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Microbiological and enzymatic studies during the development of an 'Iru' (a local Nigerian indigenous fermented condiment) like condiment from Bambara Nut [*Voandzeia subterranea* (L) Thours]

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ABSTRACT

Fifteen isolates were obtained from a-96 h spontaneously fermented bambara nut to produce an 'Iru' like condiment. Pure cultures of the isolates were identified employing API 50CH strips and API CHL medium (API system, Montalieu, Vercieu, France). Their identities were confirmed as *Bacillus subtilis*, *Bacillus licheniformis* and *Bacillus pumilus*. The total microbial counts showed that the microbial load increased significantly from 3.2 x 10^5 ± 0.11 cfu/g at 0 h to 9.2 x 10^7 ± 0.20 cfu/g at 96 h ($p \leq 0.05$). The investigation into enzymatic activities during product development revealed that the amylase activity fluctuated, by increasing significantly from 0.33 ± 0.1 x s unit g/dry wt at 0 h to 0.39 ± 0.04 x s unit g/dry wt at 60 h which finally decreased to 0.32 ± 0.08 x s unit g/dry wt at the end of fermentation. Proteinase activity showed that at 0 h an activity of 0.22 ± 0.09 x s unit g/dry wt was recorded, which increased significantly to 0.24 ± 0.06 x s unit g/dry wt at 24 h. This value decreased significantly to 0.21 ± 0.03 x s unit g/dry wt at 48 h which increased significantly to 0.28 ± 0.08 x s unit g/dry wt at 96 h. However, the lipase which was observed at 0 h to show an activity of 0.50 ± 0.05 x s unit g/dry wt increased significantly to 0.80 ± 0.06 x s unit g/dry wt at 72 h and decreased to 0.70 ± 0.08 x s unit g/dry wt at the end of production period.

Keywords: condiment, microbiology, enzyme, Bambara nut, 'Iru', fermentation

INTRODUCTION

Bambara nut (*Vigna subterranea* (L) Verdc. Syn. *Voandzeia subterranea* (L) Thours) belongs to the family leguminosae and subfamily papilionoideae (Goli and Ng, 1988; Goli *et al.*, 1991; Heller *et al.*, 1995). Despite its adaptability to harsh conditions, bambara nut have lost importance in many parts of Africa due to introduction and expanded production of groundnut (Kay, 1979) In recent years, there had been renewed interest in the cultivation of the crop because of its ability to resist drought conditions and to yield a reasonable crop when grown on such poor soils where groundnut have failed to grow (Kay, 1979).

Cereals and other plant foods may contain significant amount of toxic or antinutritional substances. In this regards, legumes have been reported, particularly as rich sources of natural toxicans including protease inhibitors, amylase inhibitors, metal chelates, flatus factors, haemagglutinnis, sapronins, cyanogens, lathyrogens, tannis, allergens, acetylenic furan and isoflavonoid phytoalexins which limit utilization of legume (Pariza, 1996; Haard *et al.*, 1999) . These toxic components were documented to interfere with digestive processes involving enzymatic degradation.

Bambara nut remains one of the most neglected crop in terms of research programme (Swanevelder, 1998). However, empirical evidence and fragmentary research results suggest it is a crop with great potentials (Swanevelder, 1998). In Africa this crop was described as the second most important food legume and the third most important crop after maize (Swanevelder, 1998).

Bambara nut and cowpea were reported as important source of proteins and carbohydrates (Borget, 1989; Minka and Bruneteau, 2000). Their protein composition is over 22%, consisting of essential amino acid predominantly lysine and leucine (Minka and Bruneteau, 2000). Due to their protein content, they are used occasionally as substitutes for meat or fish (Campbell–Platt, 1980).

Despite the nutritional potential of this pulse and coupled with its resemblance to soybeans both in texture and colour, there is a death of information on the extensive utilization of bambara nut for the production of fermented foods in sub-Saharan Africa.

This study was carried out to investigate the microbiological and enzymatic aspect of the development of an 'Iru' like condiment from this pulse.

MATERIALS AND METHODS

Collection of samples

Bambara nut (Sokoto white cultivar (SW) was purchased from Bodija market Ibadan, Nigeria. The healthy seeds

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were selected and stored inside a sterile nylon bag and kept in the refrigerator at 4 °C.

