

FERMENTATION OF SINGLE AND MIXED SUBSTRATES OF LIMA BEAN SEEDS (*Phaseolus lunatus*) AND AFRICAN LOCUST BEAN SEEDS (*Parkia biglobosa*)

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ABSTRACT

The use of single substrate of leguminous seeds for condiment production has been shown to have deficiency and imbalance in nutrient composition. This research was designed to determine possibility of the use of Lima beans and African Locust bean seeds as mixed substrates for condiment production. African locust bean seeds, Lima bean seeds as well as their mixed substrates were fermented for 72 hours. The physicochemical properties, microbiological analysis, proximate composition and anti-nutritional factors were thereafter determined for the three substrates. Five genera of bacteria (*Bacillus subtilis*, *B. polymyxa*, *Lactobacillus casei*, *Leuconostoc mesenteroides*, *Micrococcus rubens* and *Staphylococcus aureus*) and five genera of fungi (*Aspergillus flavus*, *Penicillium expansum*, *Mucor mucedo*, *Rhizopus stolonifer* and *Saccharomyces cerevisiae*) were isolated from the fermented substrates in this study. Bacterial counts (5.8 to 7.8 Cfu/g) and fungal counts (2.4 to 3.5 Sfu/g) observed in mixed substrates were lesser than their single substrates. Moreover, steady increases were observed in protein (36.86 – 40.48%), fat (7.58 – 13.60%), moisture (8.74 – 9.07%) and ash (1.69 – 2.81%) contents of the mixed substrates as fermentation days progressed. Reductions in the anti-nutritional contents were also observed in the mixed substrates. This study showed an improvement in the nutritional quality and reduction in the anti-nutritional contents of the blended African locust bean seeds and Lima bean seeds when explored as mixed substrates, and thus, could improve the use of Lima bean seeds which are among the underutilized legumes.

Keywords: Legumes, Condiments, African locust beans, Lima beans, Nutritional quality

INTRODUCTION

Condiment production has been largely on a traditional small-scale, household basis under highly variable conditions and has not attained commercial status due to the very short shelf life, objectionable packaging materials and characteristics putrid odour (Arogba *et al.*, 1995; Akande *et al.*, 2010). The desired state of fermentation of the condiments is indicated by the formation of mucilage and overtones of ammonia produced as a result of the breakdown of amino acids during fermentation (Omafuvbe *et al.*, 2004).

The conventional substrates for condiment production are diverse and each can be produced from more than one raw material, with further proof that almost any edible plant material can be subjected to fermentation (Achi, 2005a). Seeds of legumes may account for up to 80% of dietary protein and may be the only source of protein for some groups of people. Quite often, seeds that are used for fermentation are inedible in their raw unfermented state, their cooked forms are eaten as meals and are commonly used in fermented form

as condiments to enhance food flavour (Oniofok *et al.*, 1996; Ogbonna *et al.*, 2001). However, only few of these leguminous seeds have been explored for consumption with preference given to cereal crops embedded with low protein and essential amino acid contents owing to their cumbersome process of preparation as well as their high anti-nutritional factors (Akande *et al.*, 2010a; Adegbehingbe *et al.*, 2014). Although, several methods have been exploited to create a balance in the nutritional contents of cereal, their protein content is yet to equal the amount present in legumes (Akande *et al.*, 2010b; Adegbehingbe *et al.*, 2014)

High protein, fat and carbohydrate are the major constituents required when processing legumes into condiments while the fermenting microorganisms must be able to metabolise them and convert them to desired end products in terms of sensory attributes as well as nutritive value. These condiments have been widely reported to serve as substitute for fish and meat in some localities (Achi, 2005b; Farinde *et al.*, 2014).

African locust bean (*Parkia biglobosa*) belongs to the family leguminosae. The fermented product of African locust bean is locally known as *iru* by the Yorubas of the south-western and *darwadawa* by the Hausas of the northern Nigeria. The seeds of locust bean are nutritionally deficient and unpalatable for consumption (Oladunmoye, 2007; Akande *et al.*, 2010).

