

Resident microbial load, toxigenic potential and possible quality control measures of six imported seasoning powders on the Ghanaian market

Abstract

Assessment of resident microbiological contamination of six imported seasoning powders on the Ghanaian market were studied using three media DRBC, OGYE and PCA. The presence of moulds and yeasts was compared on two media (DRBC and OGYE) at 30°C for 7 days and bacteria on PCA at 35°C for 48 hr. Determination of elemental compositions and mycotoxin analyses (aflatoxins and ochratoxins) were also analysed using AAS spectrometry and HPLC technique respectively. As a means of eliminating resident microbial load in the seasonings, gamma irradiation doses of 0, 2, 5, and 10 KGy were applied at an absorbed dose rate of 1.683 KGy/hr. seven genera of fungi (*Aspergillus*, *Cladosporium*, *Penicillium*, *Absidia*, *Eurotium*, *Rhizopus* and *Paecilomyces*) were isolated from the samples. *Aspergillus* species (*A. candidus*, *A. flavus*, *A. niger*, *A. oryzae*, *A. sulphureus*) predominated over the other species followed by *Penicillium* (*P. corylophilum*, *P. aurantiogriseum*); *Cladosporium* (*C. herbarum*, *C. macrocarpum*) and *Rhizopus* (*R. oryzae*, *R. stolonifer*). The percentage occurrence profile of resident fungi changed after 6 months storage. Species of bacteria encountered varied from one seasoning to the other but included *Acinetobacter pittii*, *Bacillus funiculatus*, *B. subtilis*, *Filifactor villosus*, *Lactobacillus equi*, *L. malferment*, *L. quasseri* and *Pseudomonas orientalis*. *Lactobacillus* species predominated over the other bacteria genera encountered. All the seasonings contained variable high to low contents of Fe, Na, K, Ca and Mg. The heavy metals occurred in low or trace amounts namely Cu, Zn, Pb, Cr, Ni, Co, Ar and Hg. Benny shrimp powder contained high concentration of K (4240 mg/l) and Na (424.20 mg/l), while Onga chicken seasoning contained high Na (13,700 mg/l); Remie chicken seasoning powder contained 104.82 mg/l of Fe. Aflatoxins (B1, B2, G1 and G2) and ochratoxin A were not detected in all the test samples but there was the presence of Monosodium Glutamate. Gamma irradiation dose of 5-10 KGy decontaminated the samples.

Keywords: seasoning powders, bacterial, fungal, aflatoxins, ochratoxin a, monosodium glutamate, elemental composition

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Abbreviations: GMP, good manufacture process; HACCP, hazard analysis of the produce at the critical control point; ICMSE, international commission on microbiological specification for food; RTC, radiation technology centre; AAS, atomic absorption spectrometry; HPLC, high pressure liquid chromatography; ANOVA, analysis of variance; ISO, international standards organization; WHO, world health organization

Introduction

Seasonings are substances such as salt, pepper, spice or herb added as flavour to food. These seasonings also improve aroma, taste, colour and pungency. They can be used in the fresh state, dried, powdered or mixed ingredients. Seasoning powders are mixtures of seasoned flavouring components such as salt, sugar, spices etc. hence spices can be considered as a sub-set of seasoning.¹

Most of the raw materials used in preparing seasoning powders are cultivated in warm and humid tropics and some in temperate climates where a variety of fungal and bacterial contaminants are resident in stored raw material. The post-harvest handling operations

of gathering ingredients for manufacture of seasoning powders begin in the field level of picking raw materials with their attendant field microflora, drying to acceptable moisture content levels (i.e. sun-drying, oven drying or open) subject to the vicissitude of the weather). Other basic operations such as harvesting, transporting, threshing, drying, cleaning packaging and storage processing of seasoning are potential sources of contamination with pathogens which can lead to an increased number of food-borne infections and intoxications.²⁻⁴

If, Good Manufacture Process (GMP) and Hazard Analysis of the Produce at the Critical Control Point (HACCP) is presumed to have been carried out, the sterilization/pasteurization process should keep the product stable in quality within the stipulated expiry date. However, opening the packaging may breach the integrity of the ideal conditions for extension of shelf-life and so even under refrigeration; the product may be predisposed to contamination. The liberalized economy in developing countries such as Ghana and impact of the media advertisement has resulted in the influx of many goods especially food products in addition to our locally manufactured ones such as Maggi onion cubes, Maggi Shrimp cube, Royco Beef cube,

Royco Shrimp cube etc. to mentioned but a few.⁴ Some of the recent brands in the Ghanaian market are Benny Beef stock, Benny Crayfish powder stock, Remie Chicken seasoning powder, Napa Valley Jollof rice seasoning, Onga seasoning powder and Indomie Seasoning powder.

In Ghana, assessment of microbiological contamination on some selected spices have been reported by Caulley; Painstil; Addo; Appenteng, Ahene, Odamtten and Owusu, Yankey to mention but few. These researchers recorded the occurrence of different fungal and bacterial species in fresh and powdered dehydrated samples. They recorded the following fungal species *Aspergillus flavus*, *A. fumigatus*, *A. nidulans*, *A. alutaceus*, *A. glaucus*, *A. niger*, *Cladosporium herbarum*, *Fusarium sp.*, *Penicillium digitatum* and *Trichoderma viride*. These species showed five patterns of infection *A. niger*, *A. fumigatus* and *A. nidulans* displayed a continuous decline in population during the three months storage period. On the one hand, some bacterial species recorded included *Enterobacter cloacae*, *E. amnigenus*, *E. sakazakii*, *Flavobacterium sp.*, *Chromobacterium violaceum*, *Pseudomonas putida*, *P. aeruginosa*, *P. cepacia*, *Acinetobacter sp.* and *Serratia plymuthica*, *Aeromonas salmonicida* and *Pseudomonas*.^{4,5}

