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Full Length Research Paper

Growth and extracellular enzyme production by microorganisms isolated from Ugba - an indigenous Nigerian fermented condiment

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Studies were conducted to isolate and identify the fermenting microorganisms of 'Ugba', an indigenous Nigerian fermented food condiment. The isolated microorganisms were screened for amylase, protease and lipase production, the activity and specific activity of the enzymes were also determined. The effect of pH and temperature on the activity of the enzymes was also studied. The following groups of organisms were identified as among the major fermenting organisms isolated from fermenting 'Ugba'; *Bacillus* species, *Proteus* species, *Staphylococcus* species, *Micrococcus* species and *Pseudomonas* species. *Bacillus*, *Staphylococcus* and *Proteus* were positive for amylase production. *Bacillus subtilis*, *B. licheniformis* and *Staphylococcus* species exhibited the highest potential for amylase production. Maximal amylase activity was achieved within the pH range of 6 to 7, while the optimum temperature range for the enzyme activity was between 50 to 60°C. Twelve isolates identified as *Bacillus subtilis* (Bs_1, Bs_2, Bs_3, Bs_4, Bs_5, Bs_6 and Bs_7) and *Bacillus lichenformis* (Bl_1, Bl_2, Bl_3, Bl_4 and Bl_5) were identified as among the major fermenting organisms. These isolates tested positive for protease enzyme production with the highest protease activity shown by Bs_3 (18.48 ul/ml) and the least by Bl_2 (4.21ul/ml) and Bl_3 (4.28 ul/ml). Highest protease activity (18.48 ul/ml) was recorded at the end of the third day of incubation. The optimum temperature for protease activity was observed to be within 35 to 40°C, while the optimum pH range for the enzyme activity was recorded at pH 7 to 8. Screening of the isolated organisms for lipase production revealed that *Micrococcus* species, *Pseudomonas* species and a strain of *Bacillus subtilis* (Bs_2) were able to produce lipase enzyme. The study evaluated the potential of the strains in starter culture development for fermentation of African foods.

Key words: Ugba, fermentation, amylase, protease, lipase, enzyme activity.

INTRODUCTION

Ugba is a product of alkaline fermentation of oil bean (*Pentaclethra macrophylla*) seeds which are utilized by the Ibos and other ethnic groups in southern Nigeria as a delicacy and food flavoring. It constitutes an important nutritional contribution mainly as a source of protein and plays an economical, social and cultural role among the Ibos in the eastern part of Nigeria.

Published investigations on the biochemical changes during Ugba fermentation have shown that proteolysis is the main activity leading to a pronounced increase of free amino acids (FAA) such as lysine (Odunfa and Oyeyiola, 1985; Njoku and Okemadu, 1989). Published studies on the microbiology of the fermentation of African oil bean seeds have identified *Bacillus* species as the main micro-organismsresponsibleforitsfermentation.Thepredominant.

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species is *Bacillus subtilis*, but other species like *B. pumilus, B. megaterium, B. lichenformis* have also been found (Ogueke and Aririatu, 2004).

Ogueke and Aririatu (2004) noted that the *Bacillus* species and *Proteus* species are proteolytic and dominate during the fermentation process and therefore are responsible for the observed increased free amino acids recorded during production of the product

Though proteolysis have generally been observed to be the main activity during the production of Ugba and the *Bacillus* species identified with this proteolytic activity, no detailed screening of the various fermenting organisms of Ugba for protease enzyme production have been reported in literature.

Most fermented foods in Nigeria are produced at household level and hygiene is a major concern. Their fermentation process is usually by spontaneous culture method where the inoculum is by chance. This has almost always led to the problem of inconsistent product quality and other attendant problems. The problem of occurrence and growth of pathogens in most of these fermented food products cannot be ruled out as the general hygienic conditions of the processors, the equipment used, water and other raw materials cannot be said to be free of potential pathogens.

The use of starter cultures have generally been recognized as one major way of ensuring product consistency and to a reasonable extent eliminate the problem of foodborne pathogens (Eman, 2009). Unfortunately, however, no lactic acid bacteria (LAB) starter cultures are commercially available yet for small scale processing of traditional African foods. The potential of starter cultures for fermentation on a household scale for most of our traditionally fermented foods has not yet been fully explored. A starter culture is applied to improve a fermentation process, be it a lactic, alcoholic or the other types of fermentation. The old tradition of using a portion of a fermented product to start a new batch resembles the principle of starter cultures in an empirical sense. However, most commercial starter cultures originated from those food substrates to which they are applied today.

The oil bean seed is mainly composed of proteins 42, lipids 43 and carbohydrates 15% (Odunfa and Oyeyiola, 1985; Njoku and Okemadu, 1989; Ogueke and Aririatu, 2004). Selection of any organism(s) as starter culture(s) for the production of Ugba will therefore depend on the ability of such organism(s) to degrade these major components of the oil bean seed. Degradation of carbohydrate, lipid and protein requires that the organism must possess the capacity to produce amylase, lipase and protease enzymes which are required for carbohydrate, lipid and protein metabolism respectively.

The aim of this work therefore was to study in detail the ability of the different microbial isolates from fermenting African oil bean seeds to produce protease, lipase and amylase enzymes and to determine the activity of these enzymes. The information obtained will be used in other to select starter cultures for the production of Ugba.

