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RESEARCH ARTICLE

Effect of fermentation on nutrient composition and anti-nutrient contents of ground Lima bean seeds fermented with *Aspergillus fumigatus*, *Rhizopus stolonifer* and *Saccharomyces cerevisiae*

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Abstract

The research compared Lima bean seeds, an underutilised legume, fermented with single cultures of *Rhizopus stolonifer*, *Aspergillus fumigatus* and *Saccharomyces cerevisiae*. Ground Lima bean cotyledons were fermented in calabashes with single starter cultures of the organisms for five days. All the anti-nutrient factors reduced significantly in the starter culture fermented samples than the naturally fermented samples while variations were observed in their mineral contents but highly significantly lower than the unfermented samples. Starter fermented products contained higher moisture content, crude protein content, fat contents and ash content, and lower crude fibre and carbohydrate than the naturally fermented samples. *Saccharomyces cerevisiae* fermented sample had the highest protein content and the lowest moisture and carbohydrate contents. *Saccharomyces cerevisiae* fermented sample and the naturally fermented sample were not significantly different with respect to colour, texture and odour, and were more acceptable. These findings showed *S. cerevisiae* is a good candidate for the fermentation of Lima bean seeds with respect to protein improvement and organoleptic qualities.

Introduction

Lima bean seeds are among the leguminous plants that are underutilised in Nigeria. It is a potential supplement or even a substitute for the expensive soy meal and groundnut meal which constitute the major portion of conventional protein sources used in composite livestock feeds. The shift from this plant to other legumes such as soybean and groundnut was not unconnected with its extended cooking period resulting into the wastage of scarce and expensive fuel (Jimoh *et al*., 2011). Also Lima bean seeds contain anti-nutritional factors such as hydrogen cyanide, phytic acids, saponin, oxalate, tannin, trypsin inhibitor activity haemagglutinin activity (Ezeagu and Ibegbu, 2010). These anti-nutritional factors have been observed to inhibit absorption of nutrients and their subsequent utilisation and assimilation by animals. Besides, they cause some level of damages to some organs such as liver, kidney and spleen (Emiola *et al*., 2007).

Several efforts have been made to reduce anti-nutrient contents of Lima bean seeds and some other plants. The techniques used include soaking, boiling, toasting, germination and fermentation (Nwosu, 2010). Most of these techniques have been reported to reduce the anti-nutrient factors to considerate levels. However, apart from fermentation, most of the nutrient contents were reduced particularly the protein contents when processed with these techniques. The need to search for new alternative protein sources other than protein of animal origin which are more expensive is of great concern (Akinmutimi and Ezea, 2006). Fermentation serves as a means of enhancing the nutrient content of foods through biosynthesis of vitamins, essential amino acids and degradation of anti-nutrients (Nwosu, 2010).

Several researches have been carried out on some indigenous fermented foods such as ugba and tempeh to enhance their technological advancement and standardization by the use of some strains as starter cultures (Azeke *et al*., 2011; Nwagu *et al*., 2011). Controlled fermentation enriches nutritive value of foods with respect to shelf-life, hygienic quality, nutrient improvement. and anti-nutrient reduction (Adeyemi *et al*., 2012). This study was to ferment Lima bean seeds with *Aspergillus fumigatus, Rhizopus stolonifer* and *Saccharomyces cerevisiae* which were isolated from the naturally fermented seeds (Adegbehingbe, 2013).

Materials and Methods.

Preparation of Lima Bean Cotyledons Samples for inoculation

Fresh and healthy Lima bean seeds were sorted and washed with clean water, drained and soaked in hot water (110±10^{o}C) for 2 hours to enhance easy removal of the testa from the cotyledons. The cotyledons were washed and rinsed with several changes of sterile water (1:5), cooked for 6 hours and finally dried using a drying cabinet at 60^{o}C for 48 hours.

