# Evaluating the Effects of Gamma Irradiation and Storage Period on the Phytochemical Stability of Some Selected Spices on the Ghanaian Market

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***Research Article***

***Abstract:*** The aim of this study was to assess the effect of gamma irradiation treatment and storage period on the phytochemical stability of some spices (*Eugenia caryophyllata*, *Xylopia aethiopica* and *Aframomum melegueta*) on the Ghanaian market. The radiation processing was carried out on dose levels of 0, 5, 10 and 20kGy over a period of four months. The irradiated and control samples were analyzed for Total flavonoids, Total Phenolics, Vitamin C and DPPH radical-scavenging activity for both aqueous and ethanolic extracts with the exception of Vitamin C. The study revealed that gamma irradiation did not compromise on the innate phytochemical stability of the spice samples; however storage period showed significant effects on the phytochemical indices of the spice samples.

***Keywords:*** Irradiation, doses, Storage, Phenolic, Flavanoids, Antioxidants, Vitamin C.

## Introduction

Spices are used as ingredients in food, alcoholic beverages, medicine, perfumery, cosmetics, and colouring. Spices and herbs are used in foods to impart flavour, pungency and colour. They also have antioxidant, antimicrobial, pharmaceutical and nutritional properties. In addition to the known direct effects, the use of these plants can also lead to complex secondary effects such as salt and sugar reduction, improvement of texture and prevention of food spoilage (Pamplona-Roger, 2004). The basic effects of spices when used in cooking and confectionery can be for flavouring, pungency and colouring. They are also used to make food and confectionery more appetizing and palatable. Some spices, such as turmeric and paprika, are used more for imparting an attractive colour than for enhancing taste. Because of their antioxidant and antimicrobial properties, spices have dual function, in addition to imparting flavour and taste; they play a major role in food preservation by delaying the spoilage of food. Many herbs and spices have been used in cosmetics, perfumery and beauty and body care since antiquity (Farrell, 1990). Not only are spices used as food flavourings and seasonings to improve the flavour, but they may also be used as traditional medicines (Srinivasan, 2005 and Gao *et al*., 2000). Many spices have been recognized to have medicinal properties and possess many beneficial effects on health, such as antioxidant activity, digestive stimulant action, anti-inflammatory, antimicrobial, hypolipidemic, antimutagenic, anticarcinogenic potential, etc. Antioxidants are also known to play an important role in stabilizing lipid peroxidation and to inhibit various types of oxidizing enzymes (Laughton et al., 1991 and Cos *et al*., 1998) In Ghana, spices play a key role in the preparation of local dishes due to their benefits such as the ability to enhance food flavour when added in small amounts. Studies have shown that the major problems associated with spice production are high microbial contamination. Microbial contaminations are generally considered a potential hazard to public health due to their toxicity, mutagenicity, teratogenicity and carcinogenicity (Richard, 2000). Spices are the primary sources of alimentary intoxication when added to foods in which pathogenic growth is favourable. Spices are cultivated in various areas of the world, mainly in developing countries where food sanitation is often poor as compared to the situation in western countries. The production environment in the cultivation areas cause storage problems which lead to an increased number of food-borne infections and intoxications. Irradiation doses of 3-25 kGy have been shown to be reliable for improving microbial safety and the decontamination of dried aromatic herbs, spices and vegetable seasonings with a maximum overall average absorbed dose of 10 kGy (Buckenhuskes & Rendlen, 2004) However, conflicting reports have emerged about the effects of irradiation on the stability of some phytochemical parameters. This study therefore seeks to ascertain the effects of irradiation on some phytochemical parameters of spices in order not to compromise on the innate nutritional qualities of the spices. The results gathered from this work would also bridge the knowledge gap that presently exists concerning

 

the effect of irradiation on the phytochemical constituents of spices on the Ghanaian market.

**Objectives**

- To examine the effects of the irradiation doses on some selected phytochemical constituents of the spice samples.
- To evaluate the effect of storage on total flavonoids, total Phenolics, Vitamin C contents and DPPH radical-scavenging activity of the spice samples.

## Methodology

### Sampling

Samples of spices were obtained from some major retail markets in the Accra metropolis and blended to obtain a homogenous unit.