MATERIALS AND METHODS

Laboratory preparation of ugba

The traditional method of preparing Ugba was employed in the laboratory to ferment the product. The processing of the large brown glossy seeds of the African oil bean to obtain 'Ugba' involved the following; the oil bean seeds were boiled in an autoclave at a temperature of 121°C and a pressure of 15 pounds per square inch (psi) for 1 h to soften the hard brown testa (shell). The shells were removed and the kernels washed, drained and rewashed with cold water several times. The washed cotyledons were cut into long thin slices. These slices were mixed with salt, wrapped in small packets with leaves and lightly tied. These small packets were placed in a basket to ferment at room temperature for 3 days to yield 'ugba'.

Isolation and characterization of organisms responsible for fermentation of ugba

Samples were collected daily from the fermenting African oil bean seeds and serial dilutions of the samples were prepared. The dilutions were plated out on nutrient agar and tryptone soy agar using the spread plate method. Based on the cultural and morphological characteristics of the organisms, different isolates were selected and purified by streaking on the same media as used in the isolation process. Characterisation and identification of the isolates were done based on the cultural and morphological features of the isolates on the plates, sugar fermentation and biochemical tests carried out and the use of API 50 CHB Kit.

Screening of isolates for enzyme production

Amylase

The plate assay technique was employed in the screening of the isolates for amylase production. Nutrient agar medium with 1% inclusion of soluble starch was prepared, the isolates were inoculated on each plate and incubated for 48 h at 37°C. At the end of the incubation period, the plates were flooded with iodine solution and observed for colour change around the growth of each isolate. A change of colour from the usual blue – black colour of iodine on starch to light yellow indicated a positive result, while a blue – black colour indicated a negative result.

Protease

The plate assay technique was employed in screening the isolates for protease enzyme production. Nutrient agar with 1% inclusion of casein was prepared, the isolates were smeared on the plates of the medium and incubated at 37°C for 48 h. The plates were observed for clear zone at the end of the incubation period. Clear zone around each isolate indicated a positive result while no clear zone signified a negative result.

Lipase

The same organisms isolated from fermenting African oil bean seeds were screened for the production of lipase enzyme. The plate assay technique was also employed in the screening exercise. These organisms were inoculated on a tributytrin agar medium and incubated at 37°C for 72 h. These organisms were observed for a clear zone around each isolates daily throughout the incubation period. A clear zone around the isolates indicated a positive result, while no clear zone indicated a negative result.

Determination of activity of enzyme produced by the isolates

Amylase

Isolates that tested positive for amylase production were selected and subjected to further screeing to determine the amylase activity. The Ramakrihna et al. (1982) method was employed in carrying out the test. The different isolates were grown in a shaker incubator at a revolution speed of 200 rpm for 72 h in a growth medium composed as follows; bacteriological peptone 10 g, magnessium sulphate 0.5 g, soluble starch 10 g, iron sulphate 0.5 g, potassium dihydrogen phosphate 1.0 g, ammonuim sulphate 1.0 g and distilled water 1000 ml.

At the end of the production period the enzyme was extracted by centrifugation at 5000 rpm for 30 min. The extracted crude enzyme was assayed for its activity by reacting the following; 1 ml of the crude enzyme, 1 ml of 1% (w/v) starch solution, 0.1 ml of citrate buffer solution (pH 4.5) and incubated the mixture for 1 h at 60°C in a water bath. The reaction was terminated by immersing the reaction tube in boiling water (100°C) for 2 min. 1 ml of Dinitrosalicyclic acid (DNS) reagent was added to the reaction mixture and placed in a water bath (100°C) for 5 min, cooled to ambient temperature and the absorbance taken at 540 nm on a UV spectrophotometer against a blank. One unit of amylase activity is definned as the amout of enzyme that liberated 1 micro mole of D – glucose from starch in 1 micro liter of reaction mixture under assay conditions.

Protease

Organisms that showed positive result for protease enzyme production were selected and subjected to further test to determine the activity of protease produced by them. Determination of protease activity was by Kunitz (1982) caseinolytic method. Each isolate was grown for 120 h in a protease supporting growth med-ium composed as follow; magnesium sulphate 0.5 g, dipotassium hydrogen phosphate 2.0 g, potassium chloride 0.3 g, ammonium nitrate- 10.0 g, peptone water 1.0 g, tri sodium citrate 10.0 g, distilled water 1 L, pH adjusted to 6.9 to 7.0 with 1.0 M NaOH. The cultures were incubated in a shaker incubator at a revolution speed of 200 rpm.

Samples were taken daily from each isolate till the end of the growth period and analysed for protease activity. The enzyme was extracted by centrfugation at a revolution speed of 5000 rpm. 1 ml of the enzyme extract was added to 3 ml of 0.5% solution of casein, incubated for 30 min at 37°C. The reaction was terminated by adding 5 ml of 5% solution of trichloroacetic acid. The reacting mixture was stored at room temperature for 30 min and thereafter centrifuged at 5000 rpm for 30 min. The absorbance of the supernatant was taken at 280 nm on a UV spectrophotometer. One unit of enzyme activity is defined as an increase of 0.1 in the absorbance of trichloroacetic acid (TCA) soluble casein hydrolysis of product.

Effect of temperature on enzyme activity

Amylase

The effect of temperature on the activity of amylase enzyme was assayed on starch solution (1% starch solution) at 20 to 70°C, at pH 7.0. After a 10 min incubation, amylase activity was determined at each temperature regime as described earlier.

Protease

Effect of temperature on protease activity was assayed on casein solution at the same temperature regime and pH value as used for the amylase assay. After a 10 min incubation, protease activity was determined as described earlier.