The dried Lima bean cotyledons (500g) were washed with 2000 ml of water containing 8g sodium metabisulphite (4% w/v) and rinsed with several changes of sterile distilled water. The sample was ground using a blender sterilized with 90% ethanol and rinsed with sterile distilled water and then cooked for six hours and allowed to cool.

3.5.1 Preparation of inoculum for the controlled fermentation of Lima bean seeds

Aspergillus fumigatus, R. and *S. cerevisiae* which were among the dominant fungi previously isolated from naturally fermented Lima bean seeds (Adegbehingbe, 2013) were used as single starters for the fermentation of the Lima bean seeds. The cultures were subcultured from their respective potato dextrose agar slants and inoculated into freshly prepared potato dextrose agar plates by streak plate technique and incubated at 25°C for 48 hours. An inoculum from the plate of each fungus was aseptically transferred into a cooled sterile molten potato dextrose broth and incubated at 25°C for 48 hours.

Inoculation procedure for the controlled fermentation of Lima bean seeds

Five milllilitres each from the broth culture of each of the fungi (4x10^5 spore/ml) was aseptically inoculated into 100 ml of sterile distilled water. The suspension was then poured on 500g ground Lima bean seeds which had been cooked for six hours and then mixed with the substrate using a sterile spatula. The sample was then poured into a calabash lined with fresh banana leaves both which were sterilized with 90% ethanol. The calabashes were then covered with their lids and then sealed tightly with wraps of paper tapes. The samples were allowed to ferment for five days at ambient temperatures (27±2°C).

Proximate Composition

The proximate composition and mineral contents of the raw, naturally fermented and starter culture fermented Lima bean seeds was determined (AOAC, 2005). The parameters determined include moisture, crude protein, crude fats, crude fibre, and carbohydrate contents.

Anti-Nutrient Determination

The saponin, tannin, phytic acid, trypsin inhibitor, oxalate, saponin and cyanide of the raw and the fermented samples were determined (Bradbury *et al*., 1999; Egwaikhide *et al*., 2009).

Sensory Evaluation

The fermented samples were served 20 untrained judges to evaluate the sensory qualities (colour, sliminess, texture, odour and overall acceptability) using a five-point hedonic scale (1 and 5 representing extremely dislike and extremely like, respectively).

Statistical Analysis

Data obtained were analyzed by ANOVA and significant differences between means were compared using Duncan multiple range test (Duncan, 1955) using SAS/STAT program).

RESULTS

Significant reductions were observed in all the anti-nutrient factors of the sample fermented with starter cultures than the naturally fermented samples (Table 1). The highest and the lowest reductions in saponin content was found in *R. stolonifer* fermented sample (0.46%) and *S. cerevisiae* fermented sample (0.52%) samples respectively. Oxalate contents reduced from 1.61 mg/g to 0.86 mg/g, (0.41 mg/g), (0.39 mg/g) and (0.39 mg/g) in naturally fermented sample, *A. fumigatus* fermented sample, *R. stolonifer* fermented sample and *S. cerevisiae* fermented sample respectively. Significant reductions were more pronounced in phytic acid contents of the sample which was fermented with the single starters of *A. fumigatus* (3.18 mg/g) and *S. cerevisiae* (3.17mg/g) than the *S. cerevisiae* (3.32 mg/g). Tannin contents reduced from 0.84 mg/g in raw sample to 0.12 mg/g, 0.11 mg/g and 0.12 mg/g) when fermented with *A. fumigatus, R. stolonifer,* and *S. cerevisiae* respectively. Cyanide contents were significantly reduced from 56.76 mg/100Kg when fermented with the molds (*A. fumigatus* 12.67 mg/100Kg, *R. stolonifer* 11.56 mg/100Kg) than when fermented with *S. cerevisiae* 13.45mg/100Kg.