Lima bean (*Phaseolus lunatus*) belong to the family fabaceae. The seed is flat and about 5-12 cm long. It is known as *pakala* among the Yorubas in the south-western part of Nigeria (Cobley and Steele, 1976). Lima beans are known to be rich in digestible carbohydrate, mainly starch having a concentration of 50% or more (Paul and Southgate, 1992). They also contain appreciable amount of dietary and soluble fibre which helps to regulate blood sugar levels and lowers cholesterol, preventing constipation, digestive disorders, irritable bowel syndrome and diverticulitis (Farinde *et al.*, 2011; Heuzé *et al.*, 2015). Ojokoh and Daramola (2012) reported that these highly nutritious beans are grown for their seeds, which are eaten as vegetables.

However, there is little information on the thermal treatment on African locust bean seeds and Lima bean composites for the production of food condiments. This study is therefore aimed at determining the possible improvement on the nutritional quality of Lima beans and African locust beans seeds when used as mixed substrates.

MATERIALS AND METHODS

Sources of Materials

Seeds of Lima beans were purchased from a farmer raising them for food at Temidire-Ekiti in Ijero Local Government of Ekiti State, Nigeria, while dried African locust bean seeds were purchased at Ado-Ekiti township market in Ado Local Government of Ekiti State, Nigeria. They were transported to Microbiology laboratory of Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria for analyses.

Preparation of Substrates

Laboratory Preparation of Lima Bean Seeds

Clean and apparently healthy seeds of Lima bean were sorted, weighed, washed with clean water

and then pressure cooked for 1 hour 30 minutes. The seeds were dehulled manually, leaving the cotyledons. Three hundred grams of the cotyledons were then washed thoroughly with distilled water and boiled again for 1 hour. After boiling, the cotyledons were drained of water and kept in a plastic bucket for a period of 72 hours.

Laboratory Preparation of African Locust Bean Seeds

Healthy African locust bean seeds were sorted, weighed and then pressure cooked for 4 hours making the seeds to be tender for manual dehulling, leaving the cotyledons. Three hundred grams of the cotyledons were washed thoroughly with distilled water and pressure cooked again for 1 hour, after which the cotyledons were drained of water and kept in a plastic bucket for a period of 72 hours (Akande *et al.*, 2010).

Laboratory Preparation of Mixed Substrates

Clean and apparently healthy Lima bean and African locust bean were processed as above (pre-treatments-pressure cooking for 1 hour 30 minutes and 4 hours respectively). They were hand-dehulled leaving their cotyledons. One hundred and fifty grams each of their cotyledons were mixed together and washed thoroughly with distilled water and pressure cooked for 1 hour, after which their cotyledons were drained of water and kept in a plastic bucket for a period of 72 hours.

Microbiological Analysis of Prepared Substrates

Five grams each of mashed cotyledons of the fermenting substrates were homogenised in 45 ml of sterile distilled water to form stock solutions. The fermenting substrates were further serially diluted appropriately using ten-fold serial dilution method. Quantitative and qualitative microbial analysis of the three samples (Lima beans, African locust beans and their mixed substrates) were carried out at 24 hours interval on nutrient agar (NA), malt extract agar (MEA) for the cultivation of bacteria and fungi respectively. Growth seen on the respective media were sub-cultured to obtain pure cultures and characterized using cultural, morphological and biochemical tests (Olutiola *et al.*, 2000; Madigan *et al.*, 2002).

Temperature, pH and Total Titratable Acidity of Prepared Substrates

The temperature of the fermenting substrates were monitored on daily basis for 3 days by aseptically inserting a thermometer into the samples. The pH values of the fermenting substrates were determined daily for 3 days by mixing 5 g of the respective samples with 45 ml of distilled water as described in AOAC (1990). Total titratable acidities (TTA) of the fermenting substrates were determined daily for 3 days by homogenising 5 g of each samples in 50 ml of sterile distilled water. 25 ml of each sample was titrated against 0.1M NaOH using phenolphthalein as indicator.

Proximate Composition of Prepared Substrates

The proximate compositions of the fermenting samples were monitored on daily basis throughout the fermentation period (AOAC, 1990). The parameters determined include moisture, crude protein, crude fats, crude fibre, and carbohydrate contents.

Antinutritional Contents of Prepared Substrates

The oxalate, phytate, alkaloid, saponin and tannin

contents of the fermenting samples were also determined on daily basis for 72 hours (Egwaikhide *et al.*, 2009).