Effect of pH on enzyme activity

Amylase

The effect of pH on amylase activity was determined on starch solutions (1% starch solution and 0.006 M NaCl) at pH values 3.0 to 9.0 after 30 min incubation. Amylase activity was determined as outlined earlier.

Protease

Effect of pH on protease activity was determined on casein solution at the same pH values as used for amylase. Activity was determined as outlined earlier after 30 min incubation in a water bath.

RESULTS AND DISCUSSION

Studies on isolation and identification of the fermenting microorganisms of African oil bean seeds identified the following groups of microorganisms as being present in the fermentation of African oil bean seed into ugba; *Bacillus* species, *Proteus* species, *Micrococcus* species, *Staphylococcus* species and *Pseudomonas* species (Table 1). This aspect of the study was designed to identify the organisms responsible for the fermentation of the African oil bean seeds into ugba and utimately select starter cultures for its production.The presence of members of the coliform group which were earlier isolated and considered as possible pathogens in this study were therefore disregarded as they are unlikely to be selected as possible starter cultures for the production process.

Bacillus species have been implicated in the fermentation of most vegetable oil protein seeds like African locust bean seeds, soy bean seeds, African oil bean seed etc (Odunfa, 1981; Antai and Ibrahim, 1986; Odunfa and Oyewole, 1986; Diawara et al., 1992; Ogueke and Aririatu, 2004). Ogueke and Aririatu (2004), in their study of microbial and organoleptic changes associated with Ugba stored at ambient temperature, identified *Proteus* species as one of the predominant species isolated throughout the period of their study. *Staphylococcus* species and *Micrococcus* species have also been isolated from fermenting African oil bean seeds.

However, *Pseudomonas* species have never been reported to be associated with fermentation of Ugba or any other vegetable protein seeds fermentation. Oral reports however suggest that in some instances, this organism could be involved in the fermentation process of Ugba as cases of products occasionally having a shade of greenish colour have been cited by the local processors. Some species of Pseudomonas are noted for a greenish –gray- bluish colour production. It is therefore possible that such shade of greenish colour occasionally observed by the local processors could have been as a result of the presence of *Pseudomonas* species in such products.

Table 1. Microscopic, biochemical and physiological properties of extracellular enzymes producing organisms isolated from fermenting ugba.

Characterization test	Bacterial isolate				
	1	2	3	4	5
Gram reaction	+	-	+	+	-
Catalase	+	+	+	-	-
Casein Hydrolysis	+	+	+	-	+
Gelatin Liquefaction	+	+	-	-	+
Starch Hydrolysis	+	+	+	-	-
Voge-Proskaeuer	+	-	+	+	+
Citrate Utilization	+	+	-	+	-
Oxidase	ND	ND	ND	ND	ND
H_2S Production	+	-	-	-	+
Urease	+	+	-	+	+
Sugar fermentation					
Fructose	A	A	A	A	-
Galactose	A	-	A	-	-
Glucose	A	-	AG	A	A
Lactose	A	A	A	-	-
Maltose	A	A	A	-	A
Mannitol	A	-	A	A	A
Sucrose	A	A	A	A	-
Xylose	ND	ND	ND	ND	ND
Tentative identity	Bacillus	Proeus	Staphylococcus	Micrococcus	Pseudomonas

+ = Positive; - = Negative; ND = Not done.

The traditional method of fermentation of most African fermented foods is by chanced inoculation, which implies that one of the factors that determine the organisms to be isolated from such fermenting product will be the initial microbial content of the starting raw materials (water inclusive). *Pseudomonas* species are reported to be a natural inhabitant of water bodies. It is therefore possible that the occurrence of this species of organism in this work could have originated from the source of water used in the processing of the product. It should also be noted that when the various isolates were screened for protease production, *Pseudomonas* species isolated was found to be positive for this enzyme production. This implies that the organism is well equipped to play an active role in the fermentation process of the product since the major activity during its production has been shown to be proteolytic in nature.

Screening of isolates for enzyme production

Amylase

The screening of various isolates obtained from fermenting African oil bean seeds for their ability to produce amylase enzyme revealed that the *Bacillus* species, *Staphylococcus* species and *Proteus* species have the capacity to produce amylase (Figure 1). Most of the previous workers on the production of Ugba (Odunfa, 1981; Antai and Ibrahim, 1986; Odunfa and Oyewole, 1986; Diawara et al., 1992; Ogueke and Aririatu, 2004) have only associated the *Bacillus* species with the pro-duction of protease and by extension with the proteolytic activities associated with the production of the product. No past record has linked this group of organisms with amylase production and by extension with carbohydrate hydrolysis during the fermentation of Ugba. The result of this study has however shown that the *Bacillus* species which is one of the predominant organisms during the production of Ugba are not only capable of producing protease enzyme which play a key role in the fermen-tation process but are also active in the production of amylases. Forgarty et al. (1974) has however identified the *Bacillus* species as important sources of proteases and amylases.

