JOURNAL OF PURE AND APPLIED MICROBIOLOGY, June 2012. Vol. **6**(2), p. 589-596

Microorganisms Associated with the Natural Fermentation of Extruded Sorghum-Soya Blends

A.O.Ojokoh* and E.N. Udeh

Department of Microbiology, Federal University of Technology, P.M.B. 704, Akure, Nigeria.

(Received: 20 September 2011; accepted: 30 October 2011)

In this study six combinations of sorghum and defatted soy flour(100:0, 90:10,80:20,70:30,60;40and 50;50) coded A-F were extruded in a laboratory using single screw extruder and were fermented naturally at 37^{0}C. The bacteria isolated during the process include *Flavobacterium rigense*, *Micrococcus icristinae*, *Enterobacter spp, Staphylococcus albus, Brevibacterium spp.,Bacillus sublitis,Bacillus cereus and Bacillus brevis,* the yeast were *Saccharomyces cerevisiae, Geotrichum candidium* and *Candida utilis* while the mould were *Aspergillus niger* ,*Aspergillus fumigatus* and*Rhizopus Stolonifer.* The bacterial population of all the extrudates increased up to 48hr and decreased at 72hr of fermentation, the highest bacterial population was at 48hr of fermentation while the fungal count increased from 24hr. The pH and the titratable acidity (TTA) significantly varied during fermentation.

Key Words: Sorghum, Soyabean, Microorganisms, Extrudates, Fermentation.

Foods including cereals and legumes are processed for different reasons; one very obvious reason for processing foods is to improve their storage potential, most raw foods intended for future use are subjected to some limited or elaborate form of processing and special conditions for their extended storage provided to ensure their usefulness and wholesomeness over time with or without further culinary treatment. Foods may be processed to eliminate the inconveniences encountered by the home-maker in getting food ready for the table; convenience is a virtue of most processed foods today. Okaka (1997) noted that many persons feed away from home today than two decades ago. The practice of blending locally grown crops in Nigeria partly for aesthetic purposes and partly as nutrient supplementation has been an age long tradition. The poor starch and protein digestibility of cereals is caused by phytic acid and polyphenols that bind to enzymes in the digestive tract and thus inhibit utilization of proteins and carbohydrate; however, this adverse effect can be overcome by fermentation or extrusion(Onyango *et. al*, 2004). Fermentation is a processing technology that is low cost to be affordable by the poor sectors of the community and should address the problems of food spoilage(Omezuruike,2008). In Africa, and in other part of the world, fermented foods form an important part of the diet, Akinyele and Bello (2008), recorded that fermented foodstuffs remain key constituents of diets in many parts of Asia and Africa.

Extrusion cooking is a relatively recent form of food processing, it is one of the most

* To whom all correspondence should be addressed.
Tel.: +2348036225977
E-mail: tonyojokoh@yahoo.com

common industrial processes used to make snacks and one of the most versatile and well–established processes used in the food industry today. Ojokoh and Yimin (2009); Nwanbueze (2007); n *et al*, (1999) Onyango *et. al* (2005), reported that extrusion cooking is a versatile process for producing starch and/or protein based foods at a low cost due to more efficient use of energy and greater process control with greater production capacities. Functional ingredients such as soy and botanicals that are relatively unpalatable alone can be incorporated into new food items by extrusion, traditional foods can be further enhanced by addition of dietary fibre or other ingredients during extrusion. Extrusion may improve protein digestibility by denaturing proteins, exposing enzyme–accessible sites.