Statistical Analysis

Data obtained were analyzed by ANOVA and significant differences ($p < 5$) between means were compared using Duncan multiple range test with the aid of IBM SPSS software version 20.

RESULTS

Twenty-nine bacteria and fourteen fungi were isolated during the fermentation of Lima bean seeds, African locust bean seeds and their mixed substrates. The identities of the bacteria isolates as shown in table 1 were *Bacillus subtilis*, *Bacillus polymyxa*, *Staphylococcus aureus*, *Lactobacillus casei*, *Micrococcus rubens* and *Leuconostoc mesenteroides*. Five genera of fungi identified include *Saccharomyces cerevisiae*, *Mucor mucedo*, *Rhizopus stolonifer*, *Aspergillus flavus* and *Penicillium expansum* as shown in table 2. There was an increase in the total viable bacterial and fungal (Figure 1 and 2) counts of the three fermenting substrates as the fermentation days progresses.

Table 1: Biochemical Characteristics of Bacterial Isolates from the Fermenting Substrates

Gram staining	Catalase	Motility	Starch hydrolysis	Orthinine	Indole production	Gelatin hydrolysis	Glucose	Fructose	Lactose	Maltose	Sucrose	Mannitol	Substrate present	Suspected organism
+	+	+	+	-	-	+	AG	AG	-	A	A	AG	LM, RR, MS	<i>Bacillus subtilis</i>
+	+	+	+	-	-	+	A	A	-	A	A	-	LM, RR, MS	<i>Bacillus polymyxa</i>
+	-	-	+	-	-	+	AG	AG	A	A	A	A	LM, MS	<i>Lactobacillus casei</i>
+	+	-	+	-	-	-	AG	A	-	A	AG	-	LM	<i>Micrococcus rubens</i>
+	+	-	-	-	-	+	AG	AG	A	AG	AG	AG	LM, RR, MS	<i>Staphylococcus aureus</i>
+	-	-	+	-	-	+	A	A	-	AG	A	A	RR	<i>Leuconostoc mesenteroides</i>

Keys

+	Positive
-	Negative
A	Acid production
G	Gas production
AG	Acid and gas production
LM	Lima bean isolate
RR	Africa Locust bean isolate
MS	Mixed substrates

Table 2: Cellular Characterization of Fungal Isolates from the Fermenting Substrates

Colour	Cellular characterization	Substrate present	Probable cell
Brown	Sporangia heads appear brown in colour, with a length of 2.8-5.0 cm on MEA in six days at 25 °C with rough walled and singly developed sporangiophore with aseptate hyphae.	RR	<i>Rhizopus stolonifer</i>
Grey	Sporangia heads are grey in colour with a length of 8.08.4 cm on MEA in six days at 25 °C with smooth walled and large sized sporangiophore.	RR	<i>Mucor mucedo</i>
Green	Conidia heads appear green in colour, 3.5 -5.0 cm long on MEA in six days at 25 °C with broad thick wall and conidiophore with septate hyphae.	LM, MS	<i>Aspergillus flavus</i>
Blue	Conidia heads are blue in colour, 1.8 -2.0 cm long on MEA in six days at 25 °C with thick wall and conidiophore with septate hyphae.	LM, MS	<i>Penicillium expansum</i>
White	Yeast, conidia appear white, thalli unicellular and irregularly oval shaped, conidiophore with septate hyphae.	RR, MS	<i>Saccharomyces cerevisiae</i>