In Costa Rica, the microbiological quality of some powdered spices usually used in homes, without further thermal treatment was evaluated. Black and white and green pepper counts gave plate counts and faecal coliforms counts exceeded the International Commission on Microbiological Specification for Food (ICMSF) standards and that contamination was probably due to drying conditions and post-harvest treatment Arce (as cited in Ahene & Odamtten et al.⁴

These constituted a serious health hazard to the consumer of such spices on the market. Fungi associated with spices, herbs, codiments, cereals and dehydrated products are predominated by *Aspergillus* species majority of which strains produce mycotoxins including the potent aflatoxins Flannin and Hui.⁴ These mycotoxigenic species impart toxic metabolites during growth in food and food products making food unfit for human consumption. The mycotoxins with the most potential for human hazard are toxins of storage fungi in the genera *Aspergillus*, *Penicillium* and *Fusarium*. The most potent are aflatoxins B1, B2, G1 and G2 (produced by *Aspergillus flavus*, *A. parasiticus*, *A. nomius*). Fumonisin (*Fusarium verticilloides*), trichothecene (*Fusarium spp*); Patulin (*P. expansum*, *Paecilomyces sp.*).⁴

Odamtten⁶ reiterated that the danger of mycotoxicoses especially aflatoxicoses still exists in Africa and cannot be discounted in our health delivery system. However, in some instances despite the presence of these mycotoxigenic species in the raw spices and spice products there is self-protection against mycotoxin products by virtue of the formulation and inherent preservation offering self-protection. For example, Ahene & Odamtten et al.,⁴ showed that aniseed, rosemary, royco and maggi cubes offered self-protection against aflatoxin formation by *A. flavus* by creating condition unfavourable for toxin formation. Benny Beef powdered stock, Benny Crayfish powdered stock, Remie Chicken seasoning powder, Napa Valley Jollof Rice seasoning, Onga Chicken seasoning and Indomie seasoning are popular imported seasoning powders on the shelf of local supermarkets and shops in Ghana. Their formulation although stated on the packaging material is not known for certain by the consuming public and cannot be taken for granted. This paper report our findings on work carried out to assess the microbiological quality of these listed seasonings and provides information on the aflatoxigenic potential of the resident

Aspergillus flavus and *A. alutaceus* isolated from these products and the possible control measures via gamma irradiation energy.

Materials and methods

The packaged seasoning powders selected for the study have the following composition according to the manufacturers and specific flavour enhancers stated in this study are numbered in E numbering system are listed below:

E102: Tartrazine; E110: Sunset Yellow FCF; Orange Yellow S; E121: Orcein, Orchil; E122: Azorubine, Carmoisine; E124: Ponceau 4R, Cochineal Red A; E133: Brilliant Blue FCF; E330: Citric acid; E363: Succinic acid; E551: Silicon dioxide; E621: Monosodium L-glutamate; E627: Disodium guanylate; E631: Disodium inosinate

Materials

Benny beef powdered stock: Ionised salt, cornflour, flavour enhancers (monosodium glutamate MSG E621, E631, E627, flavouring anticaking agent (E551), colourant E122, E133, E124, Tartrazine (E102, E110).

Benny crayfish powdered stock: Ionised salt, monosodium glutamate MSG E621, cornflour, disodium inosinate, crayfish powder, approved colourant, E121, flavouring herbs, spices.

Remie chicken seasoning powder: Ionised salt, monosodium glutamate MSG E621, corn starch, sugar, vegetable fat, natural colours, spices, chicken seasoning powder.

Napa valley jollof rice seasoning: Ionised salt, monosodium glutamate MSG E621, starch, sugar, maltodextrin, tomato powder.

Onga chicken seasoning: Iodated salt, maize, flavour enhancers (E621, E631, E627), maltodextrin, sugar, anticaking agent (E551), spice extracts (turmeric, capsicum, chilli) vegetable fat, flavourants, acids (E363, E330), herbs.

Indomie seasoning: Ionised salt, flavour enhancers (E621, E631, E627) sugar, garlic powder, yeast extract, dry leek, onion powder, chicken flavour, spices.

Methods

- Microbiological enumeration:** This was done according to the method described by ISO.⁷ Fungi and bacteria in the seasoning powders were determined as prescribed using the decimal serial dilution technique up to 1:10³. 1ml aliquots of the serially diluted samples were placed in either 20ml of Dichloran Rose Bengal Chloramphenicol agar (DRBC, CM 727) or Oxytetracycline Glucose Yeast Extract agar (OGYE, Oxoid 545, for fungal count or on Plate Count Agar, PCA (Biochemika 70152 (for bacterial count). Plates containing PCA were incubated at 35°C for 48hrs which plates containing DRBC and OGYE were incubated at 28-30°C for 7 days. At the end of the prescribed incubation periods bacterial and fungal counts obtained were calculated as log₁₀CFU/g sample.
- Identification of fungi encountered:** Fungi isolated were identified by their cultural, colour morphological characteristics (microscopic and macroscopic) according to the standard reference of Samson & Barnett et al.^{8,9}
- Identification of bacterial species:** This was carried out using the novel Matrix-Assisted Laser desorption/ionization time of flight (MALDI-TOP-MS) Mass Spectrometry method prescribed by the

manufacturer.¹⁰ It is an analytical method of identification and is characterised by the fast and precise assessment of mass of molecules in a variable range of 100Da to 100KDa. About 0.1µl of Bacterial Test Standard (BTS) of *Escherichia coli* with HCl was added and allowed to dry. The prepared target plates were put in the Maldi Biotype Galaxy (Microflex) chamber according to the manufacturer's instructions using a scanner. The codes of the target plates were scanned and the names of the bacteria appeared automatically on the computer.