Sorghum (*Sorghum bicolor* L. *moench*) also know as guinea corn, is one of the major cereal grains in Africa and Asia (Amusa and Falola, 2004), it is a plant species of grass family *Poaceae*. Sorghum is an important food and feed crop in hemi and semi–arid region of the world, where it is grown under rain fed and irrigated conditions (Abdel *et al.,* 2010; Ezeaku and Mohammed 2006) and globally it is the fifth most important grain (around 60 million tons produce annually) with the US, India and Nigeria being the top three producing countries; it is the third largest crop harvested In USA (Greg 2010; Abdel *et al.,* 2010); according to Oke *et al* .(1984); sorghum occupies about 46% of the total land area devoted to cereal production in Nigeria. The Soya bean, *Glycine max* [L.] *Merrill*, is a grain legume belonging to family *Leguminosae*.It is native to East Asia (Enwere 1998)and has been grown and used as human food for nearly 5000 years in China. It is cultivated in many areas of the world, from the tropics to temperate regions, today soya account for well over half of total worldwide oil seed production, soya bean was first introduced into Nigeria about 1908, but commercial production did not commence until the have 1940s(Elegbede,1998); Soya bean–fortified products not only have more protein and mineral than their non–fortified counterparts they are considerably cheaper than other sources of high–quality protein such as fish, meat, milk, and other protein rich legumes. According to Dashiell (1987), the cost of protein, when purchased as soya bean, is only about 10-20% of the cost of protein from fish, meat, or milk. Many Nigerian now incorporate soya bean into their diets, and the Nigeria government has declared soya bean production and utilization a national priority. The objective of this study is to isolate and indentify the microorganisms responsible for the fermentation of sorghum-soya extrudates.

MATERIALS AND METHODS

The sorghum (*Sorghum bicolour*) grains was purchased from Oba's market Akure, Ondo State, while Soya beans seeds (*Glycine Max*) variety TGX1448-2E was obtained from International Institute for tropical Agriculture (IITA), Ibadan, Nigeria. All the raw materials were cleaned before subjection to processing treatment. The coarsely grind sorghum was winnowed to remove the bran, it was then milled to fine flour in the attrition mill and then sieved to fineness.

The Cleaned Soya beans seeds were first coarsely milled, winnowed to separate the coat and finished up in attrition mill. The fine flour was then sieved through a mesh. The fine flour was defatted with n-hexane in cold extraction from 20.57% fat content to 15.17%. The recovered flour was air dried and the clumps broken to fines, the flour samples from sorghum and Soya beans were Mixed at Six (6) level combinations as follows:

Sorghum (g)	Soya flour (g)
100	0
90	10
80	20
70	30
60	40
50	50

The flour blends were hydrated and processed in a laboratory single screw extruder (Hobart Corporation Germany), at screw speed of 80rpm and through a die nozzle diameter of 5mm at 110^0C. The extrudates obtained were allowed to cool and dry before fermentation. The extrudates were subjected to natural fermentation. A100g portion of each of the extrudate was weighed and 50ml of water was added, the extrudates were fermented at 30 ± 2^0C for 96hours.

Microbial analysis

Microbial population of the total bacterial and fungi were determined using nutrient agar(NA) and Potato dextrose agar (PDA) respectively,

organisms were enumerated by using appropriate serial dilution and pour plate techniques. The bacterial cultures were incubated at in inverted position at 37±2^{0}C for 24 hours while the fungi plates were incubated in an inverted position at 25±2^{0}C for 72 hours (3days). The organisms were characterized based on biochemical and morphological observations and tests (Cowan and Steel, 1990).

Physico-Chemical Analysis

pH and Total Titratable Acidity (TTA)

The pH of all the fermenting samples (extrudates)were determined at twenty–four(24) hour Interval using pocket size digital pH meter (PHC.P (R) Hanna instrument). The Total Titratable Acidity (TTA) was determined by macerating 2g of fermenting sample into 50ml of distilled water and titrating 10ml of the filtrate against 0.1M sodium hydroxide (Kirk and Sawyer,1991).

Statistical analysis

The data obtained were subjected to one-way analysis of variance (ANOVA) followed by Tukey's test multiple comparisons using SPSS 17.0 version computer software package. The values were considered significant at pd"0.05**.**

RESULTS

A total number of nineteen (19) microorganisms were isolated during the natural fermentation of sorghum-soya extrudates; these comprise of *Flavobacterium rigense* , *Micrococcus icristinae*, *Enterobacter spp.*, *Enterobacter cloacae*, *Corynebacterium cystitidis*, *Corynebacterium pilosun*, *Staphylococcus albus* ,*Brevibacterium spp.*, *Bacillus subtilis, Bacillus cereus, Bacillus brevis, Bacillus megaterium*, *Candida famata*, *Saccharomyces cerevisiae*, *Geotrichum candidium*, *Candida utilis*, *Aspergillus niger*, *Aspergillus fumigatus* and *Rhizopus Stolonifer*.