The substrate fermented with *A. fumigatus* had the highest potassium content of 741.00 µg/g while the lowest content was found in *S. cerevisiae* fermented sample (623.08 µg/g) (Table 2). The highest phosphorus was found in *A. fumigatus* fermented Lima bean seeds (176.75 µg/g) followed by *R. stolonifer* fermented sample (174.95 µg/g)while the least content was found in S. cerevisiae fermented sample (146.20 µg/g). Magnesium was significantly higher in sample fermented with *R. stolonifer* (87.88 µg/g) than those fermented *A. fumigatus* (85.05 µg/g)) and *S. cerevisiae* (81.98 µg/g) (Table 2). The highest sodium content was observed in *R. stolonifer* fermented sample (35.64 µg/g) while the lowest content was got from *S. cerevisiae* (30.35 µg/g)). Similar trend was also observed in manganese, iron and copper contents.

Moisture content increased after fermentation in all the samples with the highest and the lowest contents from samples fermented with *A. fumigatus* (3.81%) and *S. cerevisiae* respectively (36.38%) (Figure 1). Samples fermented with single starters of *S. cerevisiae* and *A. fumigatus* had the highest and the least protein contents of 24.41% and 21.23% respectively.. The highest fat content of 3.41% was obtained from *A. fumigatus* fermented sample while the lowest content was from samples fermented with *S. cerevisiae* (2.89%). The highest fibre content was found in sample fermented with *S. cerevisiae* (4.01%) while the least content was got from *A. fumigatus* fermented sample (3.31%). The highest ash content was got from *R. stolonifer* fermented sample (6.23%) while the lowest content from *A. fumigatus* fermented sample (5.73%) but higher than the control content (4.98%). The lowest and the highest carbohydrate contents were obtained from *S. cerevisiae* fermented sample (26.3%) and *A. fumigatus* fermented sample (28.22%) respectively but lower than the control (35.24%) (Figure 2).

The lighter brown colour of *S. cerevisiae* fermented sample was rated higher (2.5) and significantly higher than those fermented with single starters of *A. fumigatus* (1.5) and *R. stolonifer* (1.9) (Table 3). *Saccharomyces cerevisiae* fermented Lima bean seeds was significantly rated best (2.0) in terms of sliminess while *R. stolonifer* was rated lowest (1.5) substrate was rated best while *Rhizopus* substrate was rated lowest among the three monoculture fermented samples. However, naturally fermented Lima bean seeds were rate significantly rated higher ($p<0.05$) than the starter culture fermented lima bean seeds. The substrate fermented with single starters of A. fumigatus and *R. stolonifer* were scored same (2.6) with respect to texture while *S. cerevisiae* was scored 2.5. However, naturally fermented sample was rated best (2.8) but was however was not significantly different ($p<0.05$) from the fungal fermented samples. Ammonial odour was strongly perceived in samples fermented naturally than those fermented with the fungal cultures and scored 2.8. *Saccharomyces cerevisiae* treatment was scored 2.7 while *A. fumigatus* and *R. stolonifer* were scored 2.3 and 2.1 respectively.

Table 1: Antinutrient contents of fermented ground-cooked Lima bean seeds in calabashes using of starter cultures

Isolates	Saponin (%)	Oxalate (mg/g)	Phytic acid (mg/g)	Tannin (mg/100g)	Cyanide (mg/100Kg)
AF	**0.47± 0.01^{d}**	**0.41± .03^{c}**	**3.18± 0.05^{c}**	**0.12± 0.01^{cd}**	**12.67±0. 43^{cd}**
RS	**0.46±0.02^{d}**	**0.39±0.03^{d}**	**3.23± 0.06^{c}**	**0.11± 0.02^{d}**	**11.56± 0.98^{d}**
SC	**0.52±0.02^{c}**	**0.36±0.02^{e}**	**3.17± 0.04^{c}**	**0.12± 0.02^{cd}**	**13.45± 0.65^{c}**
NF	**0.93±0.01^{b}**	**0.86±0.04^{b}**	**8.3±0.20^{b}**	**0.45±0.02^{b}**	**25.45±0.68^{b}**
GU	**1.50± 0.02^{a}**	**1.61±0.17^{a}**	**13.9± 0.02^{a}**	**0.84± 0.03^{a}**	**56.76±1.89^{a}**

Values with the same superscript letter(s) down a column are not statistically significantly ($P>0.05$) different.