- d) Influence of radiation treatment on the survival of resident fungi and bacteria in sample:** The samples were placed in duplicate in envelopes and sent to the Radiation Technology Centre (RTC) of the Biotechnology and Nuclear Agriculture Institute of the Ghana Atomic Energy Commission Kwabenya, Accra for gamma irradiation treatment at 0,2,5 and 10KGy. Irradiation was carried out in air at dose rate of 1.683KGy/h (Gamma Conveyor Irradiator, Budapest, Hungary). The absorbed doses were ascertained by Fricke Dosimetry measured spectrophotometrically at 303nm wavelength in the peak of the absorption spectra.
- e) After irradiation the decimal serial dilution technique up to 1:10³ was used to estimate surviving population and the incubation temperature and media, fungi and bacteria are described above. Surviving fungi and bacteria were identified as spelled out above.
- f) Determination of elemental composition of samples:** The following elements were analysed for their presence and concentration [iron (Fe), sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), zinc (Zn), lead (Pb), chromium (Cr), nickel (Ni), cobalt (Co), mercury (Hg) and arsenic (As)] using an Atomic Absorption Spectrometry AAS technique (Model 240FS, Vienna, Austria) following the instructions prescribed by the manufacturer.
- g) Mycotoxin analyses of samples:** The aflatoxins (by *Aspergillus flavus*) and Ochratoxin A (by *A. alutaceus*) levels in the samples were determined using the High Pressure Liquid Chromatography HPLC (Model Agilent 1260 Series, USA) at the Food Research Institute CSIR, Ghana following the methods outlined by Pons.¹¹ The samples were treated differently; one batch was the unadulterated seasonings incubated at 85% ERH for 72h to induce fungal growth (control) and the other batch of the same samples were spiked with 2.5x10³ spores/µl of either *A. flavus* or *A. alutaceus* and stored at 85% ERH for 72h before analysis for mycotoxins.
- h) Statistical analysis:** The results, where appropriate were analysed statistically using Stat-graphics plus Analysis of Variance (ANOVA) to evaluate data at p<0.05 level of significance. Differences between means were determined using Least Significant Test LSD.

Results

A. Microbiological quality of the six dehydrated seasoning powders

The population of fungi in the six dehydrates powders varied depending on the medium used in the isolation. The fungal population varied from a low 0.20log₁₀ CFU/g to 2.54log₁₀ CFU/g sample (Table 1). Generally, Onga chicken seasoning and Benny Beef stock seasoning powder harboured more fungal population. The population of fungi resident in the samples isolated on OGYE was higher by 0.25-1.36log₁₀ cycles than on DRBC.

Total aerobic bacterial population varied from 0.50log₁₀ CFU/g sample (Indomie Seasoning Powder) to 1.40log₁₀ CFU/g sample (Napa Jollof Seasoning Powder).

Table 1 Microbiological quality of the indicated seasoning products plated on either DRBC or OGYE (for fungi) and PCA (for bacteria)

Type of Seasoning	Total microbial population (log ₁₀ CFU/g) on		
	DRBC (Fungi)	OGYE (Fungi)	PCA (Bacteria)
Benny Beef Stock	1.48	2.39	1.3
Remie Chicken	0.2	0.4	1.3
Napa Valley Jollof	1.48	2	1.4
Onga Chicken	1.18	2.54	0.2
Benny Shrimp Stock	1.3	1.54	1
Indomie Powder Seasoning	1.18	1.48	0.5

In all the seasoning powders seven genera of fungi (*Aspergillus*, *Cladosporium*, *Penicillium*, *Eurotium*, *Rhizopus* and *Paecilomyces*) were isolated from the two media. *Aspergillus* species (*A. candidus*, *A. flavus*, *A. niger*, *A. oryzae*, *A. sulphureus*) predominated over the species encountered followed by *Penicillium* (*P. corylophilum*, *P. aurantiogriseum*=*P. verrucosum* var *cylophilum* and *Cladosporium* (*C. herbarum*, *C. macrocarpum*) and *Rhizopus* (*R. oryzae*, *R. stolonifer*). Three other genera encountered were *Absidia*, *Eurotium*, and *Paecilomyces* with single species (Table 2). The percentage occurrence profile of the resident fungal species changed after 6months storage (Table 3). For example, occurrence of *A. flavus* in Benny shrimp stock powder changed from 14.3% to nil in 6months; in Napa Jollof Seasoning, it changed from initially none to 22.2% in 6months; in Onga chicken seasoning percentage occurrence of *A. flavus* changed from none initially to 12.6% in 6months.

sulphureus population in Benny Shrimp Stock seasoning changed from an initial 14.3% to nil in 6months (Table 3); Indomie seasoning powdered harboured 77.8% of *P. aurantiogriseum* but reduced to 36.2% in 6months. Population of the same fungus in Onga chicken seasoning powder changed from initial of 23.1%-46.2% in 6months; in Benny Shrimp Stock, population declined from 35.7% to 26.1% in 6months (Table 3).

Bacterial species isolated from the six samples initially and after 6months storage are presented in Table 4. About 3-7 bacterial species were resident initially in all the seasoning samples. But this changed to nil to seven in 6months depending on the type of sample Table 4.

The species of bacteria isolated were *Acinetobacter pittii*, *Bacillus funiculatus*, *B. subtilis*, *Filifactor villosus*, *Lactobacillus equi*, *L. malferment*, *L. quasseri* and *Pseudomonas orientalis*. *Lactobacillus* species predominated over the genera encountered (Table 4).

B. Influence of gamma irradiation on the microfloral population

Increasing irradiation doses from 0-10KGy proportionately decreased the fungal and bacterial population in all the samples. A dose of 5-KGy eliminated the bacterial floral while it left a residual of *Rhizopus oryzae* mycoflora in all the samples (Table 5) (Table 6).

C. Elemental composition of the seasoning powders

All the seasonings were high in iron content (3.6-104.82mg/l),

Sodium (43.4-13700mg/l), Potassium (0.5-4240mg/l), Calcium (104.82mg/l) (Table 7). (0.088-2.105mg/l), and Magnesium (0.035-0.37mg/l).