Protease

Investigation conducted earlier on isolation and identification of organisms responsible for the fermen-tation of African oil bean seeds into ugba shows that among the major groups of organisms responsible for the fermentation of Ugba are the *Bacillus* species, *Proteus* species, *Staphylococcus* species, *Micrococcus* species and *Pseudomonas* species. Recorded reports however show that the major group of organisms associated with

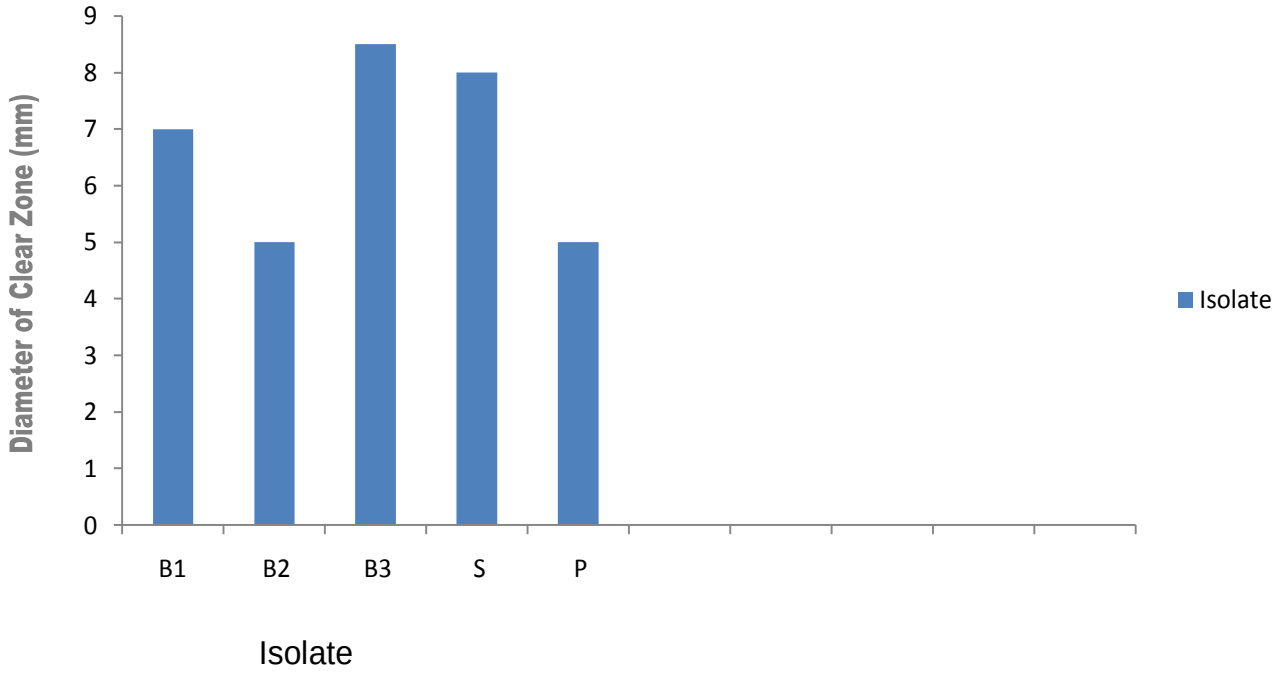

Figure 1. Production of amylase by micro-organisms Isolated from fermenting african oil bean seeds. B (1-3) = *Bacillus* spp S = *Staphylococcus* spp P = *Proteus* spp.

the fermentation of ugba are the *Bacillus* species. Therefore, the *Bacillus* species isolated in this study were subjected to further identification process to determine their strain. Result obtained shows that twelve (12) different strains of *Bacillus* species identified as *Bacillus substilis* (Bs_1, Bs_2, Bs_3, Bs_4, Bs_5, Bs_6 and Bs_7) and *Bacillus licheniformis* (Bl_1, Bl_2, Bl_3 and Bl_4) were among the *Bacillus* species isolated (Table 2) .

These different strains of *Bacillus* species*, Proteus, Staphylococcus, Micrococcus and Pseudomonas species* were screened for protease enzyme production since the major activity during Ugba production has been established to be proteolytic in nature. The result of this study shows that all the species of *Bacillus* isolated were able to produce protease enzyme. Also, *Proteus* and *Pseudomonas* species were able to produce protease enzyme (Figure 2). Isolate Bs3 recorded the highest production rate while isolates Bl2 and Bl3 had the least production rate as expressed by the diameter of clear zone observed for each isolate.

The result obtained in this study identifies *Bacillus* species (*B. subtilis* and *B*. *licheniformis*) as one of the major groups of organisms responsible for the fermentation of ugba. Similar results have been recorded by Odunfa (1981), Antai and Ibrahim (1986), Odunfa and Oyewole (1986), Diawara et al. (1992) and Ogueke and Aririatu (2004). These workers observed that the fermentation of ugba and other related vegetable proteins is usually by alkaline fermentation brought about by the activities of *Bacillus* species. Similar observations have been recorded with the fermentation of Iru, Dawadawa, Soumbala, Afiyo and Ogiri where the same group of organisms has been implicated in their production (Obeta, 1983; Odunfa, 1985; Njoku et al., 1989; Diawara et al., 1992; Omafuvbe et al., 2004; Ogunshe et al., 2007).

Odunfa and Oyeyiola (1985), Njoku and Okemadu (1989) and Ogueke and Aririatu (2004) reported that the major activity taking place during fermentation of Ugba is proteolysis. They associated the *Bacillus* species with this proteolytic activity. The result of this work which identifies all the *Bacillus* species isolated from fermenting Ugba as being able to produce protease enzyme is therefore in agreement with their findings

Lipase

The screening of isolates for lipase production showed that *Micrococcus* species, *Pseudomonas* species and a strain of *Bacillus subtilis* (Bs_2) were able to produce lipase enzyme. These isolates have been selected based on the diameter of clear zone expressed on the culture plate by them and will be subjected to further studies.

Determination of enzyme activity

Amylase

Table 3 shows the activity of amylase produced by the various fermenting microorganisms of fermenting African oil bean seeds. Highest activity of the enzyme was recorded after two days of fermentation. Further increase in the period of fermentation did not result in a corresponding increase in amylase production and by extension

Table 2. Microscopic, biochemical and physiological properties of *Bacillus* species isolated from fermenting African oil bean seed.

