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Antimicrobial Effects of Sodium Benzoate on the Growth, Survival and Aflatoxin Production Potential of Some Species of *Aspergillus* in Garri During Storage

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Abstract: The antimicrobial effect of different concentrations (0.6, 0.4 and 0.2%) of sodium benzoate (SB) on the growth, survival and aflatoxin production potentials of *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus fumigatus* in packaged garri (2kg/pack) during storage at ambient temperature (30±2°C) was investigated. A decrease of 4.5, 2.5, 1.9; 4.5, 2.4, 1.8 and 4.6, 2.6 and 2.0 folds were recorded in the viable count of *A. niger, A. flavus* and *A. fumigatus*, for 0.6, 0.4 and 0.2% SB treated samples respectively. The effectiveness of SB against the various species of *Aspergillius* was in the order 0.6% > 0.4% > 0.2%. However 1.2 and 1.7 folds increase in the viable count of *A. niger, A. flavus* and *A. fumigatus* were recorded in the non treated samples (controls). No aflatoxin was detected in all the samples treated with different concentration of SB. However, aflatoxin was detected in the samples inoculated with *Aspergillus flavus* although unspecified and irregular florescence was noticed in other samples (controls).

Key words: Sodium benzoate, aflatoxin production, *Aspergillus*, Garri, cassava roots

Introduction

Garri, produced from fermented cassava roots (Manihot-esculenta-crantz) is consumed by several millions of people in the rain forest belt in West Africa. Its acceptability cuts across the multi-ethnic and socio-economic classes, making it the commonest meal amongst the rich and the poor (Ekundayo, 1984; Oyeniran, 1984).

Garri production is cumbersome and deteriorate rapidly in storage, mainly due to mould activities and other biological agents. The presence of *Aspergillus* variables such as *Aspergillus niger, Aspergillus flavus, Aspergillus chieverliere* and *Aspergillus fumigatus* and their determinant role in garri quality and stability at the market level and during storage have been reported (Ekundayo, 1984; Oyeniran, 1984; Ogiehor, 2002). Efforts to reverse this trend, that is controlling the biodeteriorative activities of these moulds with the use of antimicrobial agents (food preservative) has received very little attention (Ogiehor, 2002).

Furthermore, the implication of *Aspergillus* species in aflatoxin production, especially in food items and the public health implication is well documented (Aspilin and Carnaghan, 1961; Hesseltine *et al.,* 1970; Patten, 1981; Ibeh *et al.*, 1991). The effects of plant extracts, extract of spices and salts of sodium on aflatoxin production by some species of *Aspergillus* in some foods and other medium are available in literatures (Thomas and Peter, 1987; Masood and Ranjan, 1990; Kumar and Prasad, 1992; Patkar *et al.*, 1993), but such information, with respect to garri is unavailable.

In line with the foregoing, the present work was undertaken to study the effects of SB on the growth, survival ad aflatoxin production potential of some species of *Aspergillus* following deliberate contamination (challenge test) of garri samples packaged in Hessian bags and held at 30±2°C.

Materials and Methods

Garri Sample and Treatment with SB: Cassava tubers (roots) were obtained from the open market in Benin City, Nigeria and processed into garri according to the scheme developed by Adeyemi and Balogh (1985) with slight modification as reported by Ogiehor (2002). The processed garri was divided into three sub-groups and treated with different concentrations (0.2, 0.4 and 0.6%) of SB (BDH, England) according to the method described by Ogiehor *et al.* (1998) and packaged in Hessian bags (2kg/pack) and held at ambient tropical temperature of 30±2°C.

Inoculation of Garri Samples: Stock of *Aspergillus niger, Aspergillus flavus* and *Aspergillus fumigatus* earlier isolated from garri during storage and identified using cultural and morphological characteristics (Bounds *et al.*, 1993; Samson and Reenen - Hoekstra, 1988) was reconstituted according to the method described by Harrigan and McCance (1976). The inoculum

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concentration and size was determined by pour plating on malt extrat agar (Oxoid, Basingstoke, UK). The garri samples treated as indicated above were inoculated using 1ml of 48hrs old broth culture of *A. niger, A. flavus* and *A. fumigatus.* After thorough mixing, the bags were sealed using hand sealing machine,(super master, Japan) adopting standard aseptic and safety precautions. These were kept in the laboratory at ambient tropical temperature of 30±2ºC and monitored at weekly intervals for microbiological and aflatoxin quality.

Microbiological Analysis: The viable count of the various garri samples were determined and enumerated as described by Vanderzannt *et al.* (1992). Weighed 10g of each sample was aseptically added to 90ml of 0.1% (w/v) sterile peptone water and allowed to stand for 3 minutes with occasional stirring. This was serially diluted (1: 10) and the viable count enumerated by pour plate method on malt extract agar (Oxoid, Basing stoke, Uk) which was prepared and incubated according to the manufacturers instructions. The colonies that developed were enumerated and expressed as log_{10} colony forming unit per gram (cfu/g).