Table 1. Frequency of occurrence of microbial isolate during fermentation of Sorghum-Soya Extrudate

	Sources of isolation					
Microbial Isolate	A	B	C	D	E	F
Flavobacterium rigense +	-		-	-	-	-
Micrococcus icristinae	-	+	-	+	-	-
Enterobacter cloacae	-	+	+	-	-	-
Enterobacter spp.	-	-	+	-	-	-
Corynebacterium cystitidis -	-	-	+	+	-	
Corynebacterium pilosun	-	-	-	-	+	-
Staphylococcus albus	-	-	-	-	-	+
Brevibacterium spp.	+	-	-	-	-	+
Bacillus subtilis	_	+	+	+	-	-
Bacillus cereus	-	-	-	-	+	+
Bacillus brevis	-	-	-	-	-	+
Bacillus megaterium	+	-	-	-	-	-
Candida famata	+	-	-	-	-	-
Saccharomyces cerevisiae -	+	+	-	-	-	
Geotrichum candidium	-	-	-	+	-	-
Candida utilis	+	+	-	-	-	-
Aspergillus niger	-	-	+	+	-	-
Aspergillus fumigatus	-	-	-	-	+	-
Rhizopus stolonifer	-	-	-	-	+	+

Key:

+ = Present; - = Absent

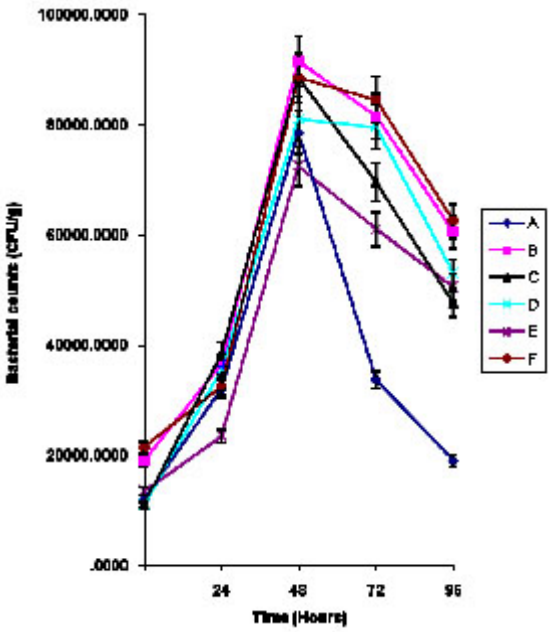
A= 100g Sorghum flour sample=90g and 10g Sorghum-Soya blend

C=80g and 20g Sorghum-Soya blend, D=70g and 30g Sorghum-Soya blend

E=60g and 40g Sorghum-Soya blend, F=50g and 50g Sorghum-Soya blend

Total bacteria populations during the fermentation process

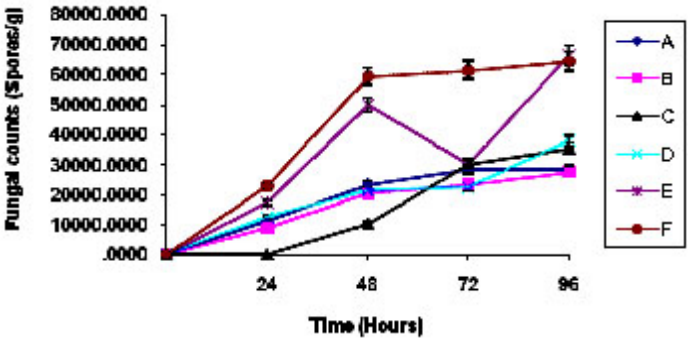
The changes in bacterial population during the natural fermentation of sorghum-soya extrudates at different time interval are represented in Fig. 1, bacterial population of all the extrudates (A-F) increased from 0 hour to 96 hour. The initial bacteria count for extrudate at 0hour was 1.2×10^4 cfu/g and increased to 3.2×10^4 cfu/g and 7.9×10^4 cfu/g at 24 hour and 48 hour respectively, followed by a sharp decrease to bacteria load of 3.4×10^4 cfu/g and 1.9×10^4 cfu/g at 72 hour and 96 hour



Key:

RF= Raw Flour, EUF=Extruded Unfermented, EF= Extruded Fermented
A= 100g Sorghum flour sample=90g and 10g Sorghum-Soya blend
C=80g and 20g Sorghum-Soya blend, D=70g and 30g Sorghum-Soya blend
E=60g and 40g Sorghum-Soya blend, F=50g and 50g Sorghum-Soya blend