Key:

Af = *Aspergillus fumigatus*, **Sc = *Saccharomyces cerevisiae*,**
Rs = *Rhizopus stolonifer*, **GU= Ground-unfermented sample**
NF= naturally fermented

Table 2: Mineral contents of fermented ground-cooked Lima bean seeds in calabashes using starter cultures.

Isolate	K(µg/g)	P(µg/g)	Mg(µg/g)	Na(µg/g)	Mn(µg/g)	Fe(µg/g)	Cu(µg/g)
AF	741.00±19.5^{b}	176.75±7.54^{b}	85.05±6.91^{b}	32.98±0.46^{c}	1.02±0.03^{b}	5.70±0.07^{b}	0.70±0.02^{b}
RS	697.79±16.26^{c}	174.95±7.54^{b}	87.88±4.90^{ab}	35.64±6.51^{b}	1.03±0.02^{b}	5.77±0.07^{b}	0.72±0.01^{b}
SC	623.08±28.74^{e}	146.20±10.12^{d}	81.98±1.54^{c}	30.35±1.14^{d}	0.98±0.01^{c}	5.42±0.06^{c}	0.63±0.02^{c}
NF	657.90±22.75^{d}	152.67±5.57^{c}	82.47±4.64^{c}	29.09±2.34^{e}	0.97±0.02^{c}	5.52±0.04^{d}	0.61±0.02^{c}
GU	901.41±32.45^{a}	187.86±5.63^{a}	93.56±3.54^{a}	42.76±2.37^{a}	1.17±0.06^{a}	7.14±0.08^{a}	0.91±0.02^{a}

Values with the same superscript letter(s) down a column are not statistically significantly (P>0.05) different.

Key:

AF = *Aspergillus fumigatus*, RS = *Rhizopus stolonifer*,
SC =*Saccharomyces cerevisiae*, NF, Naturally fermented sample,
GU= Ground-unfermented sample

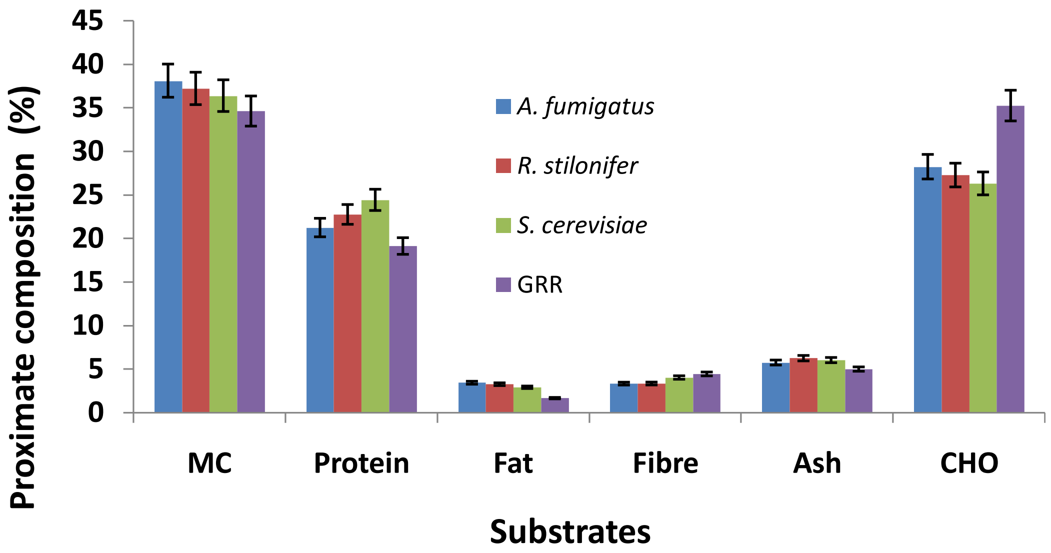

Figure 1: Proximate composition of Lima bean seeds fermented with fungi
Legend: MC= Moisture content, CHO= Carbohydrate content, GRR= Naturally fermented sample

