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Antibacterial Activity of Irradiated Powdered *Tetrapleura tetraptera* Fruit and the Moisture Sorption Isotherm of the Whole Fruit

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To assess the microbial load and antibacterial activity of irradiated *T. tetraptera* fruit and the appropriate equilibrium relative humidity for the storage of irradiated fruit of *T. tetraptera*.
Methodology: The whole fruit was analyzed for aerobic mesophiles count and moisture sorption isotherm was determined; while the powdered samples were analyzed for antibacterial potency.
Results: Irradiating the samples reduced the microbial load significantly. A dose of 10 kGy eliminated all microflora from the products while a dose of 5 kGy reduced the initial microbial count from $1.1x10^4$ to 80 CFU/g (i.e. 93% reduction). The net gain of moisture by fruits stored at 55% to 75% ERH was minimal and no growth of fungi was observed on the fruits. ERH above 75% reintroduce some microbes.

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Conclusion: Irradiation completely eliminated the microflora at 10 kGy and substantially reduced the antibacterial ability of *T. tetraptera* fruit against the bacteria strains studied. The irradiated and unirradiated *T. tetraptera* fruits were better stored up to 75% equilibrium relative humidity.

Keywords: Tetrapleura tetraptera fruit; moisture sorption isotherm; irradiation; microbial; antibacterial.

1. INTRODUCTION

Spices, medicinal and aromatic herbs are well known for their useful antimicrobial phytochemical constituents. However, these plant materials are often highly contaminated by microbes. As with many other agricultural products, spices and herbs may be exposed to a wide range of microbial contamination during pre-and post-harvest. Such contamination may occur during processing, storage, distribution, sale and/or use [1]. Having been dried material from plant origin, spices are commonly heavily contaminated with xerophilic storage moulds and bacteria [2]. Various conventional methods of sterilization and microbial load reduction including fumigation with gaseous ethylene oxide or propylene oxide and application of steam [3] have been used. These methods are however, recognized as less safe and are now prohibited or restricted in most countries [4]. Microbial contamination in raw plant materials is a very common problem for which gamma irradiation process is nowadays the most used technique for micro-organisms reduction [5]. Extracts of *Tetrapleura tetraptera* fruit are used as spices in soup and have been suspected to have antibacterial activities [6]. While gamma irradiation can reduce the microbial load, the procedure can inadvertently reduce antimicrobial potency of *Tetrapleura tetraptera* fruit. Moreover, inappropriate equilibrium relative humidity (ERH) at which the fruit is stored can promote microbial reinfection. Hence, the aim of this work was to assess the microbial load and antibacterial activity of the irradiated *T. tetraptera* fruit and the appropriate equilibrium relative humidity for the storage of irradiated fruit of *T. tetraptera*.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

Dried fruits of *Tetrapleura tetraptera* were bought from a local market in Madina, Accra - Ghana, and were identified by the Botany Department of the University of Ghana. They were divided into two batches of which the first batch was washed, oven dried at 50°C overnight, cut into small pieces and ground into a powder with a hammer mill. The ground powders were stored separately in air-tight containers and kept in a cool, dry, dark place. Ten grams each of the *T. tetraptera* powder and whole fruits were packed in different polythene bags and irradiated with gamma rays from Cobalt 60 source at 5 and 10 kGy at room temperature at the Radiation Technology Centre of GAEC. The dose rate was 2 kGy/hr and the dose delivered was confirmed by Fricke's dosimeter. The samples were then stored in the fridge at 4°C awaiting subsequent analyses.

2.2 Preparation of Plant Extract

The dried fruit samples were ground and extracted with methanol in a soxhlet apparatus. The solvent was removed using rotary evaporator under reduced pressure at temperature below 50°C. The resulting crude extracts were stored at 4°C until used. Stock solutions and serial dilutions of the plant extracts were prepared in dimethyl sulphoxide (DMSO) [7,8].

2.3 Antimicrobial Activity Test

Antibacterial activity was detected by the disc diffusion method [9] for Gram positive and negative bacteria (*Staphylococcus aureus*, *Salmonella typhimurium* and *Escherichia coli*). Chloramphenicol (30 µg) and distilled water were used as positive and negative controls, respectively [10]. Experiments were run in triplicate and the results represent mean values of the three measurements.

2.4 Aerobic Mesophiles Count (TVC) Analysis

Total mesophilic aerobic bacteria count was performed according to the method specified by the International Commission on Microbiological Specifications for Foods [11]. Plate Count Agar was used as the medium and colony counter used for the counting. Incubation was carried out at 30°C for 3 days. Yeast and mold count were performed using the pour plate method. Dichloran Rose Bengal Chloramphenicol Agar

was used as a medium. Incubation was performed at 25℃ for 5 days.