Keys

- LM Lima bean substrate
- RR Africa Locust bean substrate
- MS Mixed substrates

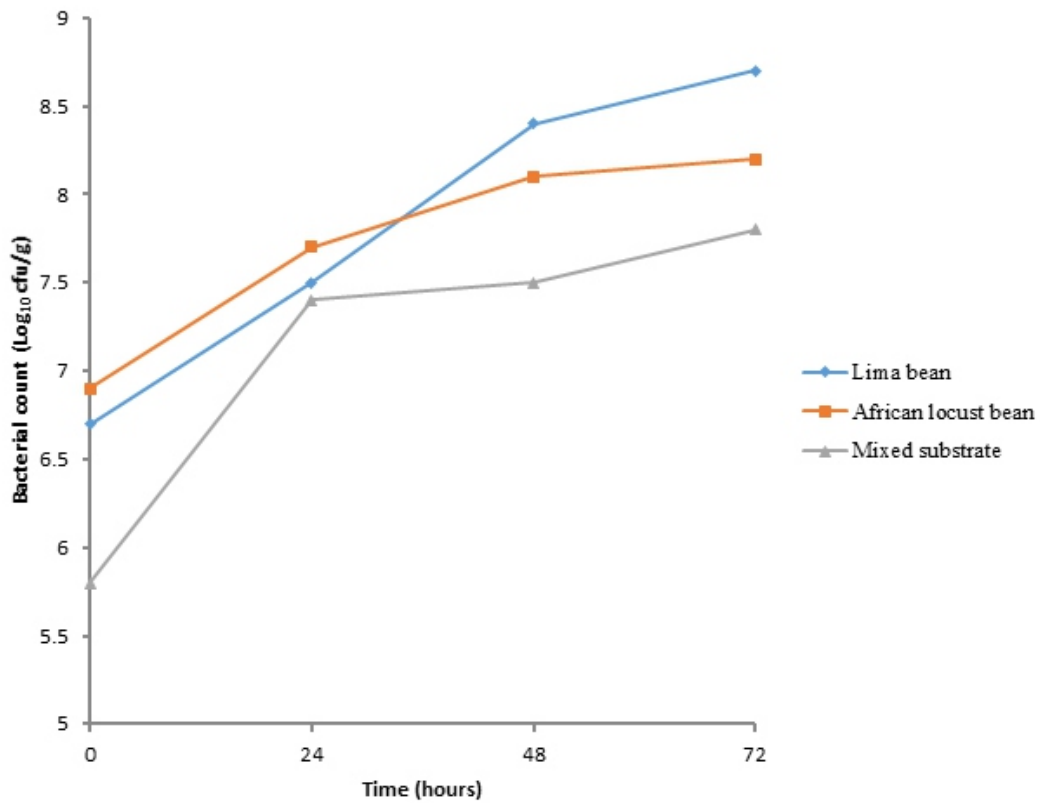


Figure 1: Total Viable Bacterial Counts of the Fermenting Substrates

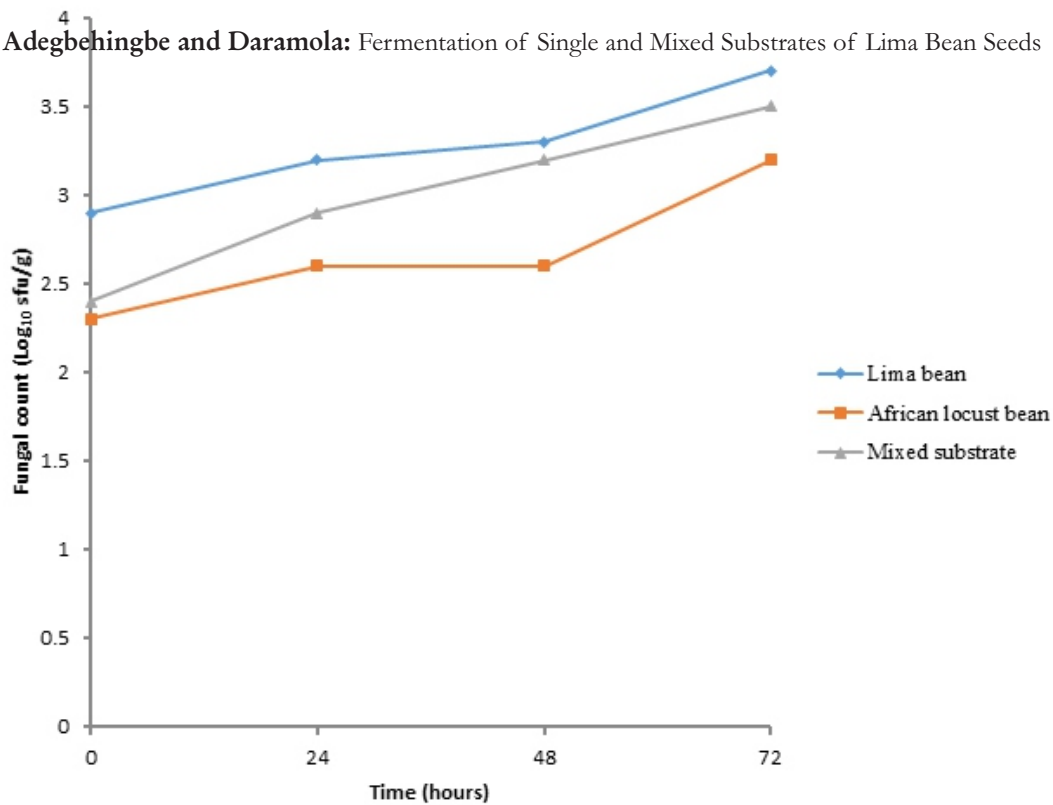


Figure 2: Total Viable Fungal Counts of the Fermenting Substrates

The occurrence of the microorganisms as shown in table 3 showed that *Bacillus subtilis*, *Bacillus polymyxa* and *Staphylococcus aureus* were the only identified microbial isolates represented in the three fermenting substrates while other microorganisms appeared once or twice in any of the substrates. The pH values of the fermenting Lima bean seeds and mixed substrates decreased from 5.78 to 4.63 and 5.74 to 5.20 respectively while fermenting African locust bean seeds pH increased from 5.44 to 6.35 (Figure 3). Figure 4 showed the temperature values of the fermenting substrates. Temperature of the fermenting Lima beans seeds increased from 26 °C to 31 °C with similar value range observed in African locust bean seeds and the mixed substrates ranging from 25 °C to 33 °C and 26 °C to 31 °C respectively with increase in fermentation days. Figure 5 reveals the

