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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 ($30\pm 2^\circ\text{C}$) 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 *Aspergillus* 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 floescence 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 and aflatoxin production potential of some species of *Aspergillus* following deliberate contamination (challenge test) of garri samples packaged in Hessian bags and held at $30\pm 2^\circ\text{C}$.

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 $30\pm 2^\circ\text{C}$.

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 extract 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 $30\pm 2^{\circ}\text{C}$ 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 Vanderzant *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 $60 - 80^{\circ}\text{C}$) in a separating funnel 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 B₁, B₂, G₁, G₂ (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 $4.72\pm 0.2 \log_{10}$ cfu/g to $1.05\pm 0.1 \log_{10}$ cfu/g, $1.80\pm 0.2 \log_{10}$ cfu/g and $2.48\pm 0.2 \log_{10}$ 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 $4.74\pm 0.1 \log_{10}$ cfu/g to

$1.08\pm 0.1 \log_{10}$ cfu/g, $1.90\pm 0.1 \log_{10}$ cfu/g and $2.60\pm 0.2 \log_{10}$ cfu/g (Table 2); and from $4.81\pm 0.2 \log_{10}$ cfu/g to $1.03 \log_{10}$ cfu/g , $1.84\pm 0.2 \log_{10}$ cfu/g and $2.41\pm 0.2 \log_{10}$ 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 $8.41\pm 0.2 \log_{10}$ cfu/g, $8.198\pm 0.1 \log_{10}$ cfu/g and $8.25\pm 0.3 \log_{10}$ cfu/g was detected for *A. niger*, *A. flavus* and *A. fumigatus* at the end of the storage period (56 days) after initial decrease from $4.72\pm 0.2 \log_{10}$ cfu/g to $3.44\pm 0.3 \log_{10}$ cfu/g (*A. niger*); $4.74\pm 0.1 \log_{10}$ cfu/g to $3.58\pm 0.3 \log_{10}$ cfu/g (*A. flavus*) and $4.81\pm 0.2 \log_{10}$ cfu/g to $3.64\pm 0.3 \log_{10}$ cfu/g (*A. fumigatus*) respectively in the control samples.

Aflatoxin B₁ and B₂ were detected in the sample inoculated with *A. flavus*, although various type of unspecified fluorescence were observed under the UV light for *A. niger* and *A. 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 aflatoxin 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 findings 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; Efiuvewwere and Efi, 1999).

Alteration of the homeostasis (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

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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 <i>Aspergillus</i>	Control	concentration of SB		
		0.6	0.4	0.2
<i>A. niger</i>	NS	-	-	-
<i>A. flavus</i>	+(B ₁ B ₂)	-	-	-
<i>A. fumigatus</i>	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,

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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 detriogens 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.

References

- Adeyemi, M.O. and E. Balogh, 1985. Processing of indigenous fermented foods. *Nig. Food J.*, 3: 31-34.
- Asplin, F.D. and R.E.A. Carnaghan, 1961. The toxicity of certain groundnut meals for poultry with special reference to their effect on ducklings and chicken. *Vet Pec.*, 73: 215-219.
- Beuchat, L.R. and A.K. Hathcox, 1996. Inhibitory effects of sucrose tatty acids and ester alone and in combination with ethylenediamintera acetic acid and other organic acids on viability of *Eschenchia coli* 0157H7. *J. Food Microbiol.*, 13: 213-215.
- Bogl-Sorensen, L., 1993. Description of hurdles. In: food and preservation by combined process. Leistrer L. and Gorris, G M. (eds) Final reports. Flair concerted Action number 7 subgroup B, Europe, pp: 7-24.
- Bounds, H.C., F.M. Boyd and J.R.A. Nafmah, 1993. Laboratory exercise in General Microbiology (1st ed.) Cambridge University Press, pp: 20-66.
- Beuchat, L.R., M. Rocelle, S. Clavero and C.B. Jacquette, 1997. Effects of Nissin and Temperature on survival, growth, and Enterotoxin production characteristics of Phychotrophic *Bacillus cerues* in Beef Gravy, *Applied and Environment Microbiology*, 63: 1953-1958.
- Efiuvwevwere, B.J.O. and E.U. Efi, 1999. Antimicrobial effects of butylated hydroxyamisole alone or in combination with potassium sarbate on quanlity changes in palm oil co-inoculated with *Aspergillus flavus* and *Bacillus spp.* *J. Sci. Food and Agri.*
- Ekundayo, C.A., 1984. Microbial spoilage of packaged garri in storage. *Microbial. letters*, 23: 217-228.
- Gould, G.W., 1988. Interference with homoeostasis; in: Homeostatic mechanisms in microorganisms Whittenbury, R, Gould, G.W, Banks, J.F and Board J.F (Eds). *FEM Symposium*, 44: 220-228.
- Gould, G.W., 1989. Mechanisms of Action of Food Preservation procedures. Elsevier Applied science London UK.
- Harrigan, W.F. and M.E. McCance, 1976. Laboratory methods in foods and Dairy microbiology Academic press, London, 416 pp.
- Haussinger, D.E., 1988. PH homeostasis: Mechanisms and control. Academic press London, UK 479 pp.
- Hesseltine, C.W., O.C. Shotwell, M. Smith, J.J. Ellis, E.E. Vandergraft and G. Shanon, 1970. Production of various aflatoxins by strains of *A. flavus* proceedings of the first U.S- Japan conference on toxic microorganisms, the U.S department of interior, pp: 202-210.
- Ibeh, I.N., N. Uraih and J.I. Ogonor, 1991. Dietary exposure to afaltoxin in Benin City, Nigeria a possible public health concern international Journal of Food Microbiology.
- Kumar, S. and G. Prasad, 1992. Efficacy of medicinal plant (*Andrographis peniculata*) extract on aflatoxin production and growth of *Aspergillu flavus*, letters in Applied Microbiology, 15: 131-132.
- Leistner, L., 1978. Hurdle effects and energy saving. In: Food quality and nutrition Dowery, W.K (ed.) Applied Science Publishers. London, UK, pp: 553-557.
- Leistner, L., 1994. Further developments in the utilization of hurdle technology for food preservation. *J. Food Engineering*, 22: 411-422.
- Masood, A. and K.S. Ranjan, 1990. The influence of fungitoxicants on growth and aflatoxin production by *Aspergillu flavus*. *Letters in Applied Microbiology*.
- Ogiehor, I.S., U.B. Owhe-Ureghe and I.O. Momodu, 1998. Microbial, chemical and organoleptic quality of palm wine preserved by combinations of preservatives. *Nig. J. Microbiol.*, 12: 63-68.
- Ogiehor, I.S., 2002. Extension of Shelf life of garii by combinations of Preservative factors. Ph.D thesis, University of Benin, Benin City Nigeria, 189pp.
- Ogunrinola, O.A. D.Y.C. Fung and I.J. Jeon, 1996. *Eschevichia Coli* 0157: 117 growth in laboratory media as affected by phenolic antioxidants. *J. Food Sci.*, 61: 1017-1020.
- Oyeniran, J.O., 1984. Mould development in garri during storage in polyethylene and Hessian bags. *Nigeria stored products Research Institute* ,11: 93-99.
- Patkar, K.L., C.M. Usha, H.S. Shely, N. Pastor and J. Lacey, 1993. Effect of spice essential oils on growth and aflatoxin B₁ production by *Aspergillus flavus*. *Letters in Applied microbiology*, 17: 49-51
- Patten, R.C., 1981. Aflatoxin and Disease. *American J. Trop. Med. Hyg.*, 30: 422-425.
- Samson, R.A. and Reenen-Hoekstra ES Van, 1988. Introduction to food borne fungi, 2nd ed. Central bureau voor Schimmel Cultures; Baur.
- Thomas, J.M. and K.G. Peter, 1987. Sodium bicarbonate reduces viability and alters aflatoxin distribution of *Aspergillus Parasiticus* in Czapek's Agar. *Applied and Environmental Microbiology*, 53: 2303-2307.
- Vanderzant, C. and Splitts to esser, D.F., 1997. Compendium of methods for the microbiological examination of foods, 3rd ed. Am. Public Health Association, Washington DC. 596 pp.