

Effect of storage time on microbial quality of some spices and dried seasonings

A. ADU-GYAMFI

Department of Food Science & Radiation Processing, Biotechnology and Nuclear Agriculture Research Institute, P. O. Box LG80, Legon, Ghana

ABSTRACT

The effect of storage time on the microbial quality of some spices and dried seasonings (SDS) (*dawadawa*, pepper, ginger, shrimp and fish powders) was studied over a 12-month period. Microbial load and profile of irradiated and unirradiated SDS were assessed at 0, 6 and 12-month periods. The range of total variable counts (TVCs) were initially determined at 0.81-4.53 and 4.65-8.51 \log_{10} cfu g^{-1} for irradiated and unirradiated SDS, respectively; those for mould and yeast counts (MYCs) were determined at 0-1.74 and 1.55-3.35 \log_{10} cfu g^{-1} , respectively. Generally, TVCs were not significantly affected ($P < 0.05$) by the 6 and 12-month periods, but MYCs were significantly reduced ($P < 0.05$) after the storage periods in some SDS. Microbial profile, mainly dominated by *Bacillus* spp., *Lactobacillus* spp., *Clostridium* spp., *Aspergillus* spp. and *Penicillium* spp., was stable after the 6 and 12-month periods for all the SDS. However, the profile was consistently more diverse on *dawadawa*, pepper and ginger powders. No adverse change in microbial quality of irradiated and unirradiated SDS was observed at the end of the storage periods.

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RÉSUMÉ

ADU-GYAMFI, A.: *Effet de la durée de stockage sur la qualité microbienne de quelques épices et les assaisonnements séchés.* L'effet de la durée de stockage sur la qualité microbienne de quelques épices et les assaisonnements séchés (EAS) (*dawadawa*, le poivre, le gingembre, la crevette et la farine de poisson) étaient étudiées au cours d'une période de 12-mois. La quantité et le profil microbien de EAS irradié et non-irradié étaient évalués au cours de périodes de 0, 6 et 12-mois. La variation de quantités viables totales (QVT) était au départ déterminée à 0.81-4.53 et à 4.65 - 8.51 \log_{10} cfu g^{-1} pour EAS irradié et non-irradié respectivement, et celle de quantités de moisissures et de levures (QML) était déterminée respectivement à 0-1.74 et 1.55-3.35 \log_{10} cfu g^{-1} . En général, QVT n'étaient pas considérablement influencées ($P < 0.05$) par les périodes de 6 et 12 mois, mais QML étaient considérablement réduites ($P < 0.05$) après les périodes de stockage de quelques EAS. Le profil microbien surtout dominé par *Bacillus* spp., *Lactobacillus* spp., *Clostridium* spp., *Aspergillus* spp. et *Penicillium* spp. était assez stable après les périodes de 6 et 12-mois pour tous les EAS. Le profil était cependant invariablement plus divers sur *dawadawa*, le poivre et la farine de gingembre. En général, aucun changement négatif de la qualité microbienne de EAS irradié et non-irradié n'était observé à la fin de périodes de stockage.

Introduction

Spices and herbs consist of dried leaves, flowers, buds, fruits and bark or rhizome of various plants. Spices and herbs, together with dried seasonings, serve as condiments that contribute immensely to the odour and flavour of foods. Their essential oils also have anti-bacterial properties that have been used in preserving foods. The total world production of spices and herbs in 1997 was 4.5

million tonnes of which 95 per cent was produced in developing countries (Wilkinson & Gould, 1996; Pearson, 1976; Eiss, 2001; CBI, 1999). These products are widely used, especially in the industrial and catering sectors that account for over 60 per cent of the usage in developed countries.

The most important quality attributes of spices, herbs and dried seasonings (SHDS) are

their cleanliness and flavour properties. These qualities are influenced by their source and origin, chemical nature, and processing conditions (Kneifel & Berger, 1994; Lewis, 1984; DeBoer, Speigelenberg & Jansen, 1985). The increased use of SHDS, especially in most freshly cooked and processed foods, has heightened concern for their sanitation (Fig. 1). Aside insects, extraneous matter and defective materials, national and international hygiene standards further indicate they should not contain pathogenic microorganisms; and that total viable counts (TVCs) must be lower than 10^6 cfu g⁻¹, and mould and yeast counts (MYCs) less than 10^4 cfu g⁻¹ (Lewis, 1984; Ghana Standards, 1997). However, like most agricultural produce, they frequently

become contaminated with pathogens such as *Clostridium* spp., *Bacillus* spp., *Staphylococcus* spp., *Salmonella* spp., *Aspergillus* spp., and *Penicillium* spp. (Kneifel & Berger, 1994; Farag, Aziz & Attia, 1995; CAST, 1996).

