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Influence of Microbial Contamination on the Antioxidant Composition and Free Radical Scavenging Effects of Fresh and Decaying Spices

[1*]Azeez, L., [1]Adeoye, M. D., [2]Ganiyu, O. T., [1]Abdulsalami, I. O., [1]Majolagbe, T. A. and [1]Lawal, A. T.

[1]Industrial and Environmental Chemistry Unit, Department of Chemical Sciences, Fountain University, Osogbo

[2] Department of Biological Sciences, Fountain University, Osogbo

Abstract

This study examined the influence of microbial contamination on the antioxidant composition and free radical scavenging effects of fresh and decaying spices: *Lycopersicon esculentum*, *Capsicum frutescens S*, *Capsicum frutescens T* and *Capsicum annum* used by food vendors. Microbial load was enumerated with potato dextrose agar (PDA) using the pour plate technique, phytochemical components were determined using Folio-Ciocalteu, Aluminium chloride, acetone-hexane methods for phenolic, flavonoids and β-carotene & lycopene respectively while free radical scavenging capacity was determined using DPPH method. Decaying *L. esculentum* had highest microbial population of 7.14 X 10^5 cfu/g and fresh *C. annum* had the lowest microbial population of 0.72 X 10^1cfu/g. Microbial population in decaying spices were generally significantly higher than fresh spices. *Aspergillus spp* were found in all decaying spices and in fresh *C. frutescens T*. *Microsporum spp* were found in all fresh spices except in *C. frutescens T* and in decaying spices except in *C. annum*. There were significant decreases ($p < 0.05$) in phenolic, flavonoid, β-carotene and free radical scavenging capacity contents of all decaying spices compared to fresh spices except for phenolic content in *C. annum* while significant decrease ($p < 0.05$) in lycopene content was obtained for only decaying *L. esculentum* compared to fresh one. Reduction in antioxidant activity and phytochemical contents could not be unconnected with the high microbial population in decaying spices because antioxidant activity and phytochemical contents significantly correlate with antibacterial activity. Thus, consumption of decaying spices as shown by our study could pose a serious health challenges due to the presence of high number of microbes detected and loss of health-protecting ability of the spices.

Keywords: Microbiological quality; moisture content; decaying spices; *Aspergillus spp*; *Micosporum spp*

Introduction

Spices are important ingredients and flavouring substances in human foods. They have been reported to possess antioxidants, anti-inflammatory, antimicrobial, cholesterol-lowering and anti-diabetic properties and have been used for medicinal purposes (Banerjee and Sarkar 2003, Bokhari, 2007, Otunola *et al.*, 2010; Witkowska *et*

Corresponding author: Tel: +234-8061538999

Email: address: azeez012000@yahoo.com

al., 2011). Red pepper (*Capsium annum*) has been used in antioxidant nutrition therapy to treat vascular diseases (Ghasemnezhad *et al.,* 2011).

Consumption of tomato has been shown to have inverse relationship with development of various diseases such as cardiovascular disease and diabetics. Spices are sources of ascorbic acid, polyphenol, flavonoid and carotenoid (Ilahy *et al.,* 2011). Synergistic combinations of these compounds have been found to be responsible for antioxidant, anti-inflammatory and antidiabetic properties of these spices (Zhou and Yu, 2006, Tapas *et al.,* 2008; Sakarkar and Kakde, 2008).

Lycopene and β-carotene have ability to scavenge singlet oxygen and detoxify free radicals. Phenolic compounds have been found to have antioxidant properties, which offer protection against oxidation of lipids, DNA and other biomolecules (Singh *et al.,* 2009; Wang *et al.*, 2011). Flavonoids have anti-inflammatory, anti-diabetic and anticancer properties. Ascorbic acid has been identified to prevent tissue damage (Olajire and Azeez, 2011; Maizura *et al.*, 2011).