## Methods

### Irradiation process

The packaged spices samples of 0.5g each (*Eugenia caryophyllata*, *Aframomum melegueta* and *Xylopia aethiopica*) were treated with different doses (0, 5, 10, 20kGy) using a cobalt-60 Gamma source at the Radiation Technology Centre (R.T.C) of the Ghana Atomic Energy Commission (G.A.E.C). The absorbed dose was determined by using Lithium fluoride photo-fluorescent film dosimeter (SUNNA Dosimeter System, UK) and the dose rate was 2.52kGy and the average dose distribution ratio was 1.03.

### Total Flavonoids Analysis

Aluminium chloride colorimetric method was utilised for determination of flavonoids (Zhishen *et al.,* 1999). 0.05ml of extract was mixed with 1.5ml of 99.9% ethanol (EtOH), 0.01ml of 1 M potassium acetate, 0.01ml of 10% aluminium chloride and 3.0ml of distilled water. The resulting mixtures were incubated for 30 minutes at room temperature and corresponding absorbance measured at 415 nm. All determinations were carried out in triplicates. A standard calibration curve was constructed using quercetin standard solutions 0.0025mg/ml, 0.005mg/ml, 0.0075mg/ml, 0.001mg/ml and 0.0125mg/ml. 0.005ml of each standard was treated in the same manner as the samples above and a calibration linear regression equation of y = 0.9056x was obtained, (where x = mg per Quercetin), R2 = 0.989, where R is the coefficient of the regression line. Total flavonoid content (TFC) was expressed as mg of quercetin equivalents (QE) /g of extract according to the formula by Chang *et al*. (2002).

### *Determination of Total Phenolic Content*

Total phenolic content (TPC) of the three spice samples was determined according to the Folin-Ciocalteau method (Kujala *et al*., 2000; Singleton *et al.,* 1999), using gallic acid as a standard. Fifty milligrams (50 mg) of the samples were dissolved in 5 ml of distilled water (50:50, v/v). 0.005ml of the extract was mixed with 3.0ml of distilled water and 0.025ml of Folin-Ciocalteau reagents (FCR). The mixtures were allowed to stand for 5 minutes, and then 0.075ml of 20% $Na_2CO_3$ was added. After incubating the resultant reaction mixtures for 30 minutes at room temperature, absorbance values were measured spectrophotometrically at 760nm using a U-VIS Spectrophotometer (Shimadzu Corporation, 1201, Kyoto, Japan).The same procedure was followed for ethanolic extracts with ethanol as the solvent. All determinations were carried out in triplicate. A calibration curve was derived using the following dilution regimes; 0.025 mg/ml, 0.05 mg/ml, 0.1 mg/ml, 0.15 mg/ml and 0.2 mg/ml from a stock solution of 10 mg/ml Gallic acid dissolved in water. 5.0ml each of these solutions were treated in same manner as the samples and a calibration linear regression equation established as y = 0.9056x+0.0012, (where x is µg/g GAE), R2 = 0.991, where R is the coefficient of the regression line, to explain the model. The total phenolic content in each extract was expressed as Garlic Acid Equivalent (GAE) in mg per gram sample using the formula below:

$$\text{Total Phenolic Content} = \frac{c \times v}{M}$$

Where $c$ = the concentration of gallic acid established from the calibration curve in mg/g;
$v$ = the volume of the extract in micro litres;
$M$ = the weight of the sample taken.

### *Determination of total antioxidant activity*

Total antioxidant activity (TAA) in spice extracts was determined using the 1, 1-diphenyl 1-2 picrylhydrazyl (DPPH) method by Blois, (1958) and Botchway *et al.* (2007) with slight modification. A solution of 0.004%µM DPPH was prepared through dissolution of 0.004g of DPPH in 100ml methanol. 200µl of the extract was added to 3.8ml of 0.004% DPPH. Concentrations of 0.2, 0.1, 0.05, 0.025, 0.020 and 0.01 mg/ml of Gallic Acid were used to plot the standard curve. The reduction or inhibition ability of DPPH radicals was determined by the decrease in its absorbance at 517nm induced by antioxidants after thirty (30) minutes incubation in the dark. Ethanol was employed as a blank and absorbance read three times for each sample. The activity of the test samples was determined as a reduction of the DPPH, which is also referred to as inhibition or quenching and defined mathematically by Hatano *et al*. (1988) as**:** % Inhibition = $(c - s) \times 100$

Where $s$ is the sample absorbance and $c$ is the absorbance of the blank. Scatter diagrams were plotted and linear regression computed as $y = ax+b$, where $y$ is the percent inhibition and $x$ is the concentration in mg/ml.