Analysis of locally produced SHDS has shown high unacceptable microbial counts for un-irradiated products (TVC, $<1.7 \times 10^8$; MYC, $<1.5 \times 10^4$ cfu g⁻¹) and low acceptable microbial counts for irradiated products (TVC, $<2.9 \times 10^4$; MYC, $<5.0 \times 10$ cfu g⁻¹) (Adu-Gyamfi, 2000). *Bacillus cereus* and *Staphylococcus aureus* have been isolated from pepper sauce and khebab spices mix of some street foods (FAO/Ghana, 1997). They can, therefore, serve as possible vectors for various microorganisms which cause

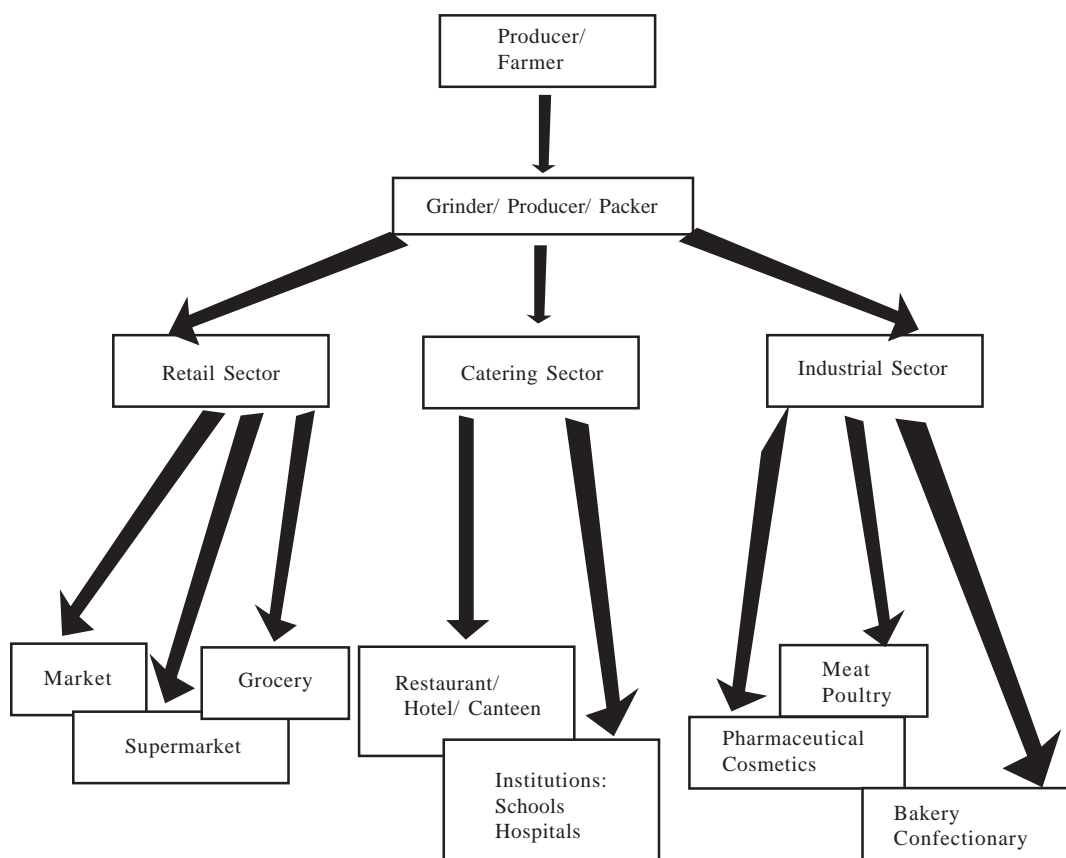


Fig. 1. Distribution channels for spices, herbs and dried seasonings.

quality and shelf-life problems in foods and subsequent health problems for consumers.

