the three barrel zones along the screw. The screw speed was maintained at 200/rpm. Two process runs were performed for each batch of flour blends. The extrudates were then oven dried at 30°C for 24 hours. They were stored at

room temperature (38±2°C) in sterile polythene bags and kept in air-tight plastic containers with cover lids.

2.6 Determination of Anti-nutrients

2.6.1 Extraction of tannin

Tannins were extracted by shaking lg of the sample in 10ml acidified methanol (l ml concentrated hydrochloric acid/100 ml methanol) in centrifuge tubes at 25°C for 20 minutes. The sample was centrifuged at 10,000 rpm for 15 minutes before pipetting Iml into a test tube. Vanillin-hydrochloric acid reagent was prepared by mixing equal portions of vanillin acidified method (8 ml concentrated hydrochloric acid /100 ml methanol). The vanillin-hydrochloric acid reagent (5 ml) was added to the sample and absorbance read in 1cm cuvettes using an ultrospec 1000 spectrophotometer at 500 nm after 20 minutes against vanillin-hydrochloric acid reagent as blank. A standard curve was prepared by adding lg of tannic acid to 100 ml acidified methanol [16].

2.6.2 Extraction of phytates

Phytates were extracted by adding 0.1 g sample to 100 ml 0.2 mol/l hydrochloric acid and shaken for 1 hour before centrifuging at 5000 rpm for 15 minutes. The supernatant (0.5 ml) was pipetted into a test tube fitted with a ground glass stopper before adding 1 ml acidic ammonium iron (iii) sulphate dodecahydrate (0.2 g NH_4 Fe $(SO_4)_2$. $12H_20$ in 100 ml 2 mol/l hydrochloric acid and made up to 1000ml with distilled water). The samples were boiled for 30 minutes then rapidly cooled to 25°C in an ice-water bath. 2 ml of 2^12^1-bipyridine solution (10g 2^12^1 bipyridine) and 10ml thioglycolic acid in 1000 ml water) was added to the test tube and the contents mixed. Absorbance was read after 1 minute using ultrospec 1000 spectrophotometer at 519 nm against distilled water. A standard curve was prepared by adding 125 mg sodium phytate to 100 ml 0.2 mol/l hydrochloric acid [16].

2.6.3 Extraction of saponin

2.5 g of the flour was dispensed in 25 ml of 20% ethanol. The suspension was heated over a hot water bath at 55°C for 4 hours with continuous stirring. The mixture was filtered and the residue re-extracted twice with another 25 ml of ethanol. The combined extracts were reduced to one quarter of the total volume by heating over water maintained at 90°C. The aqueous portion of the concentrate was extracted thrice with 20 ml of diethyl either by using a 250 ml separating funnel. The extract was purified further with 40ml of n-butanol and washed thrice with 5% aqueous NaCl. The remaining solution was evaporated at 95°C over a water bath until obtaining a constant weight. The saponin content was calculated on dry basis [15].

2.6.4 Extraction of trypsin inhibitor

0.5 g of the sample was dispensed in 50mls of 0.5M NaCl solution and shaken for 30 minutes at room temperature. The mixture was centrifuged and the supernatant was used as the extract. Assay for trypsin inhibitor activity involved mixing a portion (1 ml) of the extract with 90 mls of 0.03% Trypsin substrate (BAPA) in a test tube containing 1 ml of 0.6% Trypsin enzyme solution. After mixing, the mixture was allowed to stand for 15 minutes before its absorbance was reached at 410 nm in a spectrophotometer [17].

2.6.5 *In vitro* starch digestibility

In vitro starch digestibility as described by Miller [18] was determined by using 1% carboxymethyl cellulose in sodium acetate buffer (pH 5.5.) as substrate. 0.2 ml of the sample solution were added to 0.2 ml of the substrate solution and incubated at 37°C for 30 minutes. 0.5 ml of 3,5-dinitrosalcylic acid was added and heated for 5 minutes in a boiling water bath. The solution was allowed to cool and 10 ml of distilled water was added. The same procedure was carried out on the substrate without the addition of the enzyme solution. The absorbance was read at 540 nm.