### *Vitamin C Content*

Ascorbic Acid (Vitamin C) was determined by the Redox titration using Iodine solution. Vitamin C standard solution, iodine solution and 1% starch indicators were

prepared in accordance with AOAC, 2000. 10 drops of 1% starch indicator was added to 25ml of vitamin c standard solution in a 125ml Erlenmeyer flask. Burette was filled with 0.005mol/L iodine solution and initial volume recorded. The solution is titrated until the endpoint is reached and the final volume of iodine is recorded. Final vitamin C content was calculated from the relation: [0.005x176.12x Average Titre] mg/100

***Statistical Analysis***

Data obtained were analysed using statgraphics software version (XVI) and Microsoft excel and the mean separation were done using the Least Significant Difference (LSD) test at 95% confidence level ($p \leq 0.05$).

## Results

**Table 1:** Effect of irradiation dose on the antioxidant indices on some selected spices within the Accra Metropolis

| Sample | DOSE (kGy) | Phenolics | | Flavanoids | | % Scavenging | | Vitamin C |
|---|---|---|---|---|---|---|---|---|
| | | Aqueous | Ethanol | Aqueous | Ethanol | Aqueous | Ethanol | |
| *Eugenia Caryophyllata* | 0 | 134.25±4.19a | 78.79±1.16b | 3.50±0.01a | 3.17±0.01ab | 90.21±1.09a | 61.38±2.62a | 1.56±0.03b |
| | 5 | 129.01±4.19a | 73.22±1.16a | 3.47±0.01a | 3.15±0.01a | 92.29±1.09a | 68.26±2.62a | 1.50±0.03ab |
| | 10 | 124.94±4.19a | 76.44±1.16ab | 3.50±0.01a | 3.18±0.01b | 92.82±1.09a | 62.14±2.62a | 1.43±0.03a |
| | 20 | 125.25±4.19a | 76.63±1.16ab | 3.49±0.01a | 3.26±0.01c | 92.56±1.09a | 61.11±2.62a | 1.54±0.03b |
| *Xylopia Aethiopica* | 0 | 15.87±0.82b | 20.58±1.34c | 2.97±0.004a | 3.19±0.01b | 23.21±0.47b | 23.31±0.74c | 0.47±0.01c |
| | 5 | 11.81±0.82a | 14.82±1.34b | 2.97±0.004a | 3.15±0.01a | 19.31±0.47a | 19.05±0.74b | 0.41±0.01b |
| | 10 | 11.86±0.82a | 7.84±1.34a | 2.97±0.004a | 3.15±0.01a | 17.89±0.47a | 14.66±0.74a | 0.42±0.01b |
| | 20 | 14.73±0.82b | 14.88±1.34b | 2.96±0.004a | 3.13±0.01a | 23.18±0.47b | 21.84±0.74c | 0.38±0.01a |
| *Aframomum Melegueta* | 0 | 25.30±0.46b | 23.61±0.48a | 2.96±0.004c | 2.94±0.004a | 37.69±0.60a | 35.43±1.01a | 0.92±0.01c |
| | 5 | 22.43±0.46a | 26.52±0.48b | 2.95±0.004b | 2.95±0.004a | 38.43±0.60a | 36.33±1.01a | 0.90±0.01bc |
| | 10 | 21.98±0.46a | 26.62±0.48b | 2.93±0.004a | 2.95±0.004a | 37.96±0.60a | 35.79±1.01a | 0.87±0.01b |
| | 20 | 24.79±0.46b | 24.80±0.48a | 2.95±0.004bc | 2.94±0.004a | 37.07±0.60a | 35.51±1.01a | 0.79±0.01a |

[a-c] means ± pooled standard error; values with different letters within a column for each spice are significantly different ($p \leq 0.05$).