Treatments used by the food industry, over the years, to decontaminate SHDS include fumigation with ethylene or propylene oxide (Gephardt, 1990), ozone (Zagon *et al.*, 1992), saturated steam (Brulhart, Gysel & Emch, 1986), and gamma irradiation (Farkas, 1988; Sharma, Padwal-Desai & Nair, 1989). While losses of colour, flavour and aroma properties as well as chemical residues have rendered some of these procedures unacceptable, irradiation has been proved to be an effective decontaminating process that also preserves the sensory and organoleptic qualities of the products (Farkas, 1988; CAST, 1996; Ahmed, 1991; IAEA, 1992; ASTM, 1998). About 70,000 tonnes of these products were irradiated worldwide, and 60,000 kg irradiated locally at the Gamma Technology Centre (GTC) of Ghana Atomic Energy Commission (GAEC) in 1999 (Adu-Gyamfi, 2000).

Spices, herbs and dried seasonings are products marketed in the dried state and as such go through long storage or retail periods or both. Wide variations have been observed in product quality, which have been attributed to differences in initial bioburden and processing methods used by local producers. On analysing a total of 120 samples, wide variations of 4.0×10^2 - 1.7×10^8 and 0 - 1.5×10^4 cfu g⁻¹ were reported for total viable cells, and moulds and yeasts of unirradiated products, respectively (Adu-Gyamfi, 2000). Because effective control is lacking over product storage characteristics such as moisture content, packaging, temperature, and humidity in the local SHDS industry, it is essential that the microbial quality be monitored to detect any possible adverse changes. This could help in detecting deterioration in microbial quality during storage and in estimating shelf-life accurately in the local SHDS industry. Production of high quality SHDS would specifically improve catering services and, therefore, boost tourism in the country. It would also help local producers and exporters meet strict hygienic standards and quarantine requirements

and, therefore, improve the country's international trade in agricultural commodities.

The objective of this study was to assess the microbial quality of some locally processed spices and dried seasonings and how storage time affects the quality of these products.

Materials and methods

Experimental design

Five locally produced spices and dried seasonings (SDS) submitted for contract irradiation by producers at the GTC of GAEC were used in the study. The SDS were the dry powders of pepper, fish, shrimp, ginger and *dawadawa*. Ten grammes of each SDS were collected before and after irradiation and stored in sterile screw-capped universal bottles under ambient laboratory conditions. For each sample, microbial load and profile were initially determined and further determined after 6 and 12 months.

Irradiation treatment

Twenty grammes of each sample were packed in polythene bags and irradiated at a dose of 25 kGy from a ⁶⁰Co source at a dose rate of between 0.112 and 0.128 kGy h⁻¹ at the GTC.

Microbiological analysis

One gramme of each sample was added to 10-ml peptone water (1 % peptone water + 0.5 % NaCl) and placed on a mechanical shaker (Junior Orbit Shaker, Lab-Line Instruments, USA) for 15 min. The mixture was then left for about 5 min to allow the coarse material to settle down. Microbial load was determined on the supernatant, using standard decimal dilution and plate count methods (APHA, 1976):

1. Total viable cells were estimated on Plate Count Agar (Oxoid, UK) at 36 °C for 48 h.
2. Moulds and yeasts were estimated on Oxytetracycline (0.01 %) Glucose Yeasts Extract Agar (Merck, Germany) at 28 °C for 3 days.
3. Coliforms were estimated on Violet Red

Bile Agar (Merck, Germany) at 36 °C for 48 h.

Representative colonies from the plate counts were purified by sub-culturing and identified using morphological characteristics and biochemical tests (Gram stain, catalase test, oxidase test, motility test) with reference to *Bergey's Manual of Determinative Bacteriology* (Buchanan & Gibban, 1974), and general fungal flora according to the key of Gilman (Gilman, 1957).

Statistical analysis

Statistical Analysis of Variance with Fisher's pairwise comparison was applied to the data, using the Minitab computer software.

Results

Microbial counts

With the exception of unirradiated ginger powder that had lower counts, the TVCs of all the SDS were not significantly affected ($P < 0.05$) by the 6 and 12-month storage periods (Table 1). The range of the TVC for the irradiated SDS varied from 0.81-4.53 to 1.01-3.76 \log_{10} cfu g^{-1} , and that for the unirradiated SDS from 4.65-8.51 to 4.10-8.36 \log_{10} cfu g^{-1} after the 12-month storage period. No coliforms were detected in the samples.