The hygiene of these spices is usually informed by their microbiological quality because studies have shown that some of these spices contain microflora, which are usually of soil origin due to their harvest handling and storage method. Moulds, yeast and bacteria have been identified in spices (Schweiggert *et al.,* 2007). Some of these pathogenic microorganisms may pose serious health effects because of their ability to produce aflatoxin which has been classified as potent carcinogen, mutagen and teratogen due to its significant threat and deleterious effects on humans and animals (Cho *et al.,* 2008; Witkowska *et al.,* 2011).

Microorganisms can survive long period of time in dried spices due to their high tolerance to desiccation stress and are often resistant to heat, thus spices could provide a way for these organisms to enter into food chain and cause health defects (Seowa *et al.,* 2012; Zweifel and Stephan, 2012). Commonly identified microorganisms are *Salmonella spp., Escherichia coli, Listeria monocytogenes* and the spore-forming, toxin-producers such as *Bacillus cereus and Clostridium perfringens*. Some of these have been recognized to possess potential pathogenicity and have been implicated in food poisoning. *Salmonella spp* have been implicated to cause salmonellosis and gastroenteritis, *Aspergillus spp* to produce aflatoxin, *Escherichia coli* to cause traveller's diarrhea and *Microsporum spp* to cause diarrhea, dermatitis, headache and sore throat due to the consumption of contaminated spices (Chagas *et al.,* 2002; Abou Donia, 2008; Seowa *et al.,* 2012).

Poverty levels among Nigerians with associated increase in the prices of food stuffs, ignorance about potential health risks of being exposed to pathogenic microorganisms and loss of nutritional and health benefits inherent in spices; have made the consumption of decaying spices otherwise called "ESA ATA" in Yoruba, inevitable. This had led to the preference of quantity decaying "ESA ATA" for soup especially by food vendors who in order to maximize their profits go for quantity rather than quality spices. This study examines the influence of microbial contamination on the antioxidant composition and free radical scavenging effects of fresh and decaying spices.

Materials and Methods

Sampling

A total of one hundred and sixty (160) spices comprising fresh and decaying spices Rodo (*Capsium annum*), Sombo (*Capsicum frutescens S*), Tatase (*Capsicum frutescens T*) and Tomato (*Lycopersicon esculentum*), were bought from Sabo market in Osogbo. Antioxidant activity, phytochemical contents and microbiological quality of these spices were analyzed. Fresh spices used in this study were devoid of cuts while decaying spices had whitish spread suspected to be Mucor with attendant flies and rottenness.

Chemicals

Quercetin, Folin-ciocalteu's phenol, DPPH (2, 2-diphenyl-1-picrylhydrazyl), were all purchased from Sigma-Aldrich, Germany. Sodium carbonate, Aluminum chloride and Methanol were purchased from BDH Poole, England. Pour plate agar and peptone water were purchased from Biolab, Hungary. All the chemicals used were of analytical

grade. Deionized-Distilled water was used throughout the experiment. Jenway 6405 UV-Visible Spectrophotometer by Buch Scientific Inc.USA was used for analysis.

Extraction

The samples were rinsed with distilled water to remove sand, cut into pieces and lyophilized to remove the moisture content. Resulting dried samples were powdered using Moulinex blender. These ground samples were extracted twice with a total volume of 100 mL of 70% aqueous methanol. The mixture was shaken on an orbital shaker for 75 min at 250rpm and then filtered through Whatman No. 1 filter paper. The combined methanolic extract was then evaporated at 55°C using water bath and dried to powder in a lyophilizer.

Microbiological quality

The method of Sobukola *et al.* (2009) was used. A representative 10 g of each (fresh or decaying) spice were mixed with sterile distilled water (1:10) to prepare the initial inoculum. This was followed by serial dilutions with 1 mL of the initial inoculum being added to 9 ml of sterile distilled water in test tubes up to 10^{-6} dilution. Ten mililitres (10 mL) of the initial dilution (10^{-1}) and through each of the dilutions up to 10^{-6} were added to potato dextrose agar (PDA) using the pour plate technique. The PDA agar plates were incubated at 25°C for 72 hours after which plates were read and total aerobic mesophilic microbes enumerated using digital colony counter (Lapiz, USA). The presence of fungal spores was confirmed visually by microscopic, macroscopic and morphological inspections. This was done in triplicate. All colonies that grew on PDA were expressed in cfu/g.