The remaining heavy metals occurred in low or trace amounts namely Copper, Zinc, Lead, Chromium, Nickel, Cobalt, Arsenic and Mercury) (Table 7). Benny shrimp powder contained high concentration of K (4240mg/l) and sodium (424.0mg/l), while Onga chicken seasoning contained high amount of sodium (13780mg/l). On the other hand, Remi chicken seasoning powder had high iron content

D. Determination of total aflatoxin and ochratoxin A content of seasoning samples

The fresh seasoning samples were stored at 85% ERH for 72h to induce fungal growth and those spiked with spores of *A. flavus* and kept the same way did not contain aflatoxins B1, B2, G1 and G2 in all the test samples (Table 8). The same was true for Ochratoxin A which was not detected in any other samples (Table 9).

Table 2 Total list of mycoflora isolated from the six seasoning powders at 30°C on two different media DRBC and OGYE

S. no	Mycoflora
1	Absidia Glauca Hagem ⁵
2	Aspergillus Candidus Link ³
3	A. Flavus Link ³⁻⁵
4	A. Alutaceus Wilhelm ¹
5	A. Oryzae Cohn ³
6	Za. Niger Van Tieghem ^{1,3,4}
7	A. Sulphureus (Fres) Thom And Church ^{1-3,5}
8	A. Terreus Thom ¹
9	Cladosporium Herbarium (Pers) Link ^{4,6}
10	C. Macrocarpum Preuss ¹
11	Eurotium Amstelodanii Mangin ⁵
12	Penicillium Corylophilum Dierckx ^{4,5}
13	P. Aurantiogriseum Dierckx (P. Verrucosum Var Cyclopium) ^{4,6}
14	Paecilomyces Carneus (Duché Et Heim) Brown & Smith ²
15	Rhizopus Oryzae Went & Prinsen Geerlings ¹⁻⁵
16	R. Stolonifer Link ⁴

Key LK1, benny beef stock seasoning; LK2, remie chicken seasoning; LK3, napa valley jollof seasoning; LK4, onga chicken seasoning; LK5, benny shrimp stock seasoning; LK6, indomie seasoning

Table 3 Percentage occurrence of the indicated fungal species resident in the fresh samples and those stored for 6months at 30°C beyond the expiry date

Fungus isolated	Type of seasoning and percentage occurrence (%)											
	LK 1		LK 2		LK 3		LK 4		LK 5		LK 6	
	F	6mth	F	6mth	F	6mth	F	6mth	F	6mth	F	6mth
<i>Aspergillus flavus</i>	-	-	-	-	-	-	-	-	14.3	17.5	-	-
<i>Aspergillus niger</i>	54.5	31.4	-	29	42.9	33.5	30.8	53.8	-	56.5	-	-
<i>Aspergillus sulphureus</i>	-	-	-	45.2	35.7	15.7	-	-	14.3	-	-	-
<i>Cladosporium herbarum</i>	-	20	-	-	-	-	30.8	-	-	-	22.2	12.3
<i>Cladosporium macrocarpum</i>	31.8	-	-	-	-	-	-	-	-	-	-	-
<i>P. Aurantiogriseum</i> (= <i>p. Verrucosum</i> var. <i>Cyclopium</i>)	-	34.3	-	25.8	-	13.7	23.1	46.2	35.7	26.1	77.8	36.2
<i>Rhizopus oryzae</i>	13.6	14.3	100	-	21.4	-	7.7	-	7.1	-	-	-
<i>Rhizopus stolonifer</i>	-	-	-	-	-	-	7.7	-	-	-	-	-

Key F, fresh samples (Initial); LK 1, benny beef stock seasoning; LK 2, remie chicken seasoning; LK 3, napa valley jollof seasoning; LK 4, onga chicken; seasoning; LK 5, benny shrimp stock seasoning; LK 6, indomie seasoning

Table 4 List of Bacterial species isolated from fresh samples initially and the sample stored for 6months and plated on plate count agar at 35°C for 48hours

Bacterial species	Sample code and period of storage in Month											
	LK 1		LK 2		LK 3		LK 4		LK 5		LK 6	
	0	6mth	0	6mth	0	6mth	0	6mth	0	6mth	0	6mth
<i>Acinetobacter pitii</i>	+	+	-	-	-	-	-	-	-	-	-	-
<i>Bacillus funiculatus</i>	-	-	+	+	-	-	-	-	-	-	-	-
<i>Bacillus subtilis</i>	-	+	+	+	-	-	-	+	+	+	+	-
<i>Filifactor villosus</i>	+	+	+	+	-	-	-	-	-	-	-	-
<i>Lactobacillus equi</i>	+	+	+	+	+	-	+	+	+	+	+	-
<i>Lactobacillus malferment</i>	+	+	+	+	+	-	-	-	-	-	-	-
<i>Lactobacillus quasseri</i>	+	+	+	+	+	-	-	-	+	-	+	-
<i>Pseudomonas orientalis</i>	-	-	+	+	-	-	-	+	-	+	-	-

Key LK 1 – LK 6; +: Present; -: Absent

Table 5 Radiation response of the indicated bacteria species resident in the six seasoning powders

Types of seasoning	Bacterial species surviving and dose applied (Kgy)	Dose applied (Kgy)			
		0	2	5	10
LK 1 (Benny Beef Stock)	<i>Acinetobacter pitii</i>	+	-	-	-
	<i>B. subtilis</i>	-	-	-	-
	<i>B. funiculatus</i>	+	-	-	-
	<i>F. villosus</i>	-	-	-	-
	<i>L. equi</i>	+	+	-	-
	<i>L. malferment</i>	+	-	-	-
	<i>L. quasseri</i>	+	-	-	-
	<i>A. pitii</i>	+	-	-	-
	<i>B. subtilis</i>	+	-	-	-
LK 2 (Remie Chicken Powder)	<i>B. funiculatus</i>	+	-	-	-
	<i>F. villosus</i>	+	-	-	-
	<i>L. equi</i>	+	-	-	-
	<i>L. malferment</i>	+	-	-	-
	<i>L. quasseri</i>	+	-	-	-
	<i>P. orintalis</i>	+	-	-	-
LK 3 (Jollof Seasoning)	<i>L. equi</i>	+	+	+	-
	<i>L. malferment</i>	+	-	-	-
	<i>L. quasseri</i>	+	-	-	-
	<i>P. orintalis</i>	-	-	-	-
LK 4 (Onga Chicken Seasoning)	<i>L. equi</i>	+	+	-	-
	<i>L. quasseri</i>	-	-	-	-

Table continued...