Fig. 1. Changes in bacteria count during fermentation of sorghum-soya extrudates



Key:

RF= Raw Flour, EUF=Extruded Unfermented, EF= Extruded Fermented
A= 100g Sorghum flour sample=90g and 10g Sorghum-Soya blend
C=80g and 20g Sorghum-Soya blend, D=70g and 30g Sorghum-Soya blend
E=60g and 40g Sorghum-Soya blend, F=50g and 50g Sorghum-Soya blend

Fig. 2. Changes in fungal count during fermentation of sorghum-soya extrudates

respectively. Extrudate B exhibited an initial bacteria count of $1.9x10^4$ cfu/g at 0 hour with an increase of $3.7 x10^4$ cfu/g,$9.2x10^4$ cfu/g at 24 hour and 48 hour respectively which was followed by a decrease to $8.2x10^4$ cfu/g and $6.1x10^4$ cfu/g at 72 hour and 96 hour respectively. The bacterial count of extrudate C between 0-48 hour were $1.1x10^4$ $3.9x10^4$ and $8.9x10^4$ respectively followed by a decrease to $7.0x10^4$ cfu/g and $4.8x10^4$ cfu/g at 72 hour and 96 hour respectively. Extrudate D had the initial the initial bacterial count of $1.1x10^4$ at 0 hour and increased to $3.6x10^4$ cfu/g and $8.1x10^4$ cfu/g at 24 hour and 48 hour followed by a slight decrease to $8.0x10^4$ cfu/g at 72 hour and a sharp decrease to $5.3x10^4$ at 96 hour of fermentation. Extrudate E also increased from $1.4x10^4$ cfu/g to $2.4x10^4$ cfu/g to $7.3x10^4$ cfu/g at 0 hour, 24 hour and 48 hour respectively; followed by decrease to $6.1x10^4$ and $5.1x10^4$ at 72 hour and 96 hour of fermentation. Extrudate F had initial bacteria count of $2.2x10^4$ cfu/g which increase to $3.3x10^4$ and $8.9x10^4$ cfu/g at 0 hour, 24 hour and 48 hour respectively, followed

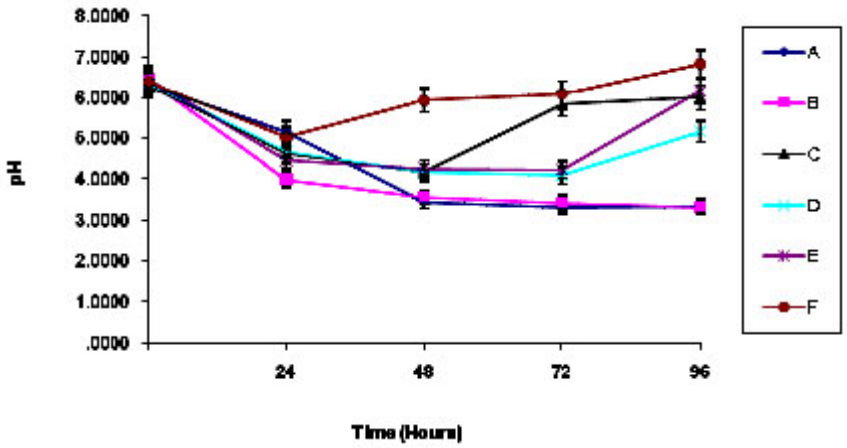
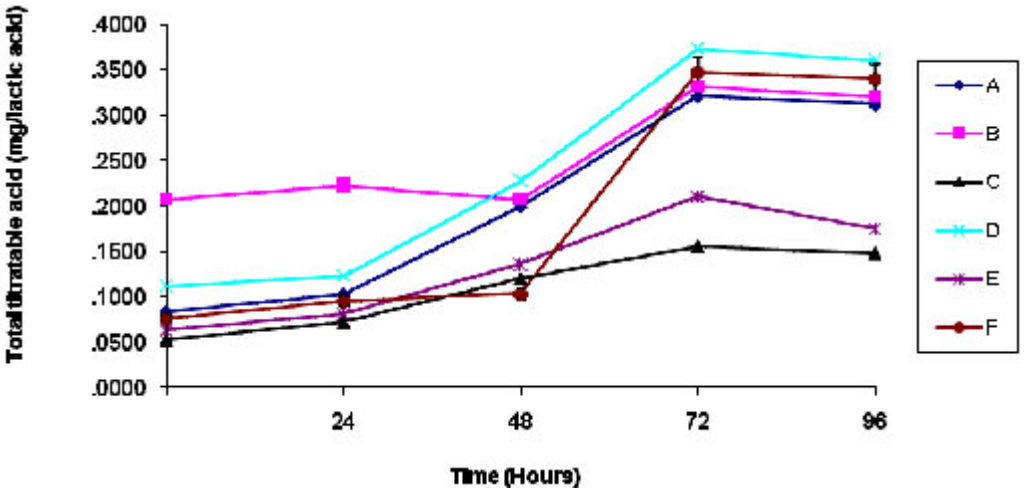