changes in the total titratable acidities (TTA) of the three fermenting substrates. Fermenting Lima bean seeds TTA decreased from 2.31% to 1.42% while African locust bean seeds and their mixed substrates increased from 0.22% to 2.96% and 1.49% to 1.82% respectively with increase in fermentation days.

The proximate composition values of the fermenting substrates showed a slight increase in the moisture content of the fermenting African locust bean seeds (6.10 to 6.89%) and the mixed substrates (8.74 to 9.07%), with a declined value noticed in the fermenting Lima bean seeds (8.96 to 8.24 %). More so, the three fermenting substrates showed an increase in their protein, fat, ash contents and a decrease in their carbohydrate and fibre contents at 72 hours of fermentation (Table 4).

Table 3: Occurrence of Microorganisms in the Fermenting Substrates

	Lima bean seeds	African locust bean seeds	Mixed substrates
<i>Bacillus subtilis</i>	+	+	+
<i>Bacillus polymyxa</i>	+	+	+
<i>Lactobacillus casei</i>	+	-	+
<i>Micrococcus rubens</i>	+	-	-
<i>Staphylococcus aureus</i>	+	+	+
<i>Leuconostoc mesenteroides</i>	-	+	-
<i>Rhizopus stolonifer</i>	-	+	-
<i>Mucor mucedo</i>	-	+	-
<i>Aspergillus flavus</i>	+	-	+
<i>Penicillium expansum</i>	+	-	+