Determination of moisture content

The method of Boulekbache-Makhlouf *et al.* (2012) was used. 10 g of each sample (both fresh and decaying spices) were placed in an oven (Genlab, India) at 105°C for 3 h. The moisture content (MC) was calculated by expressing the weight loss upon drying as a fraction of the initial weight of sample used.

$$MC = \frac{W_O}{W_i} X\ 100 \qquad 1$$

Where W_0 = loss weight (g) and W_i = initial weight of sample (g).

Determination of phenolic, flavonoid, β-carotene and lycopene contents

Total phenol content in the sample was determined using Folin-Ciocalteu method of Olajire and Azeez (2011). 0.5 mL of the methanolic extract was added to 10 mL distilled water and 2.5 mL of 0.2 N Folin-Ciocalteu phenol reagent. The mixture was allowed to stand at room temperature for 5 min and then 2 mL of 2% of sodium carbonate was added. The resulting solution was measured at 780 nm. Quercetin was used as standard for the calibration curve.

The $AlCl_3$ method of Jagadish *et al.* (2009) was used for determination of the flavonoid content of the sample extract. 1.5 mL of extract was added to 1.5 mL of 2% methanolic $AlCl_3$ solution. The mixture was vigorously shaken on orbital shaker for 5 min at 200 rpm and the absorbance was read at 367 nm after 10 min of incubation. Reagent blank using distilled water instead of sample was prepared. Quercetin was used as standard for the calibration curve.

β- carotene and lycopene were determined according to the method of Nagata and Yamashita (1992). The dried methanolic extract (100 mg) was vigorously shaken with 10 mL of acetone – hexane mixture (4:6) for 1 min. The absorbance of the filtrate was measured at λ = 453, 505, 645 and 663 nm. Contents of β- carotene and lycopene were calculated according to the following equations: lycopene (mg/100 mL) = - $0.0458A_{663}$ + $0.372A_{505}$ + $0.0806A_{453}$: β- carotene (mg/100 mL) = $0.216A_{663}$ – $0.304A_{505}$ + $0.452A_{453}$. The values are expressed as μg/g of extract.

DPPH radical scavenging capacity assay

The method of Olajire and Azeez (2011) was used. 1 mL of methanolic solution of the extract (0.2-1.0 mg/mL) was added to 4 mL of 0.1mmolL^{-1} methanolic solution of DPPH. After 30min incubation in the dark at room temperature, the absorbance was read against a blank at 517nm.

Inhibition of free radicals by DPPH in percent ($I_{(\%)}$) was calculated using equation 2:

$$I_{(\%)} = \left[\frac{(A_{control} - A_{sample})}{A_{control}}\right] x\ 100 \qquad (2)$$

Where $A_{control}$ is the absorbance of the control reaction and A_{sample} is the absorbance of the test compound.

Statistical Analysis

All results are mean and standard deviation of three replicates. Student's t-test was used to calculate the significant difference between fresh and decaying spices. Linear regression analysis was used to calculate IC_{50}. Significant differences were tested at $p < 0.05$. SPSS 15 version was used for the statistical analysis.

Results

Microbiological quality

The microbial counts and microbes detected in the fresh and decaying spices are as shown in Table 1. Microbial count was highest in decaying *L. esculentum* with the highest microbial population of 7.14 X 10^5 cfu/g and *C. frutescens S* has the lowest microbial population of 5.78 X 10^3 cfu/g. In fresh spices, *C. annum* had the lowest microbial population of 7.2cfu/g and *L. esculentum* had the highest microbial population of 20.5cfu/g. There were significant increases in microbial population in decaying spices compared with fresh spices. "*Aspergillus spp*" were found in all decaying spices and in fresh *C. frutescens T*. "*Micosporum spp*" were found in all fresh spices except *C. frutescens T* and in decaying spices except in *C. annum* (Table 2).