Fig. 3. pH variations during fermentation of sorghum-soya extrudates



Key:
RF= Raw Flour, EUF=Extruded Unfermented, EF= Extruded Fermented
A= 100g Sorghum flour sample=90g and 10g Sorghum-Soya blend
C=80g and 20g Sorghum-Soya blend, D=70g and 30g Sorghum-Soya blend
E=60g and 40g Sorghum-Soya blend, F=50g and 50g Sorghum-Soya blend

Fig. 4. Total titratable acidity (tta) variation during fermentation sorghum-soya extrudates

by a decrease to $8.4x10^4$ cfu/g and $6.3x10^4$ cfu/g respectively at 72 hour and 96 hour. Extrudate B exhibited the highest bacteria count at 48 hour of fermentation while extrudate E recorded the lowest bacteria count .

Fungal count during the fermentation of sorghum-soya extrudate

The total fungal count during the fermentation the fermentation of sorghum-soya extrudates are represented in Fig. 2, there was no fungal growth recorded at 0 hour for all the extrudates. Extrudate A had initial fungal count of $1.2x10^4$spore/g at 24 hour and increased to $2.4x10^4$ spore/g at 48 hour, which later increase to $2.9x10^4$ spore/g and maintained through 96 hour of fermentation. Extrudate B had initial count of $9.0x10^3$ spore/g at 24 hour this increased to $2.1x10^4$ spore/g, $2.4x10^4$ spore/g and $2.4x10^4$ spore/g at 48 hour, 72 hour and 96 hour. C had no count at 24 hour, it had $2.4x10^4$ spore/g, $3.1x10^4$ spore/g and $3.5x10^4$ spore/g at 48 hour, 72 hour and 96 hour respectively.Extrudate D had the lowest count of $1.2x10^4$ spore/g at 24 hour and increased through $2.2x10^4$ spore/g, $2.3x10^4$ spore/g and $3.8x10^4$ spore/g at 48 hour, 72 hour and 96 hour respectively. Extrudate E had 1.8x104 spore/g at 24 hour $5.0x10^4$ spore/g at 48 hour with sharp decrease to $3.0x10^4$ spore/g at 72 hour and later increased to $6.6x10^4$ at 96 hour. F at 24 hour had a count of $2.3x10^4$, $6.0x10^4$, $6.2x10^4$ and $6.4x10^4$ at 24 hour, 46 hour, 72 hour and 96 hour respectively.

Frequency of occurrence of bacteria during fermentation of sorghum-soya extrudates

Flavobacterium rigense was isolated only if extrudate A at 0 and 24 hour of fermentation, *Micrococcus icristinae* was isolated in extrudate B at 0 hour and D at 72 hour and 96 hour, *Enterobacter cloacae* occurred in extrudate B and C at 24 hour, *Enterobacter spp*. occurred in extrudate C at 0 hour. *Corynebacterium cystitidis* was isolated in extrudate D at 0 and 24 hour; it was also isolated in E at 24 hour. *Corynebacterium pilosun* appeared in extrudate E at 0 hour. *Staphylococcus albus* occurred in extrudate F at 0 hour, *Brevibacterium spp.* was isolated in extrudate A at 48 and 72 hour and also in F at 24 hour. *Bacillus subtilis* occurred in extrudate B and C at 48, 72 and 96 hour; and in D at 48 hour. *Bacillus cereus* was isolated in extrudate E at 48, 72 and 96 hour and in F at 96 hour. *Bacillus brevis* occurred in extrudate F at 48 and 72 hour while *Bacillus megaterium* occurred only in A at 96 hour of fermentation (table 1).