Generally, the MYCs for the irradiated and unirradiated SDS were very low after the 12-month storage period (Table 2). With the exception of *dawadawa* powder, the counts of the irradiated SDS were not significantly affected ($P < 0.05$) by the storage periods. Also, with the exception of

TABLE 1

Effect of Storage Time on Total Viable Counts of Some Spices and Dried Seasonings

Storage time (months)	Microbial counts				
	Dawadawa	Ginger	Fish	Shrimp	Pepper
0	2.95 ^a (8.51 ^a)	4.53 ^a (8.04 ^a)	1.40 ^a (4.65 ^a)	2.36 ^a (4.68 ^a)	0.81 ^a (6.80 ^a)
6	1.31 ^a (8.47 ^a)	3.55 ^a (7.70 ^b)	0.77 ^a (3.65 ^a)	2.81 ^a (3.96 ^a)	1.24 ^a (7.41 ^a)
12	1.39 ^a (8.36 ^a)	3.76 ^a (7.14 ^c)	1.01 ^a (4.86 ^a)	1.52 ^a (4.10 ^a)	1.88 ^a (7.15 ^a)

Values for unirradiated spices and dried seasonings are in brackets
Counts are expressed as \log_{10} cfu g^{-1} . Each value is the mean of three replicates
Figures in a column bearing the same superscript are not significant at 5 %

TABLE 2

Effect of Storage Time on Mould and Yeast Counts of Some Spices and Dried Seasonings

Storage time (months)	Microbial counts				
	Dawadawa	Ginger	Fish	Shrimp	Pepper
0	0.87 ^a (2.14 ^a)	1.74 ^a (3.35 ^a)	0.33 ^a (2.55 ^a)	0.57 ^a (1.72 ^a)	0.00 ^a (1.55 ^a)
6	0.00 ^a (0.33 ^b)	0.67 ^a (1.00 ^b)	0.00 ^a (0.00 ^b)	0.49 ^a (0.67 ^a)	0.33 ^a (1.79 ^a)
12	0.00 ^b (0.00 ^b)	0.72 ^a (0.72 ^b)	0.00 ^a (0.00 ^b)	0.69 ^a (0.33 ^a)	0.43 ^a (0.00 ^b)

Values for unirradiated spices and dried seasonings are in brackets
Counts are expressed as \log_{10} cfu g^{-1} . Each value is the mean of three replicates
Figures in a column bearing the same superscript are not significant at 5 %

unirradiated shrimp powder, the MYC for all the unirradiated SDS were significantly reduced ($P < 0.05$) by the storage periods. Moulds and yeasts were not detected in the powders of *dawadawa*, fish and unirradiated pepper at the end of the 12-month storage period.

Microbial profile

Fig. 2 shows that pepper and *dawadawa* powders had a more diverse microbial profile compared to fish, ginger and shrimp powders for unirradiated powders. *Aspergillus* spp., and *Penicillium* spp. were the dominant moulds while *Bacillus* spp., *Lactobacillus* spp., and *Clostridium* spp. were the bacteria contaminating SDS (Table 3). Though some differences were observed in the microbial profile of the SDS after the 6 and 12-month storage periods, no pattern could be deduced. Compared to the unirradiated SDS, the irradiated samples had few types of contaminants. No coliforms were detected in the samples.

Discussion

One of the major constraints of the spice and herb industry is difficulty in attaining consistency in product quality due to crude methods of processing. The total number of microorganisms in commercial spices generally range from 10^5 to 10^8 cfu g^{-1} (i.e. 5.0 - 8.0 \log_{10} cfu g^{-1}) (Kiss, 1982; Grecz, 1986). Previous work on SDS at the GTC indicated a TVC range of 2.60 to 8.23 \log_{10} cfu g^{-1} and that of MYC as 0-4.17 \log_{10} cfu g^{-1} for unirradiated samples (Adu-Gyamfi, 2000). These compare well with the range of 4.65 to 8.51 \log_{10} cfu g^{-1} (TVC) and 1.55 to 3.35 \log_{10} cfu g^{-1} (MYC) recorded in this study. The consistently low levels of moulds have been stressed in other works that have indicated spices to be poor substrates for fungal growth and subsequent mycotoxin production (Madhyastha & Bhat, 1984).