2.6.6 *In vitro* protein digestibility

The *in vitro* protein digestibility of the raw flour blends and extruded samples were determined according to the method of Saunder et al. [19].

250 g of the sample was suspended in 15 ml of 0.1 M HCL containing 1.5 mg pepsin (1:10,000) in a 100 ml conical flask. The mixture was incubated at 370°C for 3 hours. The mixtures were then neutralized with 0.5N NaOH and treated with 4 mg pancreatine in 7.5 ml of 0.2N phosphate buffer (pH 8.0), containing 0.005M sodium acid. The mixture solutions were incubated at 37°C for 24 hours. 10 milliliter of 10% trichloroacetic acid (TCA) mixtures were added to the mixture to stop the reaction. The

mixtures were then centrifuged at 5000 rpm for 5 minutes. 5.0 ml aliquots from the supernatant were pipetted and analysed for nitrogen content.

3. RESULTS AND DISCUSSION

The phytate content significantly ($P<0.05$) changed in all extrudates (Fig. 3). However, the change was most significant in the fermented-extruded (FE) blends. From the results, it was observed that phytic acid reduced with increase in defatted soybean supplementation. The lowest (4.944 mg/g) phytate content was observed in FE4, and highest (7.416 mg/g) in E1. The lowering of phytate enhances the bioavailability of minerals such as dietary zinc, calcium, and iron in the extrudates. In this study, phytic acid has been observed to have made these minerals unavailable.

Tannin has been reported to occur in appreciable amount in legumes. The seed coat colour of legumes has largely been associated with tannin contents. From the results (Fig. 1), tannin content decreased with increase in defatted soybean supplementation. The fermented-extruded (FE) blends recorded lower tannin content due to fermentation reduction or elimination in foods. As compared to the tannin contents of the raw flour blends, there was a significant ($P<0.05$) decrease in the tannin contents of all the extrudates. Tannin forms insoluble complexes with proteins thereby decreasing their digestibility [20]. Tannin also decreases palatability; causes damage to intestinal tract, and enhances carcinogenesis.

The saponin content was significantly ($P<0.05$) changed from 11.65-13.08% (raw flour blends) to 5.01-6.39% (extruded (E) blends), and 2.14-2.88% (fermented-extruded (FE) blends) Fig. 2. Saponin increases permeability of small intestinal mucosa cells, thereby inhibiting nutrient transport. However, they can lower plasma cholesterol concentrations.