Table 3: Organoleptic and physical properties of the fermented ground-cooked Lima bean seeds in calabashes using starter cultures

Isolates	Colour	Sliminess	Texture	Odour	Overall acceptability
AF	1.5±0.02^{c}	1.9±0.03^{c}	2.6±0.04^{a}	2.3±0.02^{b}	1.7±0.02^{c}
RS	1.9±0.03^{b}	1.7±0.02^{d}	2.6±0.02^{a}	2.1±0.04^{c}	2.1±0.04^{b}
SC	2.5±0.04^{a}	2.0±0.02^{b}	2.5±0.01^{a}	2.7±0.02^{a}	2.6±0.02^{a}
NF	2.4±0.05^{a}	2.8±0.04^{a}	2.8±0.02^{a}	2.8±0.06^{a}	2.7±0.06^{a}

Values with the same superscript letter(s) down the column are not significantly (P>0.05) different.
Key:Af = *Aspergillus fumigatus*, Sc = *Saccharomyces cerevisiae*,
Rs = *Rhizopus stolonifer*, GU= Naturally fermented sample

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DISCUSSION

Results from this study have shown that starter cultures have significant effects on fermented foods. The increase in moisture content as the fermentation progressed was in agreement with Ogueke *et al*., 2010). This is believed to be due to the hydrolytic activity of the fermenting organisms releasing moisture as part of their metabolic products.

The increases observed in protein contents of the fungal fermented Lima bean seeds agreed with Oboh and Elusiyan (2007) while fermenting cassava tubers with single starters of *S. cerevisiae and R. oligosporus* and Adeyemi, *et al*. (2012) while fermenting *Chrysophyllum albidum* seeds with *A. niger*. The increase in protein contents could be attributed to the ability of the organisms to secrete extracellular protease during the fermentation process (Erukainure *et al*., 2010). The multiplication of the fungi in the form of single cell protein could also contribute to the increase in the protein contents (Sanusi et al., 2013). Some fungi such as *A. fumigatus*, *R. oryzae* and *S. cerevisiae* have been used to enrich protein contents of fermented food products (Akindahunsi *et al*., 1999; Oboh and Akindahunsi, 2003)

The higher crude fat content of the fungi treated Lima bean seeds was in agreement with Belewu *et al*. (2010). The Increase in fat content of the fungal fermented sample could be due to the possibility of the fermenting fungi to transform the carbohydrate content of the seeds to fat (Lehninger, 1987). Besides, some fungi have been reported to synthesise microbial oil during fermentation (Oboh and Akindahunsi, 2003). It has also been reported that fungi have more lipogenic ability than lipolytic ability which could enhance ether extract content of the fungi treated sample (Sanusi *et al*., 2013). One of the possible reasons for the ability of *S. cerevisiae* to cause a higher increase in fat and protein could be that the culture condition favours the growth of S. cerevisiae more than *A. fumigatus* and *R. stolonifer.* This was similar to the findings of Oboh and Akindahunsi (2003) that *S. cerevisiae* solid substrate fermented cassava had higher protein content than *R. oryzae* fermented cassava flour. The general low lipid content of the fermented sample could enhance its usefulness as animal feed because high fat content in feed ingredients could cause difficulty in mixing the feed and also require antioxidants for preventing the feed from oxidative rancidity (Emiola *et al*., 2007).