Characterization test	Bacterial strians											
	BS_1	**BS_2**	**BS_3**	**BS_4**	**BS_5**	**BS_6**	**BS_7**	**B_{11}**	**B_{12}**	**B_{13}**	**B_{14}**	**B_{15}**
Gram reaction	+	+	+	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+	+	+
Casein hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+
Gelatin liquefaction	+	+	+	+	+	+	+	+	+	+	+	+
Starch hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+
Methyl red	+	-	+	+	-	+	+	+	+	+	+	-
Voges-Proskaeuer	+	+	+	+	+	-	+	+	+	+	+	+
Citrate utilization	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
H_2S production	+	+	+	+	+	+	+	+	+	+	+	+
Sugar fermentation												
Fructose	A	A	A	A	A	A	A	A	A	A	A	A
Galactose	A	A	A	A	A	A	A	A	A	A	A	A
Glucose	A	A	A	A	A	A	A	AG	A	AG	AG	A
Lactose	A	A	A	A	A	A	A	A	A	A	A	A
Maltose	A	A	A	A	A	A	A	A	A	A	A	A
Mannitol	A	A	A	A	A	A	A	A	A	A	A	A
Sucrose	A	A	AG	A	A	A	A	AG	A	A	A	A
Xylose	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

+= Positive; -= Negative; A= Acid production; AG= Acid production with gas; ND= Not determined; Bs= *Bacillus subtilis*; Bl = *Bacillus licheniformis.*

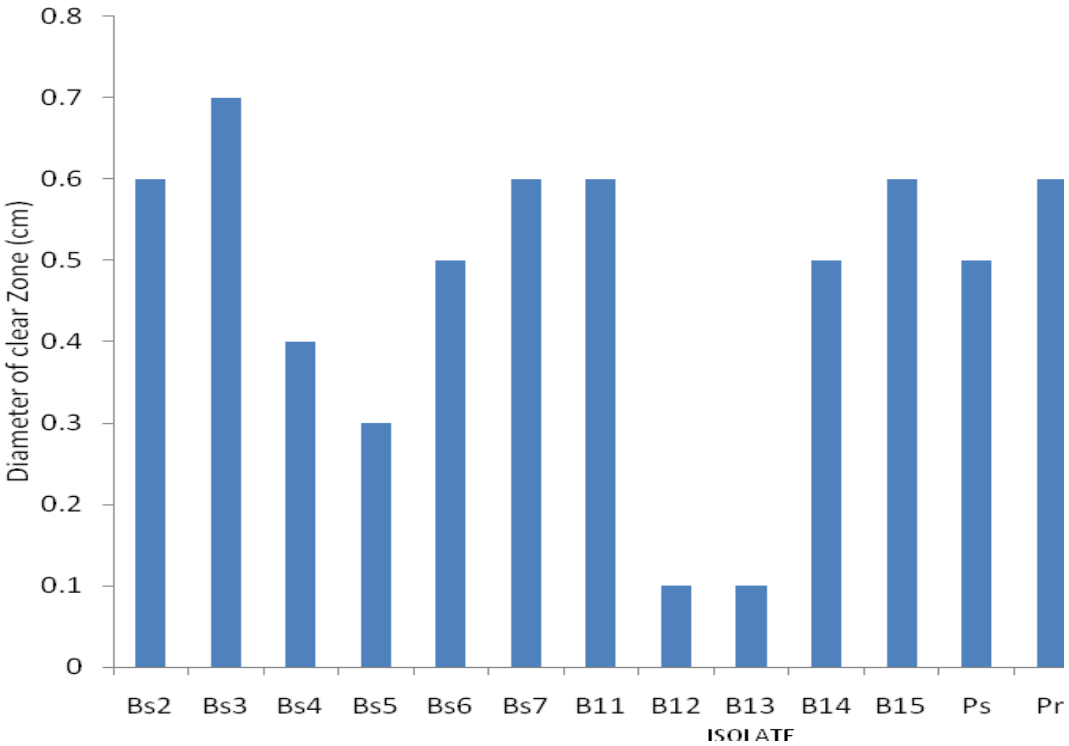

Figure 2. Rate of protease enzyme production by microorganisms isolated from ugba. Bs = *Bacillus subtilis*; Bl = *Bacillus licheniformis;* Ps = *Pseudomonas* species, Pr =*Proteus* species.

Table 3. Activity Of Amylase Produced by Micro-Organisms Isolated from Fermenting African oil Bean Seeds.

Isolate	Amylase activity (u/ml)	Specific amylase activity (u mol/mm/mg)
Bacillus subtilis (Bs_1)	19.26	1.6
Bacillus subtilis (Bs_2)	23.14	1.8
Bacillus subtilis (Bs_3)	24.06	1.5
Staphylococcus spp	20.65	0.8
Proteus	17.40	1.3