Extraction and Detection of Aflatoxin: The aflatoxin content of the various garri samples was detected and extracted according to the method reported by Ibeh *et al.* (1991). Briefly, 10g of garri sample was homogenized and added into sterile, clean erlenmeyer flask containing 40ml of methanol and water (11:9) and shaken at 2000 rpm on a mechanical shaker (Griffin and George) for 1 min. The resultant slurry was filtered through whatman number 1 filter paper. The filtrate was extracted three times each with 20ml petroleum ether (boiling point 60 - 80ºC) in a separating tunnel to remove the lipid fractions. The pooled petroleum ether extract was re-extracted with 40ml of methanol and water (11:9). The aqueous methanol extract were combined and transferred to separating funnel and extracted three times with 25ml of chloroform (BDH, England) to extract the aflatoxin present. The pooled chloroform extract was passed through bed of anhydrous sodium sulphate. The bed was re-washed with additional 20ml of chloroform. Aflatoxins were detected by thin-layer chromatography against standard aflatoxin. Aflatoxin B_1, B_2, G_1, G_2 (Aldrich chemical, Milwaukee, WI) and quantitated using a spectrophotometer (Coleman instruments).

Results

The antimicrobial effects of different concentrations of SB on the growth, survival and aflatoxin production ability of *A. niger, A. flavus* and *A. fumigatus* in garri during storage are shown in Tables 1-4.

Marked but gradual decrease from 4.72±0.2 log_{10} cfu/g to 1.05±0.1 log_{10} cfu/g, 1.80±0.2 log_{10} cfu/g and 2.48±0.2 log_{10} cfu/g were recorded in the viable count of *A. niger* for 0.6, 0.4 and 0.2% treated garri samples (Table 1). Similar trend of decrease from 4.74±0.1 log_{10} cfu/g to 1.08±0.1 log_{10} cfu/g, 1.90±0.1 log_{10} cfu/g and 2.60±0.2 log_{10} cfu/g (Table 2); and from 4.81±0.2 log_{10} cfu/g to 1.03 log_{10} cfu/g , 1.84±0.2 log_{10} cfu/g and 2.41± 0.2 log_{10} cfu/g were observed and recorded for *A. flavus* and *A. fumigatus* for 0.6, 0.4 and 0.2% treated samples respectively. The degree of antimicrobial effects of SB on the *Aspergillus* species was in the order 0.6% > 0.4% > 0.2%.

However, noticeable increase to 8.41±0.2 Log_{10} cfu/g, 8.198±0.1 log_{10} cfu/g and 8.25± 0.3 log_{10} cfu/g was detected for *A. niger*, *A. flavus* and *Afumigatus* at the end of the storage period (56 days) after initial decrease from 4.72±0.2 log_{10} cfu/g to 3.44±0.3log_{10} cfu/g (*A. niger*); 4.74±0.1 log_{10} cfu/g to 3.58±0.3log_{10} cfu/g (*A. flavus*) and 4.81±0.2 log_{10} cfu/g to 3.64±0.3log_{10} cfu/g (*A. fumigatus*) respectively in the control samples.

Aflatoxin B_1 and B_2 were detected in the sample inoculated with *A. flavus*, although various type of unspecified florescence were observed under the UV light for *A. niger* and fumigatus inoculated samples for the non SB treated samples (control). However no traces of aflatoxins was detected in all the samples treated with different concentration of SB.

Discussion

Food protection and food preservation implies putting microorganisms in a hostile environment in order to inhibit their growth or shorten their survival or cause their death and possibly repress the production of some metabolic products such as toxins. The present results (Table 1-4) show the inhibitory efficacy of SB on the growth, survival and aflatoxins production ability of *A. niger, A. flavus* and *A. fumigatus*.

The antimicrobial and the inhibitory properties of SB may have created unfavourable micro environmental conditions leading to physiological, homeostatic and metabolic distortion. The struggle to overcome the resultant hostility may create further stress and results in metabolic exhaustion leading to gradual death and subsequent decrease in the viable count of the *Aspergillus* species (Tables 1-3). These finding corroborates previous reports (Leistner, 1978; 1994; Bogl-Sorensen, 1993; Beuchat and Hathlox, 1996; Ogiehor *et al.*, 1998, Ogiehor, 2002). The effectiveness of antimicrobial agents in foods depends on a number of factors such as concentration and others. The present findings clearly demonstrate that the higher the concentration of SB the greater the antimicrobial effect exercised on the various species of *Aspergillus* (0.6% > 0.4% > 0.2%) , Tables 1-3. This supports previous reports (Gould, 1989; Ogunrinola *et al.*, 1996; Efiuvwevwere and Efi, 1999).