**Table 2:** Effect of storage period on the antioxidant indices on some selected spices within the Accra Metropolis

| Sample | Storage (Months) | Phenolics | | Flavanoids | | % Scavenging | | Vitamin C |
|---|---|---|---|---|---|---|---|---|
| | | Aqueous | Ethanol | Aqueous | Ethanol | Aqueous | Ethanol | |
| *Eugenia caryophyllata* | 1 | 154.95±2.96y | 100.51±0.82y | 2.63±0.01x | 2.08±0.01x | 89.63±0.77x | 71.32±1.85y | 1.80±0.02y |
| | 4 | 101.78±2.96x | 52.03±0.82x | 4.36±0.01y | 4.30±0.01y | 94.31±0.77y | 55.13±1.85x | 1.21±0.02x |
| *Xylopia aethiopica* | 1 | 16.46±0.58y | 16.81±0.95y | 1.99±0.003x | 2.23±0.01x | 13.97±0.33x | 15.01±0.52x | 0.65±0.01y |
| | 4 | 10.67±0.58x | 12.25±0.95x | 3.94±0.003y | 4.08±0.01y | 27.82±0.33y | 24.42±0.52y | 0.19±0.01x |
| *Aframommum melegueta* | 1 | 29.67±0.32y | 27.79±0.34y | 1.97±0.003x | 1.92±0.003x | 32.94±0.42x | 27.97±0.71x | 1.42±0.01y |
| | 4 | 17.58±0.32x | 22.98±0.34x | 3.92±0.003y | 3.96±0.003y | 42.63±042y | 43.56±0.71y | 0.32±0.01x |

[x-y] means ± pooled standard error; values with different letters within a column for each spice are significantly different ($p \leq 0.05$).

## Discussion

### Effect of Irradiation on Antioxidant Indices

***Total Flavonoids Content***

Table 1.0 shows the effect of irradiation dose on the total flavonoids contents (TFCs) of the three local spices used in the study. The highest TFC (3.26±0.01 mg/g/QE), was recorded by *Eugenia caryophyllata* while *Aframomum melegueta* registered the lowest TFC of 2.94±0.004 mg/g/QE in the ethanolic extract. The same trend was observed in the aqueous extract, *Eugenia caryophyllata* recorded the highest TFC (3.50±0.01 mg/g/QE), with *Aframomum melegueta* recording the least (2.93±0.004 mg/g/QE). TFCs in the aqueous extracts of *Eugenia caryophyllata* were higher than in the ethanolic extracts, indicating that the aqueous extraction method was more efficient than the ethanolic extraction method. This also indicates that, flavonoids in *Eugenia caryophyllata* are more soluble in water than organic solvents such as ethanol. The current findings indicate significant differences in concentrations of flavonoids in ethanolic extracts of *Eugenia caryophyllata* between doses of 5-20 kGy. The study also showed significant differences of TFC in *Xylopia aethiopica* between irradiation doses of 0-5 kGy while there were significant differences in TFC for the selected doses of 0-20 kGy for *Aframomum melegueta*. This indicates that, flavonoids in *Aframomum melegueta* are hydrophilic with varying solubilities at different doses in water.

***Total Phenolic Content***

The total phenolic contents (TPCs) of the three (3) local spices are as shown in Table 1. In

the aqueous extracts, the highest TPC was registered by *Eugenia caryophyllata* (134.25±4.19 mg/g/GAE) while *Xylopia aethiopica* had the lowest TPC of 11.81±0.82a mg/g/GAE. Similarly, *Eugenia caryophyllata* at a dose of 0kGy (78.79±1.16 mg/g/GAE) and *Xylopia aethiopica* at a dose of 10kGy (7.84±1.34 mg/g/GAE) had the highest and lowest TPCs in the ethanolic extracts, respectively. TPCs for all the spices were generally higher in the aqueous extracts than in the ethanolic. Extracts for

*Eugenia caryophyllata* and *Aframomum melegueta*, signifying that phenolic compounds in the two local spices are more soluble in water than in organic solvents such as ethanol, and thus; corroborating the reports of Owusu-Ansah (2010) who worked on moringa leaf and Ahiakpa *et al*., (2013) who worked on okro. However TPCs in *Xylopia aethiopica* were found to be more lipophilic. From the analysis of variance, statistically significant differences were observed among *Eugenia caryophyllata* for aqueous and ethanolic extracts, doses of 0 -5 kGy for *Xylopia aethiopica* in both ethanolic and aqueous extracts and doses of 0 and 5kGy and 10-20kGy for *Aframomum melegueta* in terms of TPCs. This may be attributed to genetic differences among the type of spices. The TPCs of the spices; *Aframomum melegueta, Eugenia caryophyllata* and *Xylopia aethiopica* compares well with common fruits and vegetables noted for their relatively high phenolic constituents such as apple (29.63±0.64mg/g), banana (9.04±0.32mg/g), lemon (8.19±0.35mg/g), orange (8.12±0.11mg/g), pineapple (9.43±0.15mg/g), cranberry (52.72±2.15mg/g), strawberry (16.00±0.12mg/g), pear (7.06±0.16mg/g), and grape (4.96±0.26mg/g) (Weng *et al*., 2005; USDA, 1998).