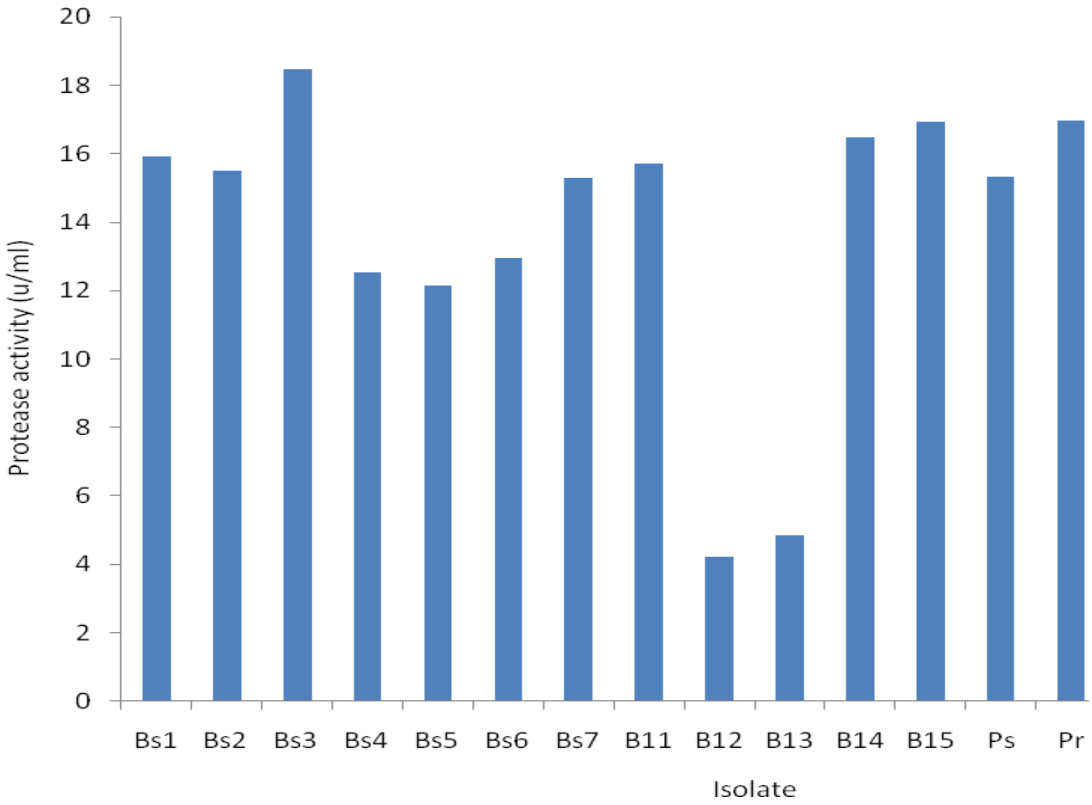

Figure 3. Activity of protease enzyme produced by various organisms isolated from fermenting Ugba. Bs = *Bacillus subtilis*; Bl = *Bacillus licheniformis;* Ps = *Pseudomonas* species*;* Pr =*Proteus* species.

its activity. The African oil bean seed contains only about 17% carbohydrate which could have been exhausted within the first two days of fermentation. This implies that further production of the amylase was not taking place after the second day of fermentation explaining the observed trend in the enzyme activity beyond two days of fermentation. Similar observation was reported by Ogueke and Aririatu (2004).

Protease

Studies on the determination of the activity of the protease enzyme produced by the various isolates from fermenting African oil bean seeds indicate that isolate Bs_3 had the highest activity while the least activity was recorded by isolate Bl_2 (Figure 3). This result followed the same trend observed earlier with regard to protease production by these isolates. There was a gradual but steady increase in protease activity with increase in the incubation period with the highest activity recorded after 72 h of incubation (Table 4). Smilar observation was made by Zotta et al. (2008) during their production of Urease production by *Streptococcus thermophilus.*

Effect of temperature on enzyme activity

Amylase

Optimum temperature for this enzyme activity was observed to be between 50 to 60°C (Figure 4). This result is in agreement with the report of Oguntimein (1993) who observed the optimum temperature range for amylase

Table 4. Changes in protease activity with increase in fermentation period

Isolate	Fermentation period (day)				
	1	2	3	4	5
BS_1	11.35	13.98	15.93	15.90	15.91
BS_2	10.96	13.12	15.49	15.38	15.45
BS_3	12.47	13.96	18.48	18.40	18.43
BS_4	9.16	11.75	12.52	12.53	12.16
BS_5	10.02	11.39	12.16	12.16	12.14
BS_6	9.65	10.78	12.96	12.97	12.96
BS_7	11.06	12.77	15.29	15.28	15.25
BL_1	11.76	13.41	15.72	15.45	15.66
BL_2	1.94	2.12	4.21	4.22	4.20
BL_3	1.86	2.40	4.86	4.48	4.44
BL_4	11.66	13.83	16.47	16.38	16.12
BL_5	12.01	13.66	16.92	16.59	15.97
Ps	8.23	9.02	12.62	14.64	14.97
Pr	6.06	8.42	13.07	15.12	4.92

Bs = *Bacillus subtilis*, Bl = *Bacillus licheniformis*, Ps =*Pseudomonas,* Pr = *Proteus.*