Moisture content

Moisture content of spices studied is presented in figure 1. Fresh *L. esculentum* (43.72%) had the highest moisture content followed by *C. frutescens T* (25.75%), *C. frutescens S* (18.62%) and *Capsicum annum* (8.7%). An increase in the moisture content was obtained for decaying *Capsicum annum* (12.9%) and *C. frutescens S* (21.42%) compared to their fresh ones while decrease in moisture level was obtained for *L. esculentum* (35.02%) and *C. frutescens T* (23.89). There were significant ($p<0.05$) decrease and increase in moisture content of decaying *L. esculentum* and *Capsicum annum* respectively compared to their fresh ones.

Table 1: Microbial profile of fresh and partially decaying spices studied.

Spice	Microbial count (cfu/g)
L. esclentum	
Fresh	2.05 ± 0.56 X 10^1
Decaying	7.14 ± 1.01 X 10^5 [(a)]
C. annum	
Fresh	0.72 ± 0.04 X 10^1
Decaying	2.18 ± 0.17 X 10^4 [(a)]
C. frutescens T	
Fresh	1.02 ± 0.08 X 10^1
Decaying	4.41 ± 0.14 X 10^4 [(a)]
C. frutescens S	
Fresh	1.24 ± 0.36 X 10^1
Decaying	5.78 ± 0.81 X 10^3 [(a)]

Mean ± standard deviation of three replicates, [(a)] significantly different from corresponding healthy spice

Table 2: Microbes detected in fresh and decaying spices

Spice	*Microsporum spp*	*Aspergillus spp*
L. esclentum		
Fresh	+	-
Decaying	+	+
C. annum		
Fresh	+	-
Decaying	-	+
C. frutescens T		
Fresh	-	+
Decaying	+	+
C. frutescens S		
Fresh	+	-
Decaying	+	+

+ Present, - Absent

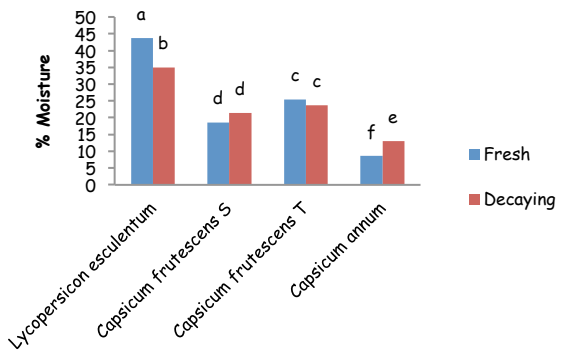

Figure 1: Moisture contents of fresh and decaying spices studied. Bars with different

Antioxidant contents

Phenolic contents of the spices studied as presented in figure 2 followed the order *C. frutescens S* (327.15 ± 24.52 mg quercetin/g of extract) > *L. esculentum* (317.24 ± 31.19 mg quercetin/g of extract) > *C. frutescens T* (267.84 ± 13.78 mg quercetin/g of extract) > *Capsicum annum* (194.13 ± 21.64 mg quercetin/g of extract) for fresh spices. Total phenolic contents of decaying spices were in the order *C. frutescens S* (208.21± 11.71 mg quercetin/g of extract) > *L. esculentum* (196.78 ± 18.57 mg quercetin/g of extract) > *C. frutescens T* (121.04 ± 15.48 mg quercetin/g of extract) > *C. annum* (101.42 ± 12.93 mg quercetin/g of extract). There were 37.97%, 47.76%, 54.81%, 36.36% reductions in phenolic conents of decaying compared to fresh spices. Significant decreases ($p < 0.05$) were obtained for decaying *L. esculentum*, *C. frutescens T* and *C. frutescens S* compared to fresh spices. No significant reduction ($p < 0.05$) was obtained for partially rotten *C. annum* compared to healthy one.