Preparation of bambara nut for fermentation

Forty grams of the beans was thoroughly washed in clean water and then soaked in distilled water (500 mL) for 24 h. The nuts were dehulled manually by pressing in between fingers. The dehulled beans were boiled in distilled water for 15 min. The boiled water was decanted and the nuts were spread on ethanol – sterilized tray to dry for 30 min and wrapped with leaves of banana (Musa – Sapientum) which had been previously washed, cleaned and surface sterilized with 75% ethanol (Odunfa, 1981). The wrapped beans were transferred into ethanol-sterilised calabash which were covered with another sterile calabash and finally placed in a sterile black polythene bag and tied firmly. The set-up was then kept on a surface sterilized laboratory bench to ferment naturally at room temperature (30 + 2 °C) for 96 h.

Isolation procedure

The medium used for isolation was nutrient agar (NA) (Lab M). One gm of the fermenting bambara nut was transferred aspectically into 10 mL of sterile saline solution in a test-tube and shaken vigorously to dislodge the associated microorganisms. The homogenate was serially diluted with sterile distilled water and 1mL (10^{-4} dilution) was taken and plated on Petri-dishes containing sterile nutrient agar. Incubation was carried out at room (30 $\pm$ 2 °C). The plates were examined for growth and microbial load determined by physical counting. The pure cultures were obtained by streaking repeatedly. All treatments were carried out in replicates. Samples were taken at 12 h interval.

Identification of organisms

Identification of the isolates was carried out using the API 5O CH strips and API CHL medium (API System, Montalieu, Vercieu, France)

Enzyme assay during Fermentation

Extraction of enzyme

Five grams of 24 h fermented samples was ground in a mortar with pestle in 20 mL distilled water. The suspension was centrifuged at 5000 rpm in a MSE high speed – 18 refrigerated centrifuge at 5 °C for 30 min. The supernatant was recovered and stored in a freezer at -2 °C.

Amylase Assay

Amylase activity was determined using DNSA method of Bernfeld (1955). The amount of reducing sugar formed was calculated from a standard curve constructed with known concentrations of maltose.

Proteinase Assay

Proteinase activity was determined employing the procedure described by Young and Woods (1977). The extracting buffer was 0.1 M sodium hydrogen phosphate solution (pH 6.5). Five mL of the extract was added to 10 mL of 2% casein solution and incubated at 35 °C for 30 min and the reaction was terminated by the addition of 10 mL of 10% trichloroacetic acid solution. The reaction mixture was filtered through Whatman No 1, filter paper and the optical density read at 275 nm using Campsec-M105 spectrophotometer

Lipase assay

This assay was carried out according to the modified methods of Somkuti and Babel (1967) A unit of lipase activity is defined as the amount of sodium hydroxide (NaOH) used in the titration to bring the reaction mixture to a pH 9.5 under the defined assay condition (Young and Woods, 1977).

RESULTS AND DISCUSSION

Bambara nut was allowed to ferment naturally for 96h. Pure cultures of the fourteen isolates obtained were identified by employing the API 50 CH strips and API CHL medium (API system Montalieu, Vercieu, France). Their identities were confirmed as *Bacillus subtilis*, *B. licheniformis* and *B. pumilus*.

The total viable microbial count recorded revealed that this parameter, increased significantly from 3.2×10^5 $\pm$ 0.11 cfu/mL at 0 h to 9.2×10^7 $\pm$ 0.20 cfu/mL at 96 h ($p \leq 0.05$).

Table 1: Total viable microbial count during fermentation

Time (h)	Microbial load (cfu/g)
0	3.2×10^5 a
12	8.0×10^5 b
24	2.0×10^6 c
36	5.0×10^6 d
48	9.0×10^6 e
60	1.1×10^7 f
72	3.0×10^7 g
84	6.0×10^7 h
96	9.2×10^7 i

Note: Means followed by the same letters are not significant by *Duncan's* Multiple Range test at 5% level of significance.

The isolation of *Bacillus* species observed in this study had been previously reported by Odunfa (1981); Popoola and Akueshi (1984); Antai and Ibrahim (1986); Ogbadu and Okagbue (1988); Sanni and Ogbonna 1991); Uzogara *et al.,* (1991); Achinehwu, (1992); Barimalaa *et al.* (1994); Ogbonna *et al.* (2001); Omafuvbe *et al.* (2002); in their various studies on condiment production from fermented vegetable proteins.