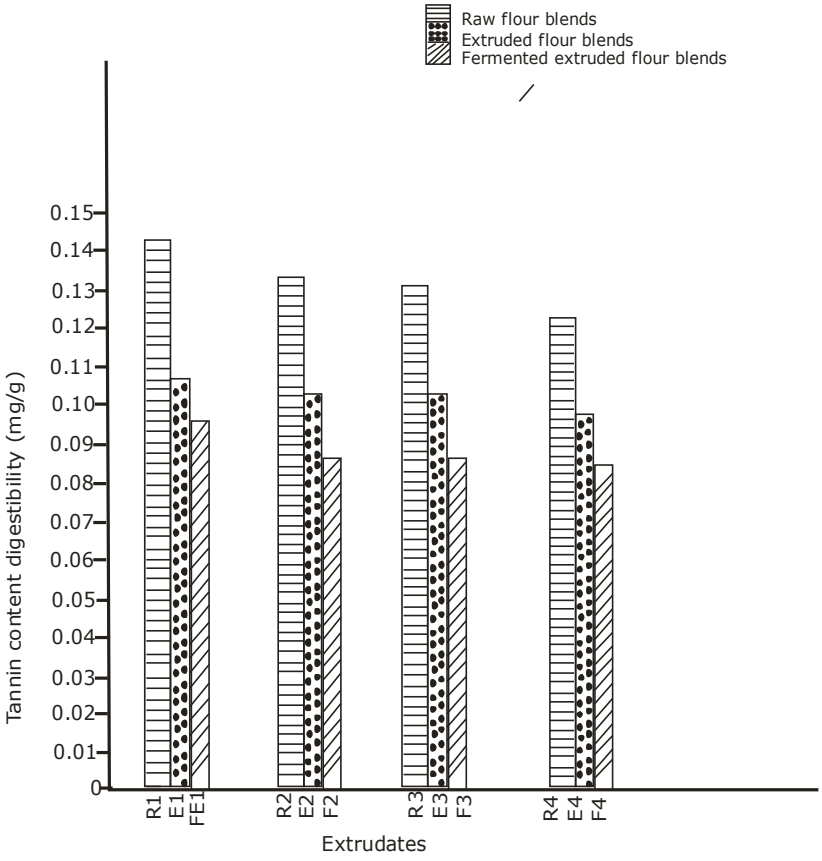

Fig. 1. Tannin (mg/g) in the raw flour blends and extrudates

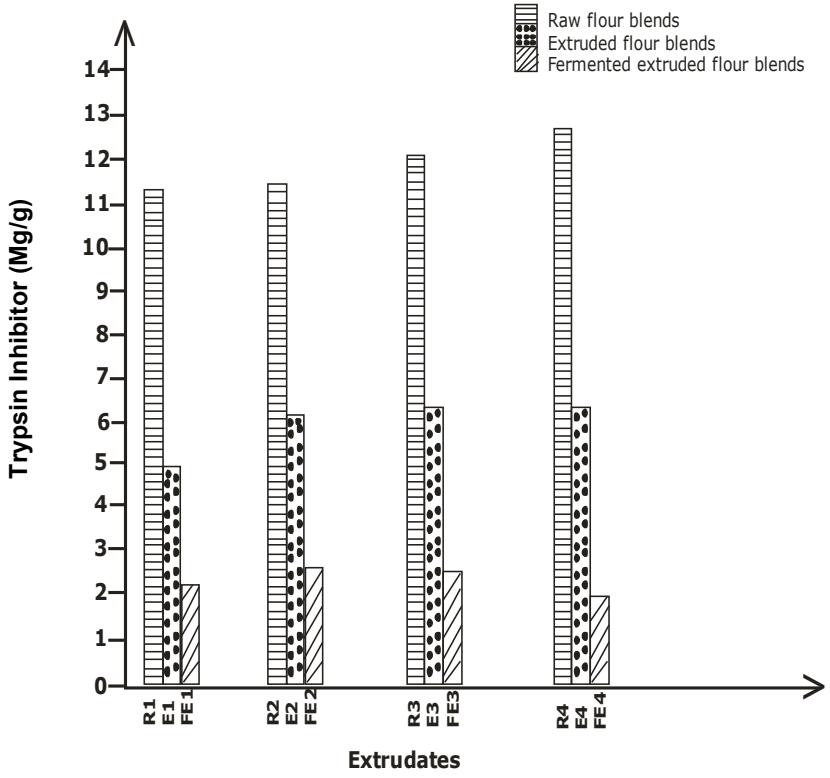

Fig. 2. Trypsin inhibitor (mg/g) in the raw flour blends and extrudates

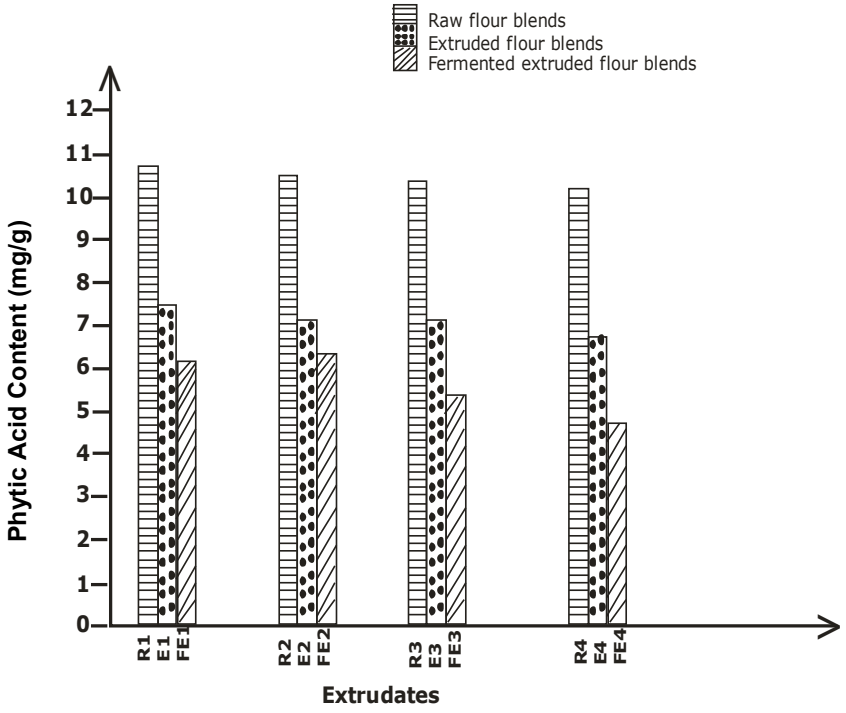

Fig. 3. Phytic acid (mg/g) in the raw flour blends and extrudates

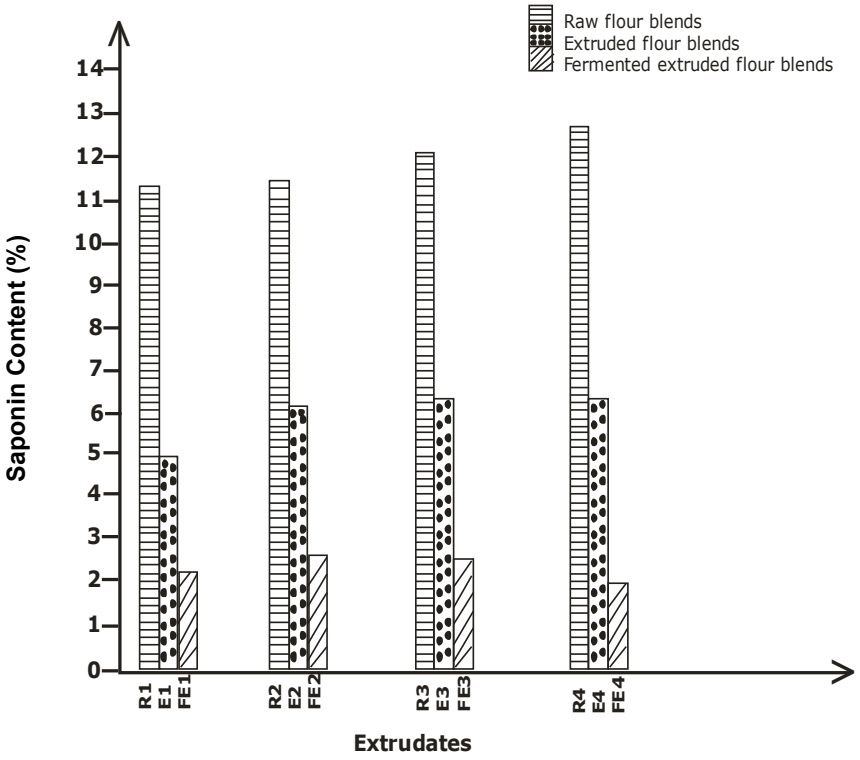

Fig. 4. Saponin (%) in the raw flour blends and extrudates

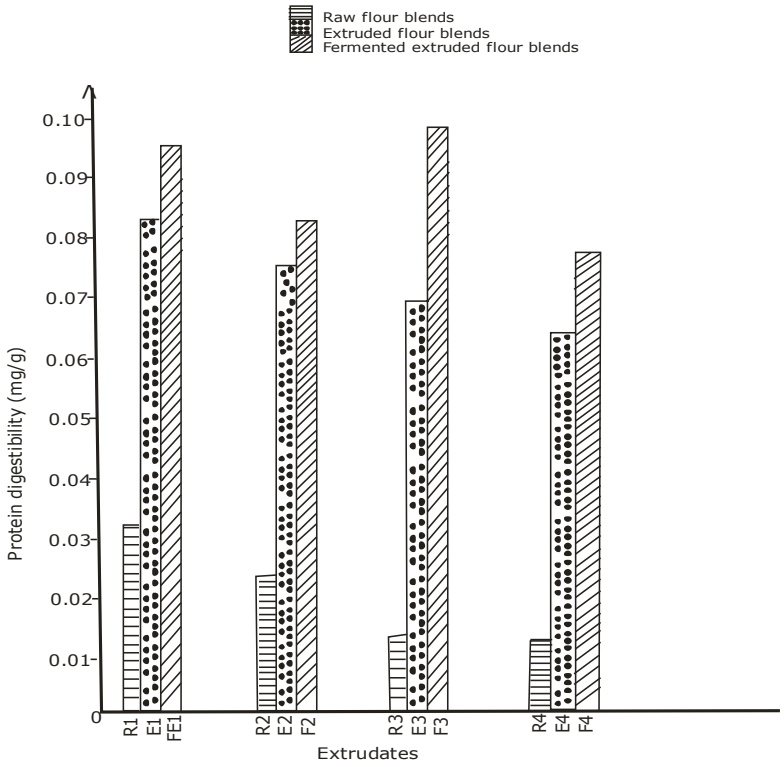

Fig. 5. *In vitro* protein digestibility of the raw flour blends and extrudates

Trypsin inhibitors are endogenous enzymes which can form stable complex with proteolytic pancreatic enzymes, i.e. trypsin and chemotrypsin. Due to complex formation, the activity of these enzymes decreases [21]. Trypsin inhibitor contents reduced changed from 50.13-56.55% (raw flour blends), to 34.12-37.56% (extruded (E) blends) and 32.98- 41.71% (fermented-extruded (FE) blends). From the results (Fig. 2), trypsin inhibitors increase with decrease in defatted soybean supplementation. Thus, trypsin inhibitor content was highest in FE1 (41.71%) and E1 (37.56%). The results showed that supplementation had more effect on the trypsin inhibitor content, than fermentation. The thermoliability of trypsin inhibitor implies that protein digestibility will not be hampered when the product is consumed as reported by Iorgyer et al. [22].

The *in vitro* starch digestibility of the fermented-extruded (FE) blends were higher than that of the extruded (E) blends. However, starch digestibility increases with increased in defatted soybean supplementation (Fig. 6). IVSD was highest in FE4 (0.4699 mg/g) and lowest in E1 (0.3186 mg/g). However, there were significant ($P<0.05$) increase in IVSD of all extrudates as compared to the raw flour blends. This agreed with the reports of Tamera et al. [23], that fermentation improves starch digestibility. Onyango et al. [24] reported that increase in IVSD by fermentation process may be attributed to the production of organic acids by the