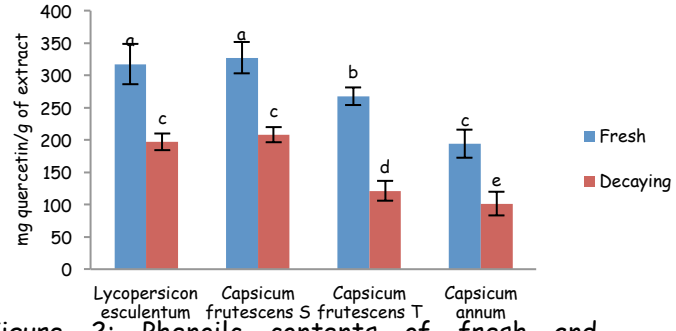

Figure 2: Phenoilc contents of fresh and decaying spices studied.

Flavonoid contents of both fresh and decaying spices are shown in figure 3. Fresh *C. frutescens S* had the highest flavonoid content with 334.14± 45.86 mg quercetin/g of extract followed by *C. frutescens T* with 295.56 ± 24.93 mg quercetin/g of extract, *L. esculentum* with 268.33 ± 31.05 mg quercetin/g of extract and *C. annum* 168.89 ± 27.73 mg quercetin/g of extract. Flavonoid contents of decaying spices range from *C. frutescens T*: 186.17 ± 10.42 mg quercetin/g of extract, *C. frutescens S*: 143.45 ± 9.07 mg quercetin/g of extract, *L. esculentum*: 113.33 ± 14.57 mg quercetin/g of extract and *C. annum*: 77.19 ± 3.92 mg quercetin/g of extract. Highest reductions were obtained for *L. esculentum* with 67.68% followed by *C. frutescens S* with 56.15%, *Capsicum annum* with 53.88% and *C. frutescens T* with 37.01%. Significant decreases ($p < 0.05$) were obtained for decaying *L. esculentum*, *C. annum*, *C. frutescens T* and *C. frutescens S* compared to fresh ones.

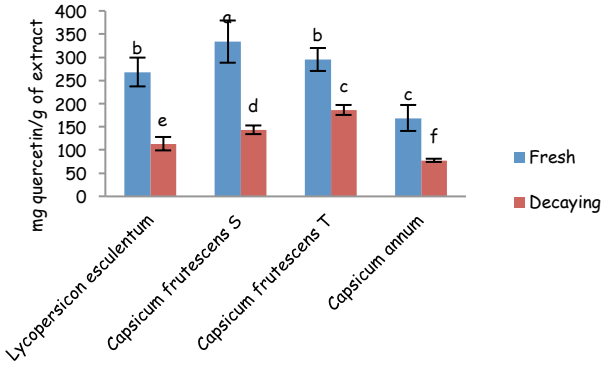

Figure 3: Flavonoid contents of fresh and decaying spices studied.

The contents of lycopene in spices studied are shown in figure 4. Fresh *L. esculentum* had highest lycopene content with 43.72± 1.87 µg/g of extract followed by *C. annum* with 34.19± 3.11 µg/g of extract, *C. frutescens S* with 24.65 ±1.48 µg/g of extract and *C. frutescens T* with 22.51±1.05 µg/g of extract. Decaying *C. annum* contained highest lycopene content with 32.78±2.49 µg/g of extract followed by *C. frutescens S* with 17.62±1.84 µg/g of extract, *C. frutescens T* with 13.50± 0.96µg/g of extract and *L. esculentum* with 14.01±2.27 µg/g of extract. *C. annum* had the lowest reduction

(4.12%) followed by *C. frutescens S* with 28.61, *C. frutescens T* with 40.01% and *L. esculentum* with 67.96%. There was significant reduction ($p < 0.05$) in lycopene content of decaying *L. esculentum* compared to fresh one. No significant decrease was obtained for lycopene contents in decaying *C. annum*, *C. frutescens T* and *C. frutescens S* compared with fresh ones.