The effectiveness of ionising radiation in reducing microbial load in SDS is well documented (Farkas, 1988; Sharma *et al.*, 1989). Irradiation doses of 10 to 20 kGy reduce TVC to acceptable national and international levels of $<10^4$ cfu g^{-1}

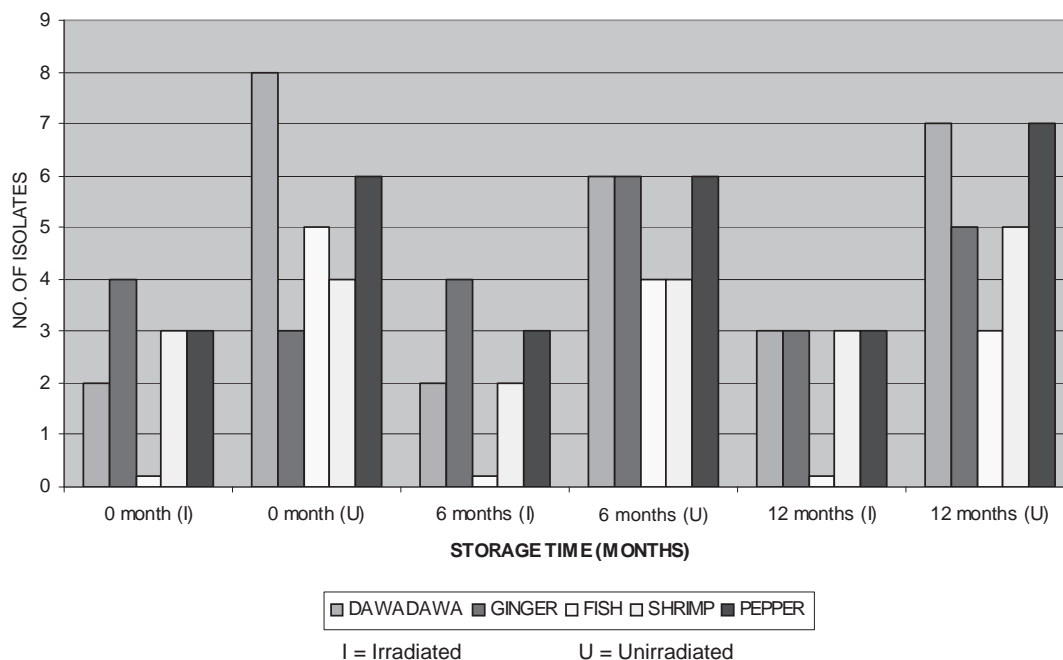


Fig. 2. Numbers of microbial isolates of species during storage.

TABLE 3

Microbial Profile of Some Spices and Dried Seasonings During Storage

Microorganism	Spices and dried seasonings				
	Dawadawa	Ginger	Fish	Shrimp	Pepper
0 month/Control					
<i>Aspergillus niger</i>	■ ◇	■ ◇		◇	■ ◇
<i>Aspergillus versicolor</i>		◇	◇		◇
<i>Aspergillus tamari</i>	◇			■	
<i>Aspergillus candidus</i>	◇				
<i>Penicillium oxalicum</i>			◇		■
<i>Penicillium cyclopium</i>	◇			◇	
<i>Rhizopus</i> spp.		■	◇		◇
<i>Cladosporium herbarium</i>	◇				
Yeasts	◇				
<i>Bacillus</i> spp.	■ ◇		◇	■ ◇	■ ◇
<i>Lactobacillus</i> spp.		■	◇		■
<i>Clostridium</i> spp.	◇	■ ◇		■ ◇	◇
6 months					
<i>Aspergillus niger</i>	◇	■ ◇		◇	◇
<i>Aspergillus versicolor</i>		◇	◇		
<i>Aspergillus wentii</i>	◇		◇		
<i>Penicillium cyclopium</i>	◇			◇	
<i>Rhizopus</i> spp.		■	◇		■ ◇
<i>Cladosporium herbarium</i>					◇
Yeasts	■ ◇				
<i>Bacillus</i> spp.	■ ◇			■ ◇	■ ◇
<i>Lactobacillus</i> spp.		■	◇		■ ◇
<i>Clostridium</i> spp.		■ ◇		■ ◇	◇
12 months					
<i>Aspergillus niger</i>	◇	■ ◇	◇		◇
<i>Aspergillus versicolor</i>				◇	
<i>Aspergillus tamari</i>	◇	◇			
<i>Aspergillus wentii</i>	◇		◇		◇
<i>Penicillium cyclopium</i>	◇			■ ◇	
<i>Geotrichum</i> spp.		◇			
<i>Rhizopus</i> spp.	◇		◇		■ ◇
<i>Cladosporium herbarium</i>					◇
Yeasts	■ ◇			◇	
<i>Bacillus</i> spp.	■ ◇	◇		■ ◇	■ ◇
<i>Lactobacillus</i> spp.		■			■ ◇
<i>Clostridium</i> spp.		■ ◇		■ ◇	