Types of seasoning	Bacterial species surviving and dose applied (Kgy)				
LK 5 (Benny Shrimp Stock)	<i>Bacillus subtilis</i>	+	-	-	-
	<i>L. equi</i>	+	-	-	-
	<i>L. quasseri</i>	+	+	-	-
	<i>B. subtilis</i>	+	-	-	-
LK 6 (Indomie Seasoning)	<i>L. equi</i>	+	-	-	-
	<i>L. quasseri</i>	+	+	-	-
	<i>F. villosus</i>	+	-	-	-

Table 6 Radiation response of the indicated fungal species resident in the six seasoning powders

Type of seasoning	Fungal species surviving and the applied dose (KGy)				
	0	2	5	10	
LK 1 (Benny Beef Stock)	<i>Aspergillus alutaceus</i>	+	-	-	-
	<i>A. oryzae</i>	+	-	-	-
	<i>A. sulphureus</i>	+	-	-	-
	<i>A. terreus</i>	+	-	-	-
	<i>Cladosporium herbarum</i>	+	-	-	-
	<i>Fusarium solani</i>	+	-	-	-
	<i>F. verticillioides</i>	+	-	-	-
	<i>Penicillium digitatum</i>	+	-	-	-
	<i>P. aurantiogriseum</i> = (<i>P. verrucosum</i> var. <i>cyclopium</i>)	+	+	-	-
	<i>Rhizopus oryzae</i>	+	-	-	+
LK 2 (Remie Chicken Seasoning)	<i>R. stolonifer</i>	+	-	-	-
	<i>Aspergillus flavus</i>	+	-	-	-
	<i>A. niger</i>	+	+	-	-
	<i>A. sulphureus</i>	+	-	-	-
	<i>C. herbarum</i>	+	-	-	-
	<i>P. aurantiogriseum</i> = (<i>P. verrucosum</i> var. <i>cyclopium</i>)	+	-	-	-
	<i>R. oryzae</i>	+	-	-	+
	<i>Aspergillus niger</i>	+	-	-	-
	<i>A. oryzae</i>	+	-	-	-
	<i>A. sulphureus</i>	+	-	-	-
LK 3 (Napa Valley Jollof Seasoning)	<i>Cladosporium herbarum</i>	+	-	-	-
	<i>P. aurantiogriseum</i> = (<i>P. verrucosum</i> var. <i>cyclopium</i>)	+	-	-	-
	<i>P. corylophilum</i>	+	+	-	-
	<i>R. oryzae</i>	+	-	-	+
	<i>R. stolonifera</i>	+	-	-	-
	<i>Syncephalastrum racemosum</i>	+	-	-	-

Table continued...

	<i>A. niger</i>	+	-	-	-
	<i>A. sulphureus</i>	+	-	-	-
	<i>C. herbarum</i>	+	-	-	-
LK 4 (Onga Chicken Seasoning)	<i>P. aurantiogriseum</i> = (<i>P. verrucosum</i> var <i>cyclopium</i>)	+	+	-	-
	<i>R. oryzae</i>	+	-	-	+
	<i>R. stolonifer</i>	+	-	-	-
	<i>Aspergillus niger</i>	+	-	-	-
	<i>A. sulphureus</i>	+	-	-	-
	<i>Cladosporium herbarum</i>	+	-	-	-
	<i>Fusarium solani</i>	+	-	-	-
LK 5 (Benny Shrimp Stock Powder)	<i>P. corylophilum</i>	+	-	-	-
	<i>P. aurantiogriseum</i> = (<i>P. verrucosum</i> var. <i>cyclopium</i>)	+	-	-	-
	<i>R. oryzae</i>	+	-	-	+
	<i>R. stolonifer</i>	+	-	-	-
	<i>A. niger</i>	+	-	-	-
	<i>A. oryzae</i>	+	-	-	-
	<i>A. sulphureus</i>	+	-	-	-
LK 6 (Indomie Seasoning)	<i>C. herbarum</i>	+	-	-	-
	<i>P. aurantiogriseum</i> = (<i>P. verrucosum</i> var <i>cyclopium</i>)	+	+	-	-
	<i>R. oryzae</i>	-	-	-	-

Table 7 Sample analysis for elemental composition of the seasoning powders

Elemental conc. (mg/l)	Types of sample: Spices					Instrument detection limit (mg/l)
	LK1 Powdered beef stock	LK2 Benny powdered stock	LK3 Napa valley joll of rice seasoning	LK4 Onga chicken	LK5 Remie chicken seasoning	
Iron	7.23	6.48	3.6	9.72	104.8	0.006
Manganese	<0.002	<0.002	<0.002	<0.002	<0.002	0.002
Coper	<0.003	<0.003	<0.003	<0.003	<0.003	0.003
Zinc	<0.001	1.26	0.96	0.54	1.2	0.001
Calcium	0.677	0.689	0.088	2.105	0.463	0.001
Magnesium	0.27	0.271	0.035	0.837	0.812	0.0003
Lead	<0.001	<0.001	<0.001	<0.001	<0.001	0.001
Chromium	1.02	0.1	0.84	2.88	1.148	0.001
Nickel	<0.001	<0.001	<0.001	<0.001	<0.001	0.001
Cadmium	0.906	<0.002	<0.002	<1.020	<0.002	0.002
Sodium	43.4	424	59.5	13700	43.8	0.01
Cobalt	1.14	<0.005	<0.005	<0.005	<0.005	0.005
Potassium	14	4240	0.5	9.1	4.7	0.01
Arsenic	<0.001	<0.001	<0.001	<0.001	<0.001	0.01
Mercury	<0.001	<0.001	<0.001	<0.001	<0.001	0.01