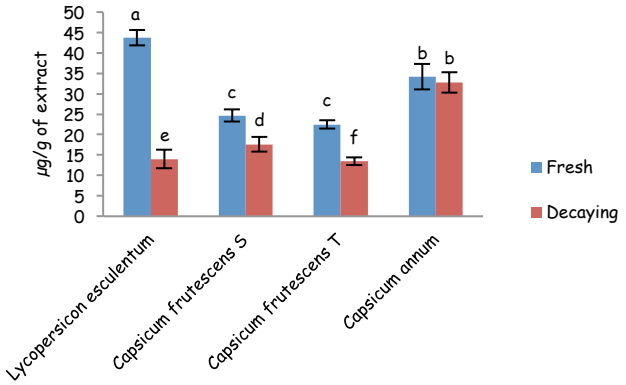

Figure 4: Lycopene contents of fresh and decaying spices studied.

β-carotene contents of fresh and decaying spices studied are presented in figure 5. They range for fresh ones in the order of *C. frutescens T* (53.92± 7.68 µg/g of extract) > *L. esculentum* (52.41± 4.06 µg/g of extract) > *C. frutescens S* (47.13± 9.62 µg/g of extract) > *C. annum* (35.61±6.54 µg/g of extract). Decaying *C. frutescens S* had the highest with 16.52± 3.87 µg/g of extract followed by *C. annum* with 15.04± 2.59 µg/g of extract, *C. frutescens T* with 11.42± 0.63µg/g of extract and *L. esculentum* with 7.61± 0.08µg/g of extract. Percentage reductions were highest in *L. esculentum* with 85.5% followed by *C. frutescens T* with 78.81%, *C. frutescens S* with 64.95% and *C. annum* with 56.71%. There were significant reductions ($p < 0.05$) in the β-carotene of all decaying spices studied compared with fresh ones.

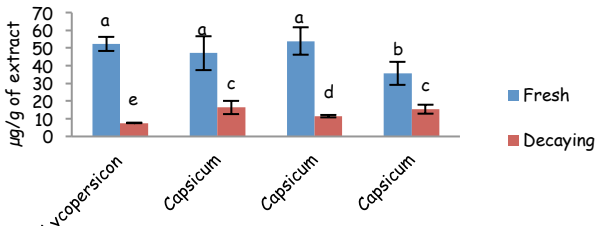

Figure 5: β-carotene contents of fresh and decaying spices studied.

Free radical scavenging activity

Free radical scavenging activity of both fresh and decaying spices determined by DPPH method as presented in figure 6 show that *C. frutescens S* had the highest free radical scavenging activity (72.14 ± 5.15 %) followed by *C. frutescens T* (67.22 ± 4.86%), *C. annum* (62.57 ± 1.32%) and *L. esculentum* (54.46 ± 6.52%) for fresh spices. Free radical scavenging activity in decaying spices were highest in *C. frutescens T* (49.86 ± 0.57%) followed by *C. annum* (26.86 ± 4.19%), *C. frutescens S* (21.76 ± 2.45%) and *L. esculentum* (19.89 ± 1.48%). There were 63.48%, 57.07%, 25.83% and 69.84% reductions in the free radical scavenging activity of decaying spices compared with fresh spices in *L. esculentum*, *C. annum*, *C. frutescens T* and *C. frutescens S* respectively. These reductions were significant ($p < 0.05$) in decaying *L. esculentum*, *C. annum* and *C. frutescens S* compared to fresh spices. No significant change ($p > 0.05$) was obtained for decaying *C. frutescens T* compared to fresh one. IC_{50} (the inhibitory concentration at which 50% of free radicals are scavenged) ranged from 0.32 mg/mL for *C. frutescens S* followed by 0.67 mg/mL for *C. frutescens T*, 0.74 mg/mL for *C. annum* and 0.83 mg/mL for *L. esculentum* for fresh spices. In decaying spices, IC_{50} ranged from 1.89 mg/mL for *C. frutescens T*, followed by 3.16 mg/mL for *C. annum*, 3.54 mg/mL for *C. frutescens S* and 4.02 mg/mL for *L. esculentum*.