Keys

- + Positive
- Negative

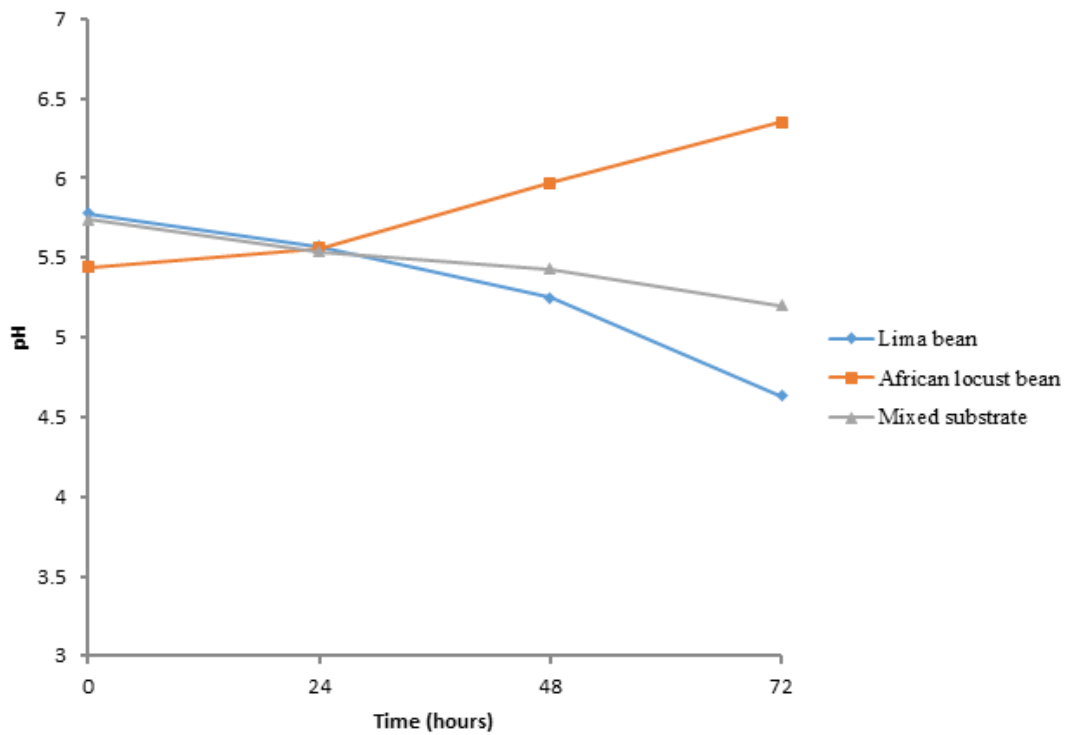


Figure 3: pH Values of the Fermenting Substrates

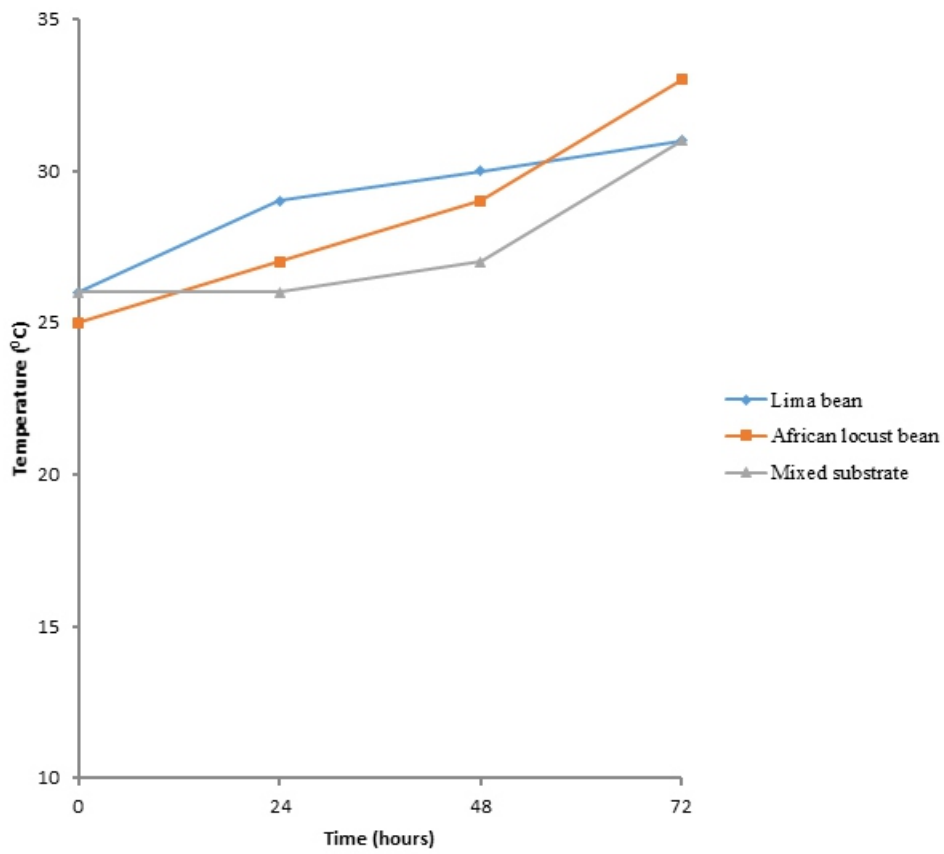


Figure 4: Temperature Values of the Fermenting Substrates

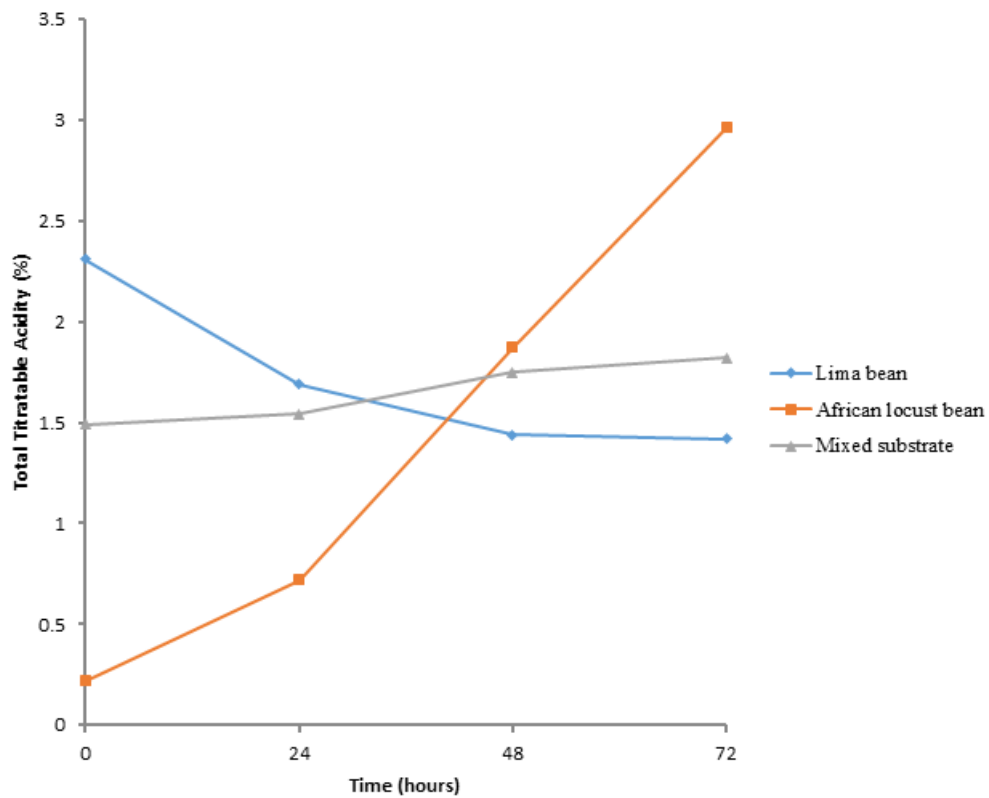


Figure 5: Total Titratable Acidities of the Fermenting Substrates

Table 4: Proximate Analysis of the Fermenting Substrates

	Moisture Content (%)		Protein Content (%)		Fat Content (%)		Ash Content (%)		Carbohydrate Content (%)		Fibre Content (%)							
	0	48	72	0	48	72	0	48	72	0	48	72						
LM	8.96 ^b	8.24 ^b	8.61 ^b	21.85 ^a	23.80 ^a	24.30 ^a	3.68 ^a	4.67 ^a	4.74 ^a	2.29 ^b	2.61 ^b	3.96 ^c	57.85 ^c	55.25 ^c	53.75 ^c	5.37 ^a	5.14 ^a	5.01 ^a
RR	6.10 ^a	6.66 ^a	6.89 ^a	45.56 ^c	46.86 ^c	47.36 ^c	21.16 ^b	25.20 ^b	26.22 ^c	1.84 ^a	2.46 ^b	2.54 ^a	17.47 ^a	11.12 ^a	10.29 ^a	7.87 ^b	7.70 ^b	6.70 ^b
MS	8.74 ^b	8.96 ^b	9.07 ^b	36.86 ^b	39.73 ^b	40.48 ^b	7.58 ^a	11.59 ^a	13.60 ^b	1.69 ^a	2.12 ^a	2.81 ^b	37.01 ^b	30.22 ^b	26.96 ^b	8.13 ^b	7.38 ^b	7.08 ^b