Frequence of occurrence of fungi during fermentation of sorghum-soya extrudates

Candida famata was isolated in extrudate A at 48 hour, *Saccharomyces cerevisiae* occurred in extrudates B and C at 48, 72 and 96 hour respectively. *Geotrichum candidium* occurred in extrudate D at 48, 72 and 96 hour. *Candida utilis* occurred in extrudates A and B at 72 and 96 hour respectively. *Aspergillus niger* was isolated from extrudates C and D at 48 and 24 hour respectively; *Aspergillus fumigatus* occurred in extrudate E at 24 hour. Rhizopus *stolonifer* occurred in extrudates E and F at 48,72 and 96 hour of fermentation (Table 1).

Changes in pH during the fermentation of sorghum-soya extrudates

The pH variations during the fermentation of sorghum-soya extrudates are shown in Fig. 3 extrudate A gradually decreased from 6.30±0.1 to 3.34±0.11, extrudate B decreased from 6.43±0.15 to 3.33±0.06. In extrudate C the initial pH was 6.3±0.00 this decreased to 5.83±0.01 at 72 hour and later increased to 6.00±0.00. Extrudate D decreased from 6.33±0.06 to 4.10±0.10 at 72 hour and increased to5.17±0.12 at 96 hour. Extrudate E decreased from 6.47±0.12 to 4.23±0.06 at 72 hour and increased to6.17±0.06 at 96 hour. Extrudate F decreased from 6.40±0.10 to 5.03±0.06 at 24 hour, it increased to5.93±0.56 at 48 hour through 6.83±0.23 at 96 hour of fermentation.

Changes in total titratable acidity(tta) during the fermentation of sorghum-soya extrudates

Variations in Titratable acidity (TTA) during fermentation of sorghum-soya extrudates are represented in Fig. 4 extrudate A had TTA of 0.0843±0.0006, this increased to 0.3213±0.002 at 72 hour and decreased slightly to 0.3213±0.002. extrudate B increased from 0.2077±0.002 to 0.2230±0.001 at 24 hour,; it decreased slightly and increased to0.3320±0.002 and finally reduced to 0.3120±0.002 at 96 hour. Extrudates C increased from 0.0523±0.002 to 0.1560±0.003 at 72 hour and decreased to 0.1483±0.001 at 96 hour. Extrudate D increased from 0.1123±0.002 to 0.3733±0.12 at 72 hour and decreased to 0.3607±0.001 at 96 hour. Extrudate E increased from 0.0637±0.001 to 0.2107±0.11 at 72 hour and decreased to 0.1757±0.001 at 96 hour. Extrudate F increased from

0.0767±0.002 to 0.3477±0.002 at 72 hour and decreased to 0.3403±0.001 at 96 hour of fermentation.

DISCUSSION

The initial low bacteria count of the extrudates was probably due to the heat treatment given to the samples before fermentation, the increase in bacteria population with time between 0-48hour could be attributed to various microorganisms adapting to the fermentation environment; and the decrease at later hour (72-96) may be due to reduction of pH which would had inhibited some microbial growth in the fermenting media. As fermentation progressed some extrudates exhibited undulating pH values though this has not been reported, but it could be related to dispersion of different molecules during extrusion cooking prior to fermentation. The lowering of pH could be probably due to the more carbohydrate composition in sorghum-soya blend extrudates which could have degraded to organic acids in extrudates A and B, while the more availability of protein constituents in extrudate E and F might have contributed to the increase in corresponding increase in pH; however, this result suggest this fermentation research to be lactic type since Ezeama and Ihezie(2006) had once reported that cereal fermentation is of the lactic type where pH of fermenting mass decrease with total titratable acidity(TTA) and vice versa , more also Omafuvbe *et al.* (2007) earlier reported that increase in pH is a common feature in the fermentation of vegetable proteins. The behavioural changes in total titratable acidity (TTA) correspond with changes pH but the undulating pattern of the total titratable acidity (TTA) in the fermenting mass may be as a result of variations in the composition of soyabean supplementation levels. The microbial flora of the fermenting media varied and heterogeneous this is similar to the findings of Efiuvwevwere and Akoma (1997) that soyabean supplemented products had a greater microbial diversity and higher microbial populations; the involvement of *Enterobacter species* may be attributed to handling and normal contaminants; the presence of *Rhizopus Stolonifer* could be due to microbial flora associated with soyabean grains.