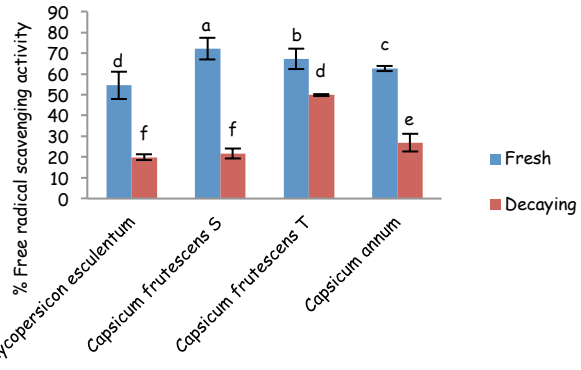

Figure 6: Free radical scavenging activity of fresh and decaying spices

Discussion

Microbiology quality

The hygiene of any spice is usually informed by their microbiological quality because studies have shown that some of these spices contain microflora, which are usually of soil origin due to their harvest handling and storage method. Studies have shown that *L. esculentum* and *Capsicum annum* are often contaminated with bacteria (Schweiggert *et al.*, 2007; Seow *et al.,* 2012; Zweifel and Stephen, 2012). Highest microbial population identified in *L. esculentum* could be due to high moisture content contained in it (Mandheel, 2005; Boulekbache-Makhlouf *et al.,* 2012). "*Aspergillus spp"* has been enumerated in many spices and implicated to produce aflatoxin. Aflatoxin is the toxic metabolite produced by "*Aspergillus spp"* and has been classified as group 1 carcinogen by International Agency of Research on Cancer (IARC) (Cho *et al.,* 2008). It usually affects spices with high moisture value. Lack of proper storage facility, improper handling after harvest and high moisture contents contained in *L. esculentum*, *C. frutescens T* and *C. frutescens S* might have made them more susceptible to aflatoxin-forming "*Aspergillus spp"*. The presence of "*Aspergillus spp"* could be the cause of decrease in the antioxidant activity and phytochemical contents as it has been previously studied to seriously reduce the quality and quantity of food (Shan *et al.,* 2007; Oyedemi and Afolayan, 2011; Bhatt and Negi, 2012; Boulekbache-Makhlouf *et al.,* 2012). "*Microsporum spp"* has been implicated to cause dermatitis, diarrhea, headache, tinea infection, pathogenic disorders and hemorrhage (Bajpai *et al.,* 2009). The presence of "*Microsporum spp"* in these spices might have affected the antioxidant activity and phytochemical contents because part would have been used for the fight against the toxic metabolites of "*Microsporum spp"* (Aboul-Enein *et al.,* 2003).

Moisture content

High moisture contents in *L. esculentum* and *C. frutescens T* could have resulted in their decay within short time of storage because high moisture content hastens food spoilage and the growth of molds and bacteria (Mandheel, 2005, Boulekbache-Makhlouf *et al.,* 2012).

Antioxidant contents

Phytochemicals are strong antioxidants that can modify metabolic activity, aid in the detoxification of carcinogens, and even influence processes in a tumor cell. Phenolic compounds are secondary metabolites in fruits and vegetables. They have been reported to exhibit antioxidant activity which allows them to scavenge both active oxygen species and electrophiles, to inhibit and chelate metal ions, to have the potential for autoxidation and the capability to modulate certain cellular enzyme activities (Huda-Faujan *et al.,* 2009; Ziecha *et al.,* 2010; EL-Gendy *et al.,* 2010). Consumption of flavonoids has been reported to reduce the incidence of cancer and studies have shown that flavonoid possess antioxidant, antimalaria, antibacterial, antidiabetic and antifungi activities (Tapas *et al.,* 2008).