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

Keys

LM Lima bean substrate
RR Africa Locust bean substrate
MS Mixed substrates

Table 5: Anti-nutritional Contents of the Fermenting Substrates

	Oxalate (mg/g)		Phytate (mg/g)		Alkaloid (%)		Saponin (%)		Tannin (mg/g)	
	0	72	0	72	0	72	0	72	0	72
LM	3.22	1.13	26.48	14.01	2.30	1.20	3.80	2.10	0.34	0.15
RR	2.28	0.95	14.47	8.24	2.10	0.80	3.20	1.60	0.21	0.11
MS	2.94	1.10	21.26	12.57	2.60	1.10	3.40	1.90	0.28	0.13

Keys

LM Lima bean substrate
RR Africa Locust bean substrate
MS Mixed substrates

DISCUSSION

The identities of bacterial and fungal isolates reported in this study had been identified by several authors. Farinde *et al.* (2014) isolated *Bacillus subtilis* and *Staphylococcus* sp. while fermenting Africa locust bean seeds and Lima bean seeds. This was also similar to the findings of Fadahunsi and Olubunmi (2010) while fermenting Bambara nut seeds. Osho *et al.* (2010) isolated *Rhizopus stolonifer* and *Aspergillus* sp. while fermenting African locust bean seeds. *Micrococcus rubens* isolated in Lima bean seeds agrees with the findings of Njoku and Okemadu (1998) while fermenting African oil beans to form *ugba*. The presence of *Lactobacillus* in Lima bean seeds and the mixed substrates fermentation were in accordance with Enujiugha (2009), while fermenting mesquite seeds and melon seeds to form *okpei* and *ogiri* respectively. In other words, microorganisms present in the mixed substrates for condiment production have been reported by Achi (2005b) as the normal flora associated with legumes when fermented for condiment production.

The increase in microbial loads in all the substrates between 0 hour and 72 hours of fermentation could be as a result of the availability and utilization of nutritional components present in the three fermenting substrates, thereby encouraging the proliferation of the microorganisms (Yabaya, 2006; Oladunmoye, 2007; Farinde *et al.*, 2014). The changes in colour of the fermented mixed substrates to light brown from cream and deep brown colour of fermented Lima bean seeds and African Locust bean seeds were in conformity with the review of Achi (2005a) and Oyarekua (2011) on legumes, suggesting the possibility of favourable colour change in fermenting substrates when mashed as a single substrates.

Steady and noticeable increase in the protein contents were recorded in all the fermenting substrates. This might be due to the release and modification of structural proteins that are integral part of the microbial cells into the substrates. In other words the fermenting microorganisms could serve as probiotics

(Oladunmoye, 2007; Olajuyigbe and Ajele, 2008; Farinde *et al.*, 2011). The significant reduction in the crude fibre and carbohydrate contents as observed in this study may be attributed to the ability of the fermenting microorganisms to produce extracellular enzymes capable of hydrolyzing and utilizing the nutrients for their metabolic activities as observed by Omafuvbe *et al.* (2004) and Oladunmoye (2007). However, excess dietary fibre in diets may alter mineral metabolism, especially when phytate is present (Sandstead, 1992). The implication of the results showed that the mixed substrates provide a reasonable proportion of the required nutrients (protein, fats and carbohydrate) which agrees with the findings of Odebunmi *et al.* (2010).

Reduction observed in the anti-nutritional contents of single substrates of Lima bean seeds and African locust bean seeds as well as their mixed substrates with increase in fermentation days were also reported by Adewumi and Odunfa (2009) and Nwosu (2012) while investigating the effect of controlled fermentation on the anti-nutritional values of common beans (*Vigna unguiculata*) and asparagus beans (*Vigna sesquipedis*). Selection of mixed substrates for condiment production could offer nutritional benefit such as increased protein contents and enhance flavours that could bring about prospects for industrialization of traditional fermented condiments.

CONCLUSION

The results from this study show that the blend of African locust bean seeds with Lima bean seeds after fermentation improved the nutrient value and reduced the anti-nutritional contents of the seeds. The improvement observed in the nutritional composition and reduction in the antinutritional contents of the samples suggests its suitability as cheap raw materials for condiment production and as alternative protein source in food supplement for less affluent individuals who cannot afford the animal-protein foods. Further studies on the work should include the use of starter cultures on the blend so as to know the main microorganisms involved in the fermentation.

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