Table 8 Aflatoxin analysis of seasoning samples with the indicated codes with or without *Aspergillus flavus* inoculation after 5days at 30°C using HPLC technique

Sample Code	Aflatoxin (µg/Kg)				
	B1	B2	G1	G2	Total
LK 1 (FRI MTR:Af)	ND	ND	ND	ND	ND
LK 2 (FRI MTR:Af)	ND	ND	ND	ND	ND
LK 3 (FRI MTR:Af)	ND	ND	ND	ND	ND
LK 4 (FRI MTR:Af)	ND	ND	ND	ND	ND
LK 5 (FRI MTR:Af)	ND	ND	ND	ND	ND
LK6 (FRI MTR:Af)	ND	ND	ND	ND	ND
LK 1 (FRI MTR: C)	ND	ND	ND	ND	ND
LK 2 (FRI MTR: C)	ND	ND	ND	ND	ND
LK 3 (FRI MTR: C)	ND	ND	ND	ND	ND
LK 4 (FRI MTR: C)	ND	ND	ND	ND	ND
LK 5 (FRI MTR: C)	ND	ND	ND	ND	ND
LK 6 (FRI MTR: C)	ND	ND	ND	ND	ND

Detection limit Aflatoxin B1 & B2: 0.15 µg/Kg, Aflatoxin G1 & G2: 0.13µg/Kg

LK 1, benny beef stock seasoning; LK 2, remie chicken seasoning; LK 3, napa valley jollof seasoning; LK 4, onga chicken seasoning; LK 5, benny shrimp stock seasoning; LK 6, indomie seasoning; (FRI MTR:Af) Samples spiked with 2.5×10^3 CFU/g sample of *Aspergillus flavus*; (FRI MTR: C) Control raw sample without *Aspergillus flavus*

Table 9 Ochratoxin analysis of seasoning samples with the indicated codes with or without *Aspergillus alutaceus* after 5days at 30°C using HPLC technique

Sample Code	Ochratoxin A (µg/kg)
LK 1 (FRI MTR:Aa)	ND
LK 2 (FRI MTR:Aa)	ND
LK 3 (FRI MTR:Aa)	ND
LK 4 (FRI MTR:Aa)	ND
LK 5 (FRI MTR:Aa)	ND
LK6 (FRI MTR:Aa)	ND
LK 1 (FRI MTR: C)	ND
LK 2 (FRI MTR: C)	ND
LK 3 (FRI MTR: C)	ND
LK 4 (FRI MTR: C)	ND
LK 5 (FRI MTR: C)	ND
LK 6 (FRI MTR: C)	ND

Detection limit Ochratoxin, 0.83µg/Kg

LK 1 -LK 6, benny beef stock seasoning; LK 2, remie chicken seasoning; lk 3, napa valley jollof seasoning; LK 4, onga chicken seasoning; LK 5, benny shrimp stock seasoning; LK 6, indomie seasoning; (FRI MTR:Aa) Samples spiked with 2.5×10^3 CFU/g sample of *Aspergillus alutaceus*; (FRI MTR: C) Control raw sample without *Aspergillus alutaceus*

Discussion

The liberalized global economy has resulted in the influx of many good as well as shoddy food products into developing countries. The agencies responsible for ascertaining the quality of those products from the exporting countries are presumed to have done due diligence to adherence of food quality standards of the International Standards Organisation. However, the survival of microbiological species in dehydrated products in a well-known phenomenon.

Although the population of resident mycoflora with low ($0.20-2.54 \log_{10}$ CFU/g sample) there were species of pathological importance resident in all the six samples (Table 2) such species included (*Aspergillus flavus*, *A. alutaceus*, *A. candidus*, *A. niger*, *A. sulphureus*, *Penicillium aurantiogriseum* and *Paecilomyces*). The preponderance of *Aspergillus* and *Penicillium* species in dehydrated foods in storage is a well-known phenomenon in the tropics and elsewhere and has been well-documented in Ghana.^{4,11,12} In this paper potential toxin-producing fungal species such as *Aspergillus candidus* (kojic acid), *A. flavus* (aflatoxins B1, B2, G1 and G2), *A. oryzae* (kojic acid), *A. sulphureus* (ochratoxins, sterigmatocystin), *Cladosporium herbarum* (epicladosporic acid), *Eurotium amstelodami* (sterigmatocystin), *Penicillium corylophilum* (griseofulvin, roquefortine C), *P. aurantiogriseum* (penicillic acid, viridicatin, ochratoxin, citrinin), *Paecilomyces* (patulin) have been isolated (Table 2).¹⁴

The health implications of consuming such contaminated seasonings are enormous in Africa for the consumer who may use an opened sachet bought for use over a long period even beyond the expiry date. The high occurrence of *A. flavus*, *A. niger*, *A. sulphureus*, *P. corylophilum*, *P. aurantiogriseum* in the sample (6.7-77.8%) kept for 6 months leave much to be desired. The seasonings were also very hygroscopic and deliquescent. For example Indomie seasoning absorbed moisture fast as even 55%ERH, (data not shown). At all the ERH's studied (55, 75, 85, 95 ERH), it took 15-20 days for equilibrium to be achieved with the surrounding (Data not shown). This implies that the seasonings were not manufactured to withstand the vicissitude of the changing fortunes of the tropical weather conditions and find purpose in being dissolved quickly in the product earmarked for seasoning. In the event of improper sealing of the package after opening, the moisture content value may increase giving the microorganism the opportunity to form and multiply even if kept under refrigeration.