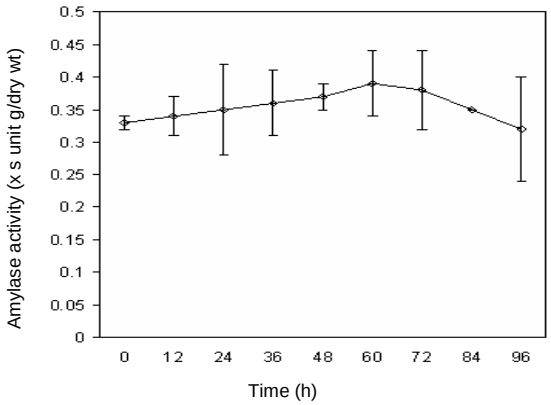

Figure 1: Changes in amylase activity during production of condiment

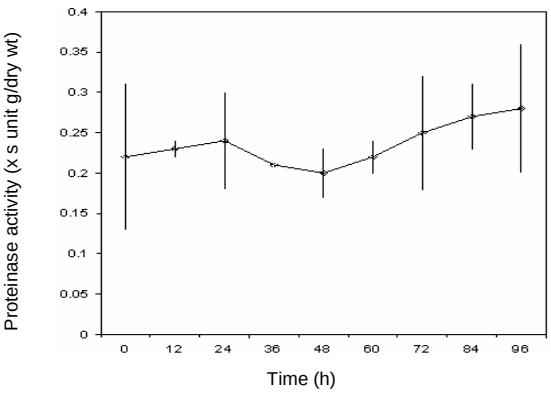

Figure 2: Proteinase activity monitoring during product development

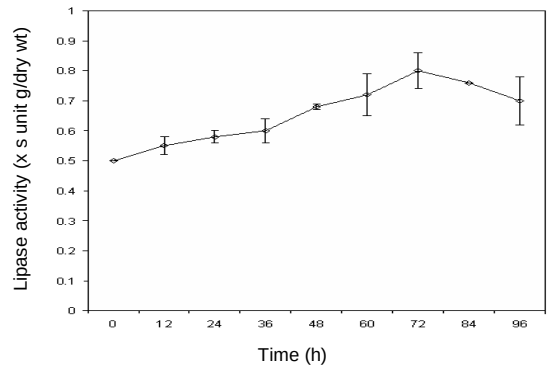

Figure 3: Lipase assay during fermentation of bambara nut

This occurrence might be due to shorter time of boiling and as well as the composition and texture of the beans. In addition, they might have originated from air, soil or normal contaminants of the calabash and leaves employed in the fermentation process.

The determination of enzymatic activities during the production period showed that the amylase activity increased significantly from 0.33 $\pm$ 0.01 x s unit g/dry wt at 0 h to 0.39 $\pm$ 0.047 x s unit g/dry wt at 60 h which finally decreased significantly to 0.32 $\pm$ 0.08 x s unit g/dry wt at the end of the fermentation period (Figure 1) ($p \leq 0.05$).

The amylase monitoring procedure revealed that the bacillus species are amylolytic (Omafuvbe *et al.*, 2000; Kiers *et al.*, 2000). The amylase activity increased to 72 h during fermentation and dropped. This observation was earlier documented by Odunfa (1981) and Omafuvbe *et al*., (2004) and substantiated this finding as resulting in increased total reducing sugar.

However, the proteinase assay showed that at 0 h an activity of 0.22 $\pm$ 0.09 x s unit g/dry wt was recorded which increased significantly to 0.24 $\pm$ 0.06 x s unit g/dry wt at 24 h. This value decreased to 0.22 $\pm$ 0.00 x s unit g/dry wt at 60 h and finally increased to 0.28 $\pm$ 0.08 x s unit g/dry wt at the end of the fermentation period (Figure 2).

This observation confirms the earlier submissions of Omafuvbe *et al.* (1999; 2000; 2004). The rapid increase in the total amino acids was adduced to be responsible for this finding (Sarkar *et al.*, 1993; Omafuvbe *et al.*, 2002; 2004).

Figure 3 shows the result of the lipase activity, which was observed at 0 h to reflect an activity of 0.50 $\pm$ 0.00 x s unit g/dry wt which increased significantly to 0.80 $\pm$ 0.06 x s unit g/dry wt 72 h and decreased to 0.70 $\pm$ 0.08 x s unit g/dry wt at the end of the fermentation process.

However the highest lipase activity was recorded at 72 h. Odunfa, (1983; 1985) reported low levels of lipase activity in *Parkia biglobosa* during dawadawa production and melon seed fermentation respectively, while Njoku and Okamadu (1989) observed minimal lipase activity during development of 'Ugba' from *Pentachletera macrophyla*. Young and Wood (1977) and Odunfa (1983; 1985) submitted that low lipase activity during fermentation of vegetable proteins has been discovered to reduce the problems of objectionable taste and development of rancidity.

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