2.5 Moisture Sorption Isotherm of the Whole Fruit

The equilibrium relative humidity for the samples was determined [12]. Two whole fruits of approximately 2 grams were weighed and placed in desiccators having specific equilibrium relative humidities at an ambient temperature of 30±1℃. The samples were placed in the upper section of each glass desiccator on wire/plastic mesh while the lower section contained the formulation of the glycerol-water mixture (Table 1). The relative humidity values in the desiccators and the temperatures were monitored using the thermo-hygrometer. The interior of the desiccators had a temperature of 29±1℃. The weight (loss or gain) of the samples was determined every 2 days by weighing the samples and graphs were drawn for the values obtained.

Table 1. Summary of the formulation of glycerine-water ratio used to establish the prescribed equilibrium relative humidity

% ERH	Volume of glycerin (ml)	Volume of water (ml)
55	75	25
65	68	32
75	58	42
85	45	55
95	22	78

2.6 Statistical Analysis

Statgraphics centurion (Version 16) statistical tool was used for the analysis of variance and mean separations. Values were represented as mean ± S.D of triplicate data. The graphs were drawn using Excel version 2013.

3. RESULTS AND DISCUSSION

3.1 Antimicrobial Properties of *Tetrapleura tetraptera* fruit as Influenced by Gamma Irradiation

This antibacterial assay was chosen because it is rapid and reliable in testing [13]. There were observable significant differences (Table 2) between extracts of the irradiated and unirradiated *T. tetraptera* fruitregarding growth inhibition of the *Staphylococcus aureus* and *Salmonella typhimurium*. There was no significant difference in the inhibition of growth of *Escherichia coli* by the extracts from the unirradiated fruit and extracts from fruits irradiated at 5kGy; however the inhibition of growth of *Escherichia coli* by extract of the fruits irradiated at 10kGy was significantly different from the extract of the unirradiated fruits (Table 2). The positive control (Chloramphenicol) had a zone of inhibition significantly higher than both the irradiated and unirradiated samples in this present work for *E. coli* and *S. aureus*. However, the extracts from the unirradiated fruits performed significantly higher than the positive control (Chloramphenicol) in the inhibition of the growth of *S. typhimurium.* In this present work, the irradiated samples generally showed the lowest bacterial growth inhibition. The antibacterial activity of some plants have been attributed to the presence of some active constituents, like phytochemicals, in extracts from those plants [14,15,16]. These constituents have been reported to interfere with cell permeability of bacteria [17,18]. Other researchers have reported on the effect of irradiation on the antibacterial activity of plant extracts. It has been reported that gamma irradiation had an effect on the antibacterial activities of leaves of *Juniperus* and Artichoke respectively [19,20]. The gamma irradiation might have inactivated these inhibitors and phytochemicals resulting in the irradiated samples having lower antibacterial ability. This present finding is contrary to the finding that irradiation significantly induced some phytochemical contents and antioxidant potential of irradiated *T. tetraptera* [21].

3.2 Microbial Load of *Tetrapleura tetraptera* Whole Fruit as Influenced by Gamma Irradiation

The microbial load of the unirradiated samples of the powdered *Tetrapleura tetraptera fruit* was very low indicating high product quality (Table 3). Irradiating the samples reduced the microbial load significantly. A dose of 10 kGy eliminated all microflora from the products while a dose of 5 kGy reduced the initial microbial count from the initial load of $1.1x10^4$ to 80 CFU/g (i.e. about 93% reduction). Gamma irradiation has been shown to be generally effective in reducing the microbial load of dehydrated ingredients, spices and dried herbal products [22,23,24,25].

Table 2. Antibacterial activity of *T. tetraptera* fruit extract against pathogenic indicator strains

Dose (kGy)	Diameter of clear zone of inhibition in mm		
	***Escherichia coli* (-)**	***Staphylococcus aureus* (+)**	***Salmonella typhimurium* (-)**
0.00	8.50±0.50^{b}	11.20±0.20^{b}	15.00±1.00^{a}
5.00	7.00±3.00^{b}	4.80±1.70^{c}	8.57±0.45^{c}
10.00	2.50±0.50^{c}	5.30±1.10^{c}	6.00±0.50^{d}
Water	–	–	–
Chloramphenicol	10.20±0.40^{a}	21.00±0.50^{a}	11.00±0.70^{b}

Means ± standard deviation with different superscripts in the same column are significantly different (p≤0.05). (-) means no measurable zone of inhibition

The initial low counts could be attributed to the fact that the samples were cleaned, as is done traditionally, with a piece of cloth. This could have resulted in the original load being reduced to the counts reported in this present work. This traditional method of making the fruits acceptable could be a viable option in instances where no other decontamination method is available. However, it must be cautioned that unless the piece of cloth is itself clean, it could serve as a source of contamination for the whole fruits. Though a dose of 10 kGy resulted in the complete elimination of all microflora, it is recommended that a dose of 5 kGy be used for decontamination of powdered *Tetrapleura* unless the aim is to use it to prepare food for immunocompromised patients where complete elimination is required.

Table 3. Aerobic mesophiles count (TVC)

Dose (kGy)	Count	Dilution factor	Pop. (CFU/g)
0.0	108.0	10^{-2}	$1.1x10^{4}$
5.0	8.0	10^{-1}	80
10.0	0.0	10^{-1}	≤10

3.3 Effects of