***Total antioxidant activity***

Table 1 shows the percentage inhibition of each extract (ethanolic and aqueous) against their of the DPPH radical scavenger for all three spices. The analysis of variance (ANOVA) showed that concentration significantly ($p<0.05$) affected percent inhibition. *Eugenia caryophyllata* registered the highest percent DPPH inhibition of 68.26±2.62a % with a correspondent antioxidant activity of 1829.58±438.00mg/g while *Xylopia aethiopica* recorded the lowest percent DPPH inhibition of 14.66±0.74 % which corresponded to the least antioxidant activity of 506.49±0.00mg/g in the ethanol extract. In general, the free radical scavenging potentials of extracts of the spices were variable but concentration-dependent. However, the ethanol extracts of the spices were better able to reduce or inhibit the free DPPH radicals than the aqueous extracts. This also suggests that compounds present in the spices have antioxidant properties, which can neutralize free radicals associated with cancer.

***Vitamin C***

Vitamin C is a water-soluble vitamin essential in the human diet because the body is unable to synthesize it. Their antioxidant properties are essential for collagen formation and help to maintain the integrity of skin, connective tissues, bone and blood vessel walls. It also facilitates the absorption of iron (Pamplona-Roger, 2004). People with elevated levels of ascorbic acid in their blood stream seem to be at a significantly reduced risk of having a stroke and low ascorbic acid has been suggested as a way of identifying those at high risk of stroke. All three spices (*Eugenia caryophyllata*, *Aframomum melegueta* and *Xylopia aethiopica*) recorded significant differences ($p\leq0.05$) in the doses of radiation exposure as well as the storage period. Gamma irradiation has been used effectively to decontaminate spices, however it has been discovered that vitamin C is quite sensitive to irradiation doses. The study revealed that vitamin C also changed with changes in radiation dose and storage time. There was a gradual decrease of vitamin C content which occurred with the increase of radiation dose and storage time. Ladaniya *et al.*, (2003) found that, doses up to 1.5 kGy caused decrease of vitamin C content of some citrus fruits. For instance, vitamin c decreased from 1.56 to 1.43 g/10 ml for *Eugenia caryophyllata*, 0.47 to 0.38 g/10 ml for *Xylopia aethiopica*, 0.92 to 0.79 g/ 10 ml for *Aframomum melegueta* as radiation dose. Antioxidant compounds, including phenolic acids, carotenoids and vitamins, are naturally present in fruits, vegetables, herbs and spices(Ali *et al*., 2008; Liu, Qiu, Ding, & Yao, 2008; Schinella *et al*., 2009; Sreeramulu & Raghunath, 2010; Vasco, Ruales, & Kamal-Eldin, 2008). It has been hypothesized that bioactive components with antioxidant capacities present in these foods may contribute to lower incidence of cardiovascular disease (Wang, Melnyk, Tsao, & Marcone, 2011). The content of these compounds varies according to the maturation stage, culture practices and processing (Faller & Fialho, 2009; Gayosso-García Sancho, Yahia, &González-Aguilar, 2011; Villa-Rodríguez, Molina-Corral, Ayala-Zavala, Olivas, & González-Aguilar, 2010).

**Effect of Storage Period on Antioxidant Indices**

Table 2.0 shows the effect of storage on antioxidant indices of the three local spices used in the study. There well significant differences in concentrations of TFCs, TPCs and Total antioxidant activity for all three spices during storage. Concentrations varied between samples stored for a month and samples shelved for 4 four months after exposure to radiation doses of 0, 5, 10 and 20 kGy. This suggests that the powdered spice samples are more sensitive to high ambient temperatures and moisture content which can affect its stability and flavour value. Loss of antioxidants in spices during storage can be attributed to many factors such as pH and acidity, sugars and sugar degradation products, oxygen, ascorbic acid, crop maturity as corroborated by These data indicate that the antioxidant properties of spices have a propensity to decrease when spice samples are stored over a long period of time. The increase in antioxidant activities following gamma irradiation may be due to the degradation of some high-molecular-weight constituents, and changing the solubility of these constituents in test solvents may give rise to the production of additional

phenolic compounds as corroborated by Khattak *et al.,* 2008.

## Conclusion

Our study clearly demonstrates that over four months, storage conditions had significant effect on the polyphenol and flavonoid contents and DPPH radical-scavenging activities in the three local spice samples. However, gamma irradiation had the potential to maintain these properties successfully. Longer storage durations were coupled with gradual decreases in the polyphenol and flavonoid contents and radical-scavenging activities of spice samples.

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