This study reveals the successful fermentation of extruded sorghum-soya flour blends, it equally documents that microorganisms involved in the fermentation are heterogeneous.

REFERENCES

1. Okaka , J. C. Cereals and Legumes storage and processing Technology, published by Data Microsystems publishers, Enugu Nig., 1997; Pp 164-181.
2. Onyango, C., Noetzold, H., Ziems, A., Hofmann, T., Bley, T.,Henle, T., Digestibility and antinutrient properties of acidified and extruded maize-finger millet blend in the production of *uji* blend. *Lebensmittel Wissenchaft and technology*, 2005; **37**: 697-707.
3. Omezuruike, I. O.: Microbiological studies on the production of Anyi-potential Condiment made from laboratory fermentation of *Samanea saman*(Monkey pod) seeds (Jacq merr),2008; http://ejeafche.uvigo.es/index.php?option=com (accessed 4th Feb., 2010).
4. Akinyele ,B.J., Bello ,B.K. Influence of salting and blanching on the proximate and antinutritional components of fermented *Termitomyces robutus*. *Nigerian Journal of Mycology*, 2008; **1**(1):94-101.
5. Ojokoh, A.O., Yimin, W. Macronutrient composition and Digestibility of extruded and fermented soya protein products. Proceeding for oral presentation and posters, Beijing China, 2009; Aug, 10-15.
6. Nwabueze T.U., Nitrogen solubility index and amino acid profile of extruded African bread fruit(*T. africana*) blends. *Nigerian food journal* 2007; **25**(1). 23-35.
7. Onyango, C., Noetzold, H., Bley, T.,Henle, T. Proximate composition and Digestibility of fermented and extruded *uji* from maize-finger millet blend. *Lebensmittel Wissenchaft and technology*, 2004; **37**: 827-832.
8. Amusa, N.A., Falola. Pre-harvest fungal infection of Sorghum (*Sorghum bicolour*) cultivars in the humid forest of agroecological zones of Nigeria. *Acta fytotechnica et zootechnica*, 2004; **7:** 7-10.
9. Abdel-Rahman I.E., Hamad, S.H., Osman, M.A., Dirar H.A. Characterisation and distribution of microorganisms associated with kisra bread preparation from three sorghum varieties in Sudan, 2010.
10. Ezeaku, I.E., Mohammed, S.G. Character association and path analysis in grain sorghum. *African Journal of Biotechnology*, 2004; **5**(14): 1337-1340.

11. Greg ,A. Nutrient composition and utilization, 2010; http://www.petfoodindustry.com/ViewArticle.aspx?id=11616
12. Oke ,B. O., Kaul, R.N., Mittal, J.P.: Physical and mechanical properties of sorghum(Sorghum bicolour) stalk harvested at various interval after grain maturity, 1984.
13. Enwere, N. J. Foods of plant origin. Published by afro-orbis publications Ltd Nig ,1998; Pp 25-134.
14. Elegbede , J. A. Legumes, Nutritional quality of plant foods . published by the postharvest Research unit, Department of Biochemistry, University of Benin, Benin City Nigeria, 1998; Pp 53-83.
15. Dashiel, I. K. (1987): An effort to promote the production and consumption of soybeans as a means of improving nutrition in Nigeria. *Soyabean research at IITA*,1987; (accessed 13th Jan.,2010).
16. Cowan, S.T., Steel, K.J. Bergy's manual of determinative microorganism 4th Ed. Published by Cambridge university press ,1990; pp 58.
17. Kirk ,R.R., Sawyer, R. Pearson's composition and analysis of foods 9Th Ed published by Singapore Longman, 1991; Pp 45.
18. Ezeama, F. C., Ihezie, I. C. Microbiological and sensory evaluations of fermented rice snacks(masa) supplemented with soybean. *Journal of Food Technology*, 2006; **4**(4): 345-349.
19. Omafuvbe, B.O., Esosuakpo, E.O., Oladejo, T.S., Toye, A.A. Effect of soaking and roastingdehulling methods of soybean on Bacillus fermentation of soy-daddawa. *American journal of food technology*, 2007; **2**(4): 257-264.
20. Efiuvwevwere, B., Akoma, O. Microbiological studies on a Nigerian maize product, kwoka, supplemented with soybean. *Journal of Food Safety*, 1997; **17**: 249–259.