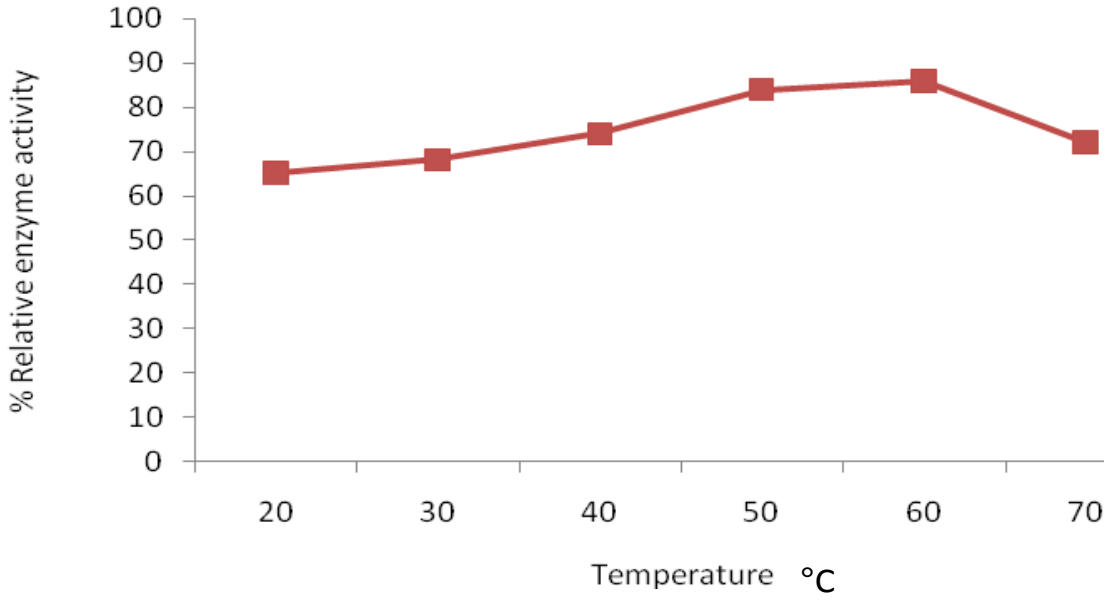

Figure 4. Effect of temperature on the activity of amylase produced by the fermenting organisms of African oil bean seeds.

activity in a cassava processing waste to be between 55 to 60°C. However, this result is lower than 76 to 95°C reported for alpha amylase from some strains of *Bacillus licheniformis* by Forgarty et al. (1974) and Pescuma et al. (2008) during whey fermentation by thermophilic lactic acid bacteria.

Protease

The optimum temperature for the activity of the protease enzyme produced by the fermenting microorganisms of African oil bean seeds was found to be between 35 to 40°C (Figure 5). This temperature range falls within the optimum temperature range recorded for the growth of the tentatively selected starter cultures.

Effect of pH on enzyme activity

Amylase

The optimum pH range for maximal amylase activity was recorded at pH 6 to 7 (Figure 6). The enzyme lacks activity

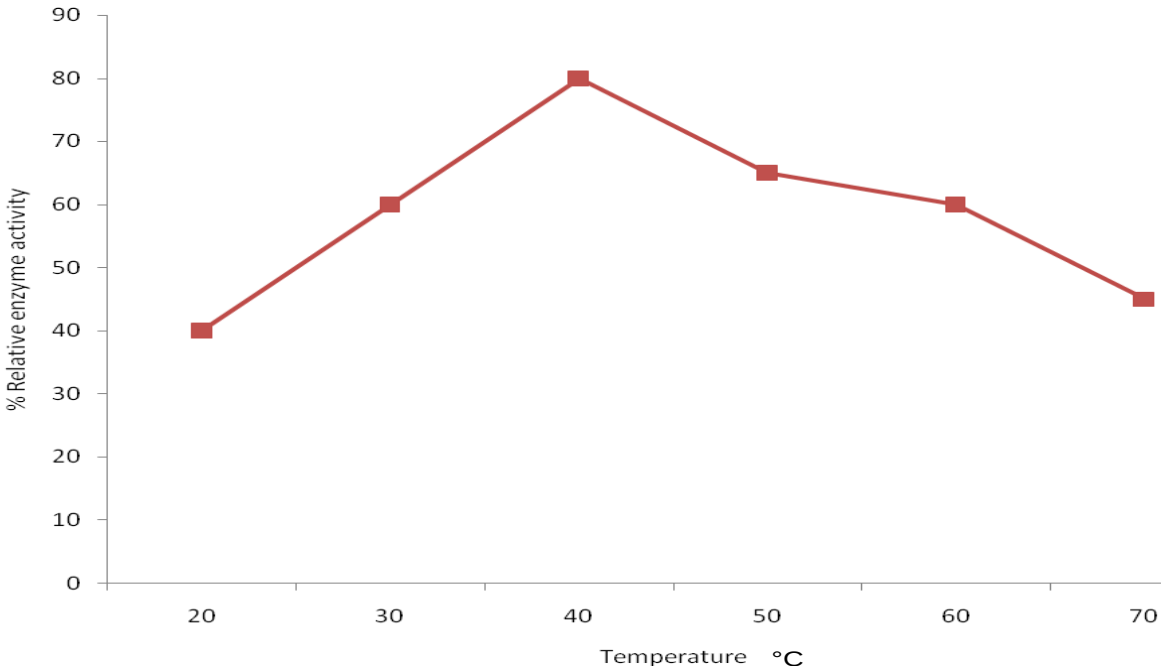

Figure 5. Effect of temperature on activity of protease enzyme produced by fermenting organisms of African oil bean seeds.