The significant decrease in fibre and carbohydrate contents of starter culture fermented sample than the naturally fermented sample was in agreement with Belewu *et al*. (2010) and Adeyemi *et al.* (2012). This could be due to secretion of extracellular enzymes such as ligninases, cellulases and hemicellulases by the fungi (Oboh and Elusiyah, 2007) which are capable of hydrolyzing carbohydrates and crude fibre into simple sugars, which the organisms could use as carbon source and transform them to other macromolecules such as proteins and fats (Oboh and Akindahunsi, 2003). Higher crude fibre is poorly digested by animals and interferes with other nutrients, thus making them unavailable for use (Adeyemi *et al.*, 2012). The ash contents which increased significantly after fermentation conforms with the findings of Sanusi *et al*. (2013) while fermenting *Jatropha curcas* seeds using various monocultures of fungi. Adeyemi *et al*. (2012) also reported increase in ash contents of *C. albidum* seeds fermented with *A. niger* spores. The significant increases in the ash contents of the starter culture fermented sample than the naturally fermented sample could be an indication that these fungi had peculiar roles they play through biosynthetic or hydrolytic mechanisms to increase the inorganic mineral elements in the sample.

General significant reductions in anti-nutrient contents observed after heat treatment of the Lima bean seeds had been observed by many in other substrates (Olajide *et al*., 2011). The decrease might be due to leaching into the cooking water (Effiong and Umoren, 2011). The subsequent reductions in the anti-nutrient contents of the fermented samples had been reported and attributed to further leaching and microbial activities (Nwosu, 2010). Besides, higher reductions in starter culture fermented samples implied that these fungi were responsible for their reductions. The variation in the level of decrease in the anti-nutrient contents of the products by each fungus indicates that the enzyme activity varies with the organisms (Oboh *et al*., 2003; Sanusi *et al*., 2013). Azeke *et al*. (2011) reported that *R. oligosporus* contained phytase which is responsible for phytate degradation.

The decrease in the phytate content of the fungi fermented samples could be attributed to secretion of phytase by the fungi thereby hydrolyzing the phytate (Oboh and Elusiyan, 2007). The phytases of many *Aspergillus* and *Rhizopus* have been characterized (Fugita *et al*., 2003). The observed decreases in tannin with increase in fermentation time agreed closely with Effiong and Umoren (2011) while monitoring the effects of multiprocessing techniques on the chemical composition of horse eye beans (*Mucuna urens*).

Reduction in cyanide observed in this study was also reported by Yan and Kin (2010) on dietary grape pomace fermented by *S. boulardii*. This showed that the fungi were capable of hydrolising the cyanide content of the seeds. Endogenous hydrolytic enzymes that occur in cyanophoric tissues may also have contributed to the hydrolysis of the cyanogens into non-toxic sugar moiety and cyanohydrin (Ravi and Padmaja, 1997).

Oxalate content was more reduced in Lima bean seeds fermented with *S. cerevisiae* than the other starters. The decrease in saponin content agrees with Erukainure *et al*. (2010) while fermenting watermelon rinds with *S. cerevisiae*.

Higher sliminess observed in the naturally fermented Lima bean seeds could be due to the presence of Bacillus species among the fermenting organisms. Foods fermented with Bacillus species are usually associated with sticky mucilage and strong proteolytic activity (Ogueke *et al*., 2010). Higher flavor rating in *S. cerevisiae* fermented foods was similar to the findings of (Medeiros *et al*., 2001). Yeasts have been reported to be a flavour enhancer in many fermented foods (Medeiros *et al*., 2001; Omemu *et al*., 2007). Various studies also suggested that yeast products and yeast culture have the ability to improve the growth performance, nutrient digestibility and health condition in animals (Kamm *et al*., 2004)

CONCLUSION

This study has shown that the nutritive value of Lima bean seeds could be upgraded if fermented with these fungi. *Saccharomyces cerevisiae* fermented Lima bean seeds showed is more promising in terms of protein improvement and organoleptic properties. Further work should be focused on strain selection for better nutritive value and also on the biological evaluation of the products.

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