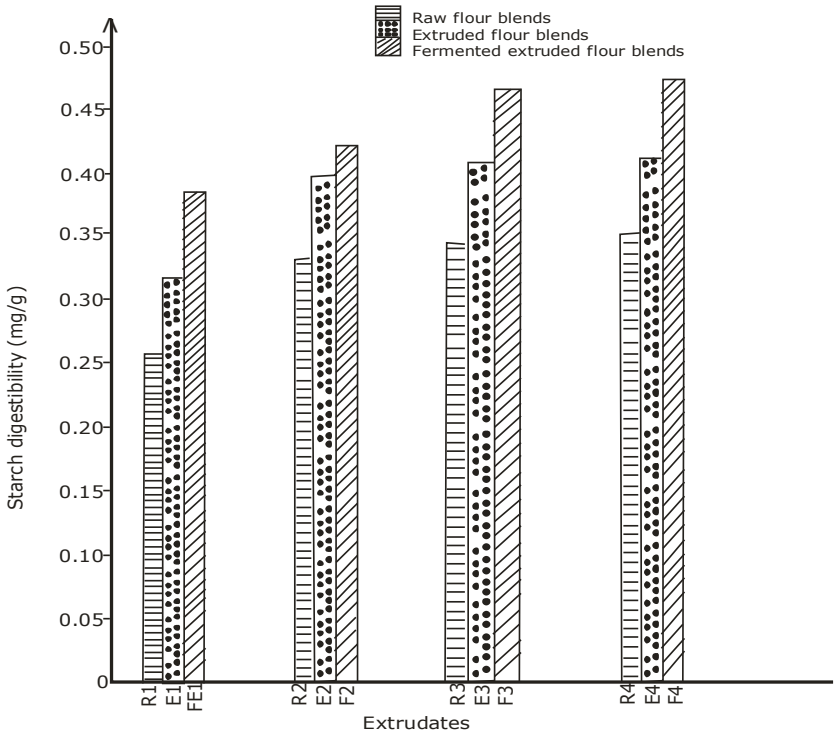

Fig. 6. *In vitro* starch digestibility of the raw flour blends and extrudates

Key:
R1 - Raw 100% millet flour
R2 - Raw 90% millet 10% defatted soybean flour
R3 - Raw 80% millet 20% defatted soybean flour
R4 - Raw 70% millet 30% defatted soybean flour
E1 - Extruded 100% millet flour
E2 - Extruded 90% millet 10% defatted soybean flour
E3 - Extruded 80% millet 20% defatted soybean flour
E4 - Extruded 70% millet 30% defatted soybean flour
FE1 - Fermented extruded 100% millet flour
FE2 - Fermented extruded 90% millet 10% defatted soybean flour
FE3 - Fermented extruded 80% millet 20% defatted soybean flour
FE4 - Fermented extruded 70% millet 30% defatted soybean flour

fermenting microorganisms which might have loosened the starch granules sites, making them available for amylolytic actions. The IVSD increased values recorded after extrusion cooking could be due to the gelatinization of granules thereby aiding efficient enzymatic attack. According to Camire et al. [25], extrusion improves starch digestibility by shearing off branches on amylopectic molecules and increasing their accessibility to amylolytic enzymes in starch granules as reported by Onyango et al. [24].

The *in vitro* protein digestibility (IVPD) is significantly higher in all extrudates than the raw flour blends (Fig. 5). Extrusion cooking improved the protein digestibility of the extrudates. The IVPD decreases with increased in defatted soybean supplementation. The improvement in the IVPD of the fermented-extruded (FE) could be attributed to the reduction of antinutrients such as phytic acid, tannin, and saponin by fermentation. Also, the increase in the protein digestibility of the FE blends could be attributed to the proteolytic action of the endogenous and microbial enzymes, this results agree with the findings of Onyango et al. [24] who reported that fermentation increases protein availability and invitro protein digestibility. The increased IVPD of the extrudates could be attributed to extrusion cooking which denatures proteins by opening up their quaternary and tertiary structures, thereby inducing polymerization, cross-linking and re-orientation to fibrous insoluble structures [24].

4. CONCLUSION

From the results of this research, it is evident that fermentation and extrusion will produce acceptable products and increase the nutritional and sensory attributes of the products. Extrusion technology provides a new exciting opportunity to processed foods.

The inclusion of fermentation in the extrusion process enhanced the nutritional qualities, as well as reduced the antinutritional components of the products. Increased nutritional qualities of the millet and defatted soybean blends through fermentation and extrusion enhanced the safety of the products which will help in improving the nutritional status of the vulnerable group of population.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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