Alteration of the homoestasis (internal environment equilibrium) of the various species of *Aspergillus* by SB may be responsible for the lack of aflatoxin observed in all the treated samples. Changes in the internal environment of microorganisms may momentarily repress or delay the mechanisms responsible for toxin production and may "switch on" with favourable micro

Table 1: The effects of SB on the viable count of *A. niger* (log_{10} cfu/g) in garri during storage at 30±2°C

Period of storage (Days)	Concentrations of SB (%)			
	Control	0.6	0.4	0.2
0	4.72±0.2	4.72±0.2	4.72±0.2	4.72±0.2
7	3.76±0.4	2.90±0.1	3.25±0.3	3.5±0.2
14	3.44±0.3	2.71±6.2	3.04±0.2	3.30±0.1
21	4.58±0.3	2.50±0.1	2.92±0.4	3.16±0.2
28	5.04±0.2	2.30±0.3	2.71±0.2	3.01±0.4
35	6.19±0.2	2.14±0.1	2.64±0.1	2.91±0.2
42	6.62±0.1	2.10±0.2	2.44±0.3	2.70±0.2
49	7.36±0.3	1.60±0.1	2.31±0.1	2.63±0.1
56	8.41±0.2	1.05±0.1	1.80±0.2	2.48±0.2

Each value is the overall mean ± standard deviation for duplicate experiments.

Table 2: The effects of SB on the viable count of *A. flavus* (log_{10} cfu/g) in garri during storage at 30±2°C

Period of storage (Days)	Concentrations of SB (%)			
	Control	0.6	0.4	0.2
0	4.74±0.1	4.74±0.1	4.74±0.1	4.74±0.1
7	3.80±0.2	2.96±0.2	3.28±0.1	3.55±0.3
14	3.58±0.3	2.76±0.2	2.91±0.3	3.36±0.2
21	4.34±0.4	2.58±0.1	2.82±0.2	3.05±0.2
28	5.16±0.3	2.41±0.4	2.66±0.2	2.96±0.1
35	6.03±0.1	2.24±0.3	2.54±0.1	2.89±0.2
42	6.74±0.2	2.11±0.1	2.41±0.1	2.80±0.3
49	7.40±0.2	1.54±0.2	2.32±0.2	2.76±0.2
56	8.19±0.1	1.08±0.1	1.90±0.1	2.60±0.2

Each value is the overall mean ± standard deviation for duplicate experiments.

Table 3: The effects of SB on the viable count of *A. fumigatus* (log_{10} cfu/g) in garri during storage at 30±2°C

Period of storage (Days)	Concentrations of SB (%)			
	Control	0.6	0.4	0.2
0	4.81±0.2	4.81±0.2	4.81±0.2	4.81±0.2
7	3.96±0.1	2.89±0.2	3.34±0.1	3.64±0.2
14	3.64±0.3	2.70±0.1	3.14±0.2	3.45±0.1
21	3.96±0.4	2.59±0.3	2.83±0.2	3.29±0.2
28	4.66±0.2	2.51±0.4	2.72±0.2	3.01±0.1
35	5.71±0.1	2.19±0.1	2.54±0.1	2.86±0.3
42	6.44±0.2	1.96±0.2	2.41±0.1	2.71±0.4
49	7.26±0.1	1.74±0.1	2.09±0.2	2.64±0.1
56	8.25±0.3	1.03±0.1	1.84±0.2	2.41±0.2

Each value is the overall mean ± standard deviation for duplicate experiments.

Table 4: The effects of SB on the aflatoxin production ability of some species of *Aspergillus* in garri at the end at the storage period (56 days)

Species of *Aspergillus*	Control	concentration of SB		
		0.6	0.4	0.2
A. niger	NS	-	-	-
A. flavus	+(B_1B_2)	-	-	-
A. fumigatus	NS	-	-	-

+ = Detected, -= Not detected, NS = Non specified.

environmental condition. However, present findings indicate irreversible hostile conditions, hence the lack of aflatoxin in all the SB treated samples even at the end of the storage periods (56 days). These findings agrees with previously documented reports (Haussinger, 1998; Gould, 1988; Masood and Ranjan, 1990; Beuchat *et al.*, 1997). Amongst the control samples, aflatoxin was detected in the sample inoculated with *A. flavus* only,

although “unspecified” and “irregular” florescence were observed in samples inoculated with *A niger* and *A. fumigatus.* This may be other forms of mycotoxins not screened for. The presence aflatoxin and others (unspecified) may be due to favorable conditions for growth and survival.

In conclusion, it is empirically clear that SB exerted high antimicrobial effects on the growth, survival and aflatoxin production ability of *A. niger, A. flavus* and *A. fumigatus,* common garri deteriogens evidenced by the results (Tables 1-4). Results can be used to develop durable and measurable indices for safe handling, shelf stability and protection of garri under tropical environment conditions.

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