Our results show that these spices are good sources of phenolics and flavonoids and the values are in agreement with what were measured by (Marinova *et al.,* 2005; Olajire and Azeez, 2011). High contents of flavonoids show that these fresh spices possess antimicrobial and antibacterial activities (Machado *et al.,* 2002). Researchers have shown that polyphenols could inhibit food pathogens, mutagenesis and carcinigenesis (Ao *et al.,* 2008; Kim *et al.,* 2009; Alsaid *et al.,* 2010). It has also been reported that total phenolic contents significantly contributed to antibacterial activity, which prevents the growth of bacateria (Shan *et al.,* 2007; Oyedemi and Afolayan, 2011; Bhatt and Negi, 2012). Boulekbache-Makhlouf *et al.,* (2012) suggested that polyphenols were responsible for antibacterial activity of *E. globulus* fruits therefore, reduction in the levels of both phenolic and flavonoid contents could have resulted from parts of them being used to inhibit the further growth of microbial population. Irradiation to kill microorganisms has been shown to lead to increased phenolic and flavonoid contents (Chianga *et al.*, 2011).

Lycopene and β-carotene act as powerful

antioxidants in humans. A diet containing moderate amounts of both has been moderate amounts of both has been associated with the prevention of cardiovascular disease and cancers of the prostate and gastrointestinal tract (Abdul-Hammed *et al.,* 2009). Carotenoids have been reported to contain antibacterial activity. Decreases in the contents of lycopene and β-carotene could be due to the contamination by microorganisms, which reduces the antibacterial activity of these spices and subsequently the carotenoid contents. β-carotene has been investigated to enhance antimicrobial activity of carrots (Hayashi *et al.,* 2012). Microbial population in *L. esculentum* and *C. frutescens T* might be responsible for higher decrease in the contents of lycopene and β-carotene as it has been previously reported that growth of microorganisms negatively associated with enhancement of carotenoids (Aboul-Enein *et al.*, 2003).

Free radical scavenging activity

Free radical scavenging activity is used to determine the health-supporting functions of food samples. The higher the antioxidant activity, the better the health benefits of such food (Ghasemnezhad *et al.,* 2011). Antioxidants protect the body from deleterious effects of free radicals by scavenging and inactivating them. They also prevent decomposition of hydroperoxides into free radicals (Huda-Faujan *et al.,* 2009, Ziecha *et al.,* 2010, EL-Gendy *et al.,* 2010). Our results show that healthy spices are good sources of antioxidants and in agreement with values reported in literature (Marinova *et al.,* 2005, Olajire and Azeez, 2011). They also show that *C. frutescens S* could scavenge free radicals the most and *L. esculentum* could scavenge the least as shown by their IC_{50}. The reason for reduction could be that some of antioxidants have been used to scavenge free radicals from toxic metabolites due to rottenness caused by microbial contamination. Free radical scavenging activity has been reported to correlate significantly with antibacterial activity, thus decrease in antibacterial activity would lead to decrease in antioxidant activity and eventually increase in microbial population, which was what was obtained for decaying spices (Shan *et al.,* 2007; Boulekbache-Makhlouf *et al.,* 2012). It has equally been reported that irradiation reduces microbial contamination and population and increases antioxidant activity (Chianga *et al.,* 2011, Musa, Ahmed *et al.,* 2011). Therefore, it could be said that microbial contamination has greatly depleted health-promoting ability of *L. esculentum*, *C. frutescens T* and *C. frutescens S,* which had more than 50% reduction in their antioxidant activity.

Conclusion

Lack of proper storage facility, improper handling, poor economic cum transportation system have had untold adverse effects on the quantity of perishable farm produce. It is obvious from this study that consumers of the decaying spices are liable to contact varying degrees of diseases. Majority of the victims are not only poor but also illiterates. However, adequate awareness should be made available to them on the health implication associated with decaying spices.

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