There has been a correlation between Hepatitis B and aflatoxins in Africa Peers et al.⁴ Liver incidence varied over a 5-fold range, and was strongly associated with estimated levels of aflatoxins Peers et al.⁴

Aflatoxins produced by *A. flavus*, *A. parasiticus*, and *A. nomius* in various food commodities including, maize, rice, barley, wheat, sorghum, groundnut and oil foods, copra, cocoa beans and some spices and condiments have attracted the attention of a number of international health authorities including WHO.¹⁵ Aflatoxins are immunosuppressive, mutagenic, teratogenic and carcinogenic in their effect with the main target organ being the liver. Evaluation of epidemiological and laboratory results by International Agency for Research in Cancer IARC⁴ showed that there is sufficient evidence in humans for carcinogenicity of naturally occurring aflatoxins. Consequently, aflatoxins are classified as Group 1 carcinogens. Acute aflatoxins in humans has been reported in Kenya¹⁵ Mozambique, Swaziland, Transkei (Van Resenberg & Schakwyk; Makaula).^{4,6}

Ochratoxins produced by *A. alutaceus* are nephrotoxins of human health importance. Co-occurrence of aflatoxin B1, Fumonisin B, Ochratoxin A and Zearalenone in cereals, dehydrated products and groundnut have been reported.¹⁶ In Ghana, Wareing et al.,¹² detected sterigmatocystin, patulin, cyclopiazonic acid, penicillic acid, tenuazonic acid, aflatoxins in kokonte (cassava flour) while Kpodo et al.,¹² found the co-occurrence of high levels of aflatoxins, ochratoxins and citrinin in fermented maize dough and fumonisins and their co-occurrence with aflatoxin in maize.

However, in our present study analysis for aflatoxins B1, B2, G1, G2 and ochratoxin A in the control and spiked samples of seasonings showed that both mycotoxins were not formed Tables 8 & 9. Presumably, the seasonings products were stable and their formulation, offered self-protection against aflatoxin and ochratoxin. A formation by *A. flavus* and *A. alutaceus* respectively. This agrees with the findings of Ahene & Odamtten et al.⁴ Four of the seasonings (Benny Beef powder stock, Benny Crayfish powder stock, Remic chicken seasoning powder, Napa Valley Jollof rice seasoning) contained unspecified amount of monosodium glutamate, MSG, in addition to unspecified ionized salt and flavor enhancers and garlic. These additives could have acted in concert to prevent mycotoxin formation. However, MSG is the sodium salt on the non-essential amino acid glutamic acid. Glutamic acid is the most abundant amino acids in human food. When glutamate is present in free form, not as a component of proteins or peptides, it has a flavour-enhancing effect and for this reason it is added to foods as it is purified monosodium salt FSANZ,¹⁷ Win,¹⁸ Adeyemo OA.¹⁹

Levels of free glutamate in foods of animal origin are generally low (beef 33mg/100g; cow's milk 2mg/100g). Higher levels are found in vegetables (30 - 200mg/100g) and in seasonings, sauces and restaurant meals.^{17,20} The debate on the safety of MSG in food consumed in the developing countries and elsewhere is the range of adverse reaction observed including the symptom complex known as Chinese restaurant syndrome (CRS) and bronchoconstriction in some asthmatic individuals. This syndrome is characterized by headaches, numbness/tingling, flushing, muscle lightness and generalized weakness.²¹ The average person in the USA consumes 0.5 - 1.0g of MSG in a day,²² comparable to an average daily dietary exposure of 0.6/day of MSG in the UK with extreme consumers having exposure of more than 2g/day e.g. in Taiwan.²²

Estimated average daily dietary MSG exposure in developing countries has been reported in the range 0.3-1.0g/day.²³ While research indicate that people differ widely in their sensitivity to MSG,²² many claim harmful effect due to it.^{18,24} It is up to national safety regulation agencies to ban or prescribe the safety limit of MSG in food although in this present paper it could have played a role in the prevention of mycotoxin formation in the six seasonings. The other interesting observation was that bacterial species isolated from the six seasoning samples varied from an initial 3-7 to none to 7 in 6 months (Table 4) depending on the type of seasoning.

Generally, the bacteria species isolated were *Acinetobacter pittii*, *Bacillus funiculatus*, *B. subtilis*, *Filifactor vilbosus*, *Lactobacillus equi*, *L. malferment*, *L. quasseri* and *Pseudomonas orientalis*. *Lactobacilli* predominated over the other bacteria encountered. The bacterial species listed are being recorded for the first time in seasonings currently on sale in Ghana and many constitute a health hazard.

Infections associated with *Acinetobacter* species include ventilator - associated pneumonia, skin and soft-tissue infections, wound infections, urinary tract infections, peritonitis, secondary meningitis and blood stream infections.²⁵ Such infections are caused predominantly by members of the *A. baumannii* complex; infections caused by other species belonging to the genus *Acinetobacter* are relatively unusual and are restricted mainly to catheter-related blood stream infections and rare outbreaks related to point-source contamination.²⁵ *Acinetobacter* may also cause purulent meningitis.²⁶

Lactobacilli on the other hand are part of the normal flora of the human mouth gastrointestinal tract and female genital tract^{26,27} and are generally regarded as safe for use in food (probiotic and to ferment food).²⁷ *Lactobacillus* infections are rare; but can occur opportunistically, especially in immunocompromised individuals.²⁶ Most deaths associated with *Lactobacillus* infections are in individual with underlying immunosuppressive conditions.