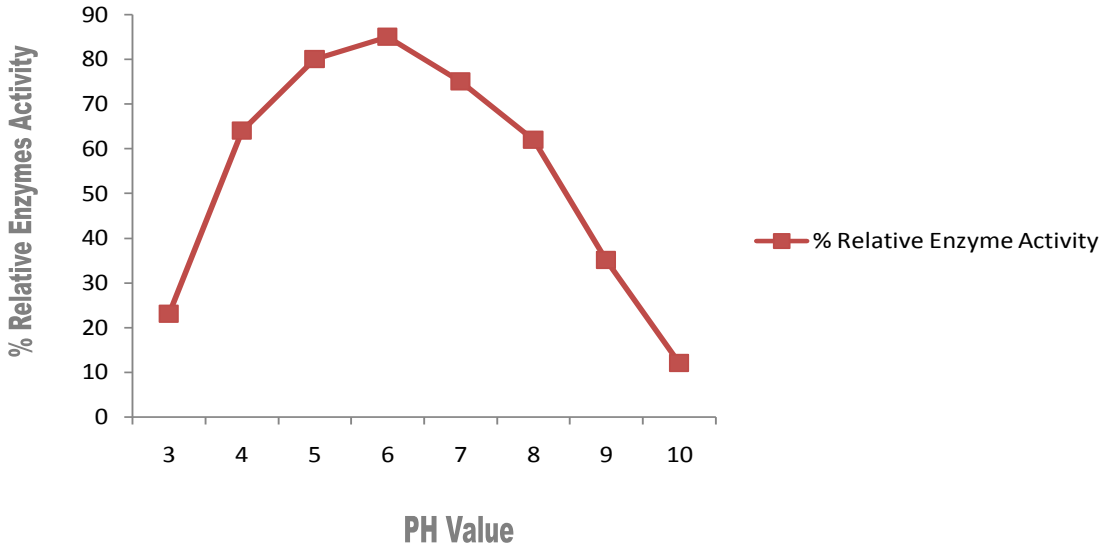

Figure 6. Effect of pH on the activity of amylase produced by the fermenting organisms of African oil bean seed.

at extreme pH values (that is below pH 3 and above pH 9). Similar results have been reported by Oguntimein (1993) in his study of growth and amylase production by *Bacillus licheniformis* isolated from cassava processing waste.

Protease

The highest activity of protease enzyme produced by the fermenting organisms of African oil bean seeds was recorded at pH range of 7 to 8 (Figure 7). This result is in agreement with the reports of most workers on the fermentation of African oil bean seeds to obtain ugba and the fermentation of other vegetable proteins (African locust bean seeds, Soy bean seeds and Melon seeds) to obtain products like iru, ogiri, daddawa, afiyo e.t.c (Antai and Ibrahim, 1986; Odunfa and Oyewole, 1986; Diawara et al., 1992; Ogueke and Aririatu, 2004). These workers

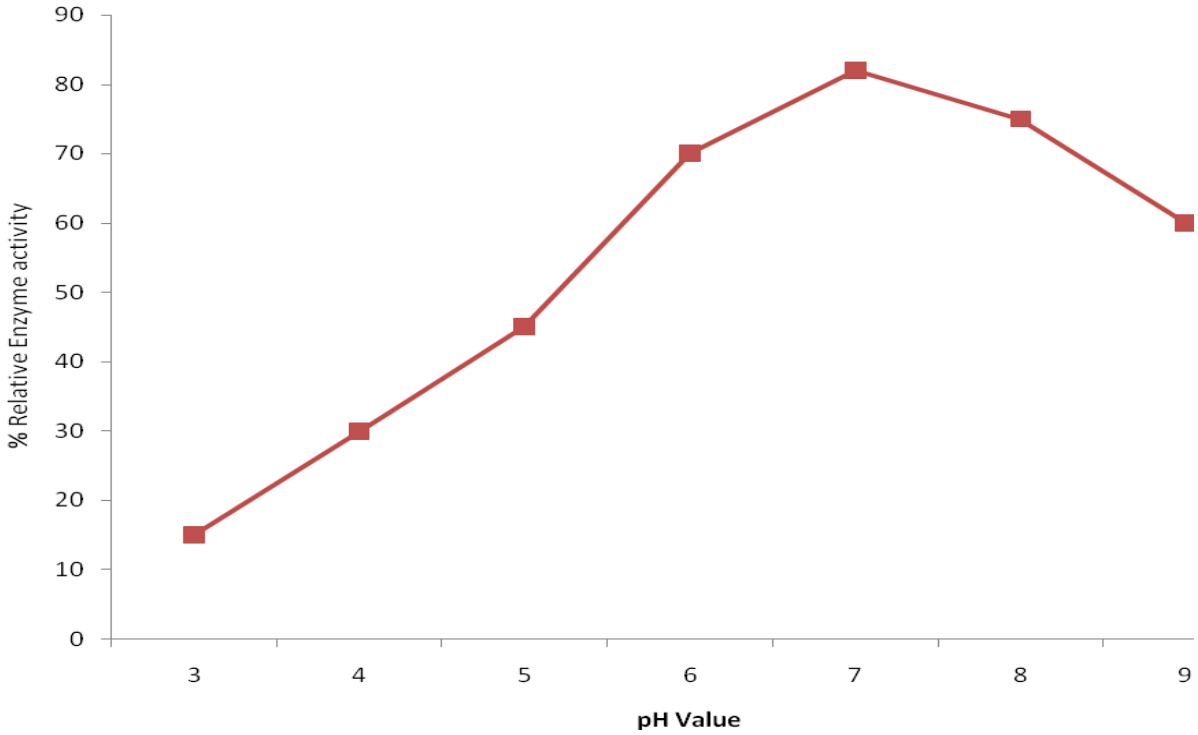

Figure 7. Effect of pH on activity of protease enzyme produced by fermenting organisms of African oil bean seeds.

observed that the fermentation process of obtaining these products takes place in a slightly alkaline medium which is what has been recorded in this work.

Conclusion

The study has shown that the *Bacillus* species, *Proteus* species, *Pseudomonas* species and *Micrococcus* species isolated from fermenting African oil bean seeds possess the capacity to produce some enzymes (amylase, protease and lipase) which are in food processing and are presumed to be important for the fermentation of Ugba.

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