■ Present in irradiated sample
◇ Present in unirradiated sample

(i.e. $4.0 \log_{10}$). This treatment, although almost equal to other commercially established processes including fumigation, is superior because it leaves no residue in the products. This study has further confirmed irradiation as an effective process in reducing the microbial load of SDS to reasonably low levels of 0.81 to 4.53 \log_{10} cfu g^{-1} (TVC) and 0 - 1.74 \log_{10} cfu g^{-1} (MYC). The effect of irradiation is also showed by the relatively less diverse microbial profile of the irradiated SDS compared to the unirradiated SDS (Fig. 2 and Table 3).

The storage time of up to 12 months did not affect TVC in the SDS, except in ginger powder in which the TVC was reduced at the end of the storage period. The latter observation was also recorded in MYC in unirradiated *dawadawa*, ginger, fish and pepper powders which had reductions after the storage periods. Because these findings were observed in irradiated and unirradiated samples, they cannot be attributed to the process of irradiation. They could possibly be due to the interplay of factors such as the nature and moisture content of the products, storage temperatures, and humidity or sensitivity of detection methods.

In their natural state, spices and herbs contain many microorganisms that can spoil foods. However, some also contain specific anti-microbial components such as anethol, eugenol and thymol that can reduce potential contamination levels of foods (Aktug & Karapinar, 1986). The predominant bacteria found in spices and herbs are mainly the aerobic sporeformers such as *Bacillus cereus*, *B. polymyxa*, *B. macerans*, *B. megaterium*, and *B. sphaericus* as well as *Clostridium* spp., whilst the fungal microflora mainly consist of *Aspergillus* spp., *Penicillium* spp., and *Rhizopus* spp. (Antai, 1988; Julseth & Deibel, 1974; Schwab *et al.*, 1982).

This study has also confirmed *Bacillus* spp., *Clostridium* spp., *Lactobacillus* spp., *Aspergillus* spp., and *Penicillium* spp. as among the dominant microbes contaminating local SDS

(Table 3). Coliforms and yeasts are rarely detected in spices and herbs, whilst mould contamination is low (Kneifel & Berger, 1994). This fact has been substantiated by the results indicating absence of coliforms and negligible presence of moulds and yeasts. The absence of coliforms eliminates the threat of potential faecal contamination that could cause serious quality problems. However, unirradiated powders of *dawadawa*, pepper and ginger consistently had high TVC and relatively more diverse profile throughout the storage periods. The observations necessitate the use of decontamination methods such as irradiation to improve the quality of the SDS.

Although most contaminants persisted in the SDS during the storage period, the microbial profile and counts were not significantly affected after 12 months. This might be attributed to favourable interaction of storage factors such as the nature and age of the product, temperature, humidity and, most importantly, packaging. Effective packaging prevented re-contamination in this study. These findings are of practical value and could assist producers in long-term storage as well as in estimating expiry dates accurately, which is an important shelf-life parameter of SDS in Ghana.

Conclusion

Spices and dried seasonings harbour many bacteria and fungi including potentially harmful organisms. The level of contamination, which is dependent on their origin, processing and chemical nature, is high for *dawadawa*, ginger and pepper powders. Irradiation is an effective way of decontaminating these products and should, therefore, be integrated into local processing protocols. Although the microbial quality of these products is stable for storage periods up to 1 year, it is necessary to ensure suitable temperatures and humidity as well as effective packaging. Ensuring consistency of quality is vital in sustaining the spice industry, a potential agro-based sector that can boost Ghana's agricultural production and exports.

Acknowledgement

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