Lactobacillus has been found present in the mouth implicated in dental caries, intestines and typically in the adult vagina.^{26,28} On the other hand, *L. maleferment* has been identified as having low pathogenic potential reinforcing the fact that it is a safe microbe.²⁹ *L. quasseri* infection is accompanied by severe inflammation of the intestinal mucosal surface. Strains of *L. quasseri* produced active bacteriocins Gasserin A which is bactericidal without causing lysis.²⁶

Bacillus subtilis is generally non- pathogenic but has occasionally caused foodborne gastroenteritis leading to food poisoning. *B. subtilis* is an endospore forming bacterium and the endospore it forms enables it to withstand extreme temperatures as well as dry environments.²⁶ This same bacterium has also been associated with septicemia, meningitis, endocarditis, pneumonia, wound infections, and other suppurative lesions, particularly as an opportunistic pathogen.²⁶

Pseudomonas can be found in many different environments such as soil, water, plant and animal tissue. Many different species of this bacterium are opportunistic pathogens which affect humans, animals and plants. For example, *P. aeruginosa* almost never infects uncompromised tissue but can practically infect any tissue which has some types of compromised defenses.²⁶ The presence of bacteria causing diarrhoea, gastrointestinal infection and meningitis etc in the seasoning kept for a long time leave much to be desired as these diseases account for an annual mortality rate of five million people worldwide.³⁰ Presence of pathogenic bacteria and potential mycotoxic fungi is not desirable for product stability and safety for human consumption.

All the six seasoning powders showed varied amount of Fe content (3.6-104mg/l), Na (43.4-13700mg/l) K (0.5-4240mg/l) (Table 7). The WHO³¹ limit for iron intake is 15mg/kg. Iron deficiency (anaemia) affects one third of the world's population but excessive intake in iron is associated with the risk of colorectal cancer.³² The presence of high potassium (4240mg/l) over sodium (424.0mg/l) was found in Benny Shrimp powder. The presence of high content of potassium over sodium in diet suggests effectiveness against hypertension. Indeed Patil & Wiafe-Kwagyan et al.^{33,34} also reported a balance between high potassium and low sodium in oyster mushrooms (*Pleurotus ostreatus* and *P. eous*) on different substrates. Manzie & Mattila et al.^{35,36} also found high potassium and low sodium concentrations in the fruiting bodies of mushrooms. Onga chicken seasoning, on the contrary, contained very low potassium (9.1mg/l) and very high sodium content (13700mg/l) (Table 7) and may be having the opposite effect of predisposing consumers to hypertension.

The remaining samples contained low traces of Cu, Zn, Ca, Mg, Pb, Co, Ni, Ar, Hg (Table 7). Heavy metals such as Cu, Mn, Pb, Zn, Ni, Co, Hg were also detected albeit in small concentrations. Some heavy metals Fe, Cu, Mn, Zn, are essential since they play an important role in the biological systems. Lead is not essential and can be toxic in trace amounts.³⁷ However, the essential heavy metals can be toxic when is excessive intake (such as being added to soups and stews as ingredients of seasonings).^{38,39} Five heavy metals were detected in the sporophore (fruiting body) of *P. eous* and *P. ostreatus* (Cu, Fe, Mn, Pb, Zn) by Wiafe-Kwagyan³⁴ in trace quantities lower than the reported permissible limit set by WHO.³¹

Copper is an essential constituent of metallo-enzyme and is required in haemoglobin synthesis and in catalysis of metabolic growth.⁴⁰ Lead is toxic even in trace levels.⁴¹ The impairment of human functions related to lead toxicity includes abnormal size and haemoglobin content of erythrocytes, hyper stimulation of erythropoiesis and inhibition of haemosynthesis Vonugopal & Wiafe-Kwagyan.³⁴ It is therefore of extreme health significance that Pb was detected in all the samples of the seasonings albeit in trace amounts. However, these values were far below the 10mg/kg limit set by WHO³¹ for Pb in plant materials. In the event of continuous consumption of foods and soups prepared with such seasonings containing Pb, the cumulative effect on the essential organs of consumers cannot be discounted or ruled out. Manganese is essential metal as it plays an important role in biological systems such as its presence in metallo proteins.³⁷ The level of <0.002mg/l detected in the seasonings would not pose immediate danger as this value falls below the toxicity limit of 1000mg/kg in plants prescribed by WHO.³¹ Interestingly, varying ranges of Mn content in mushroom have been documented as 12.9-93.3mg/kg⁴² and 14.2-67.7mg/kg⁴³ 0.116– 0.86mg/kg.³⁴

Zinc is also an essential mineral which is a component of wide range of enzymes and co-enzymes. It performs catalytic, structural and regulating roles in biosynthesis. According to Ma & Belt,³⁴ zinc contributes to about 33ppm to adult weight, and is also a constituent of many enzymes involved in the physiological functions of protein synthesis and enzyme metabolism. The WHO recommended limit for zinc in foods in 60mg/kg. The amount detected in the seasonings varies from <0.001-1.26mg/l far below their permissible level.

Conclusion

In the concluding experiment of this paper, the possible control of the resident mycoflora and bacterial in the six seasoning was studied using gamma irradiation (Tables 4–6). The WHO/FAO/IAEA Joint Expert Committee has since 1980 approved the use of gamma irradiation up to 10KGy for food preservation without any adverse effect on organoleptic and functional properties.⁴⁴ Over 64 countries are currently using this process in diverse ways to prolong the shelf-life of foods for human and livestock consumption.⁴⁴ In this present study all bacteria species were eliminated by a dose of 5KGy and above. While 10 KGy of gamma irradiation left a residual, insignificant trace of *Rhizopus* in the samples (Table 5) (Table 6). It is therefore feasible to use gamma irradiation up to 10KGy of gamma irradiation to decontaminate the seasoning powders and thus extend the shelf-life of the pre-packaged products and render them safe for human consumption.

It is well-known that both micro and macro-nutrients are required for proper growth of fungi. These mineral elements P, K, Mg, S, Na and Ca are required for growth as sporulation of fungi Wiafe-Kwagyan.³⁴ The widely studied micronutrients for the growth of fungal species

include Fe, Zn, Al, Mn, Cu, Cr, Mo,^{45–47} some of which were detected in the six seasonings used in this present study. The influence of the nutrients in the seasoning on the growth and toxin production of some of the resident predominant fungal species in the seasonings will be reported in a subsequent paper.

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Conflicts of interest

Authors declare that there is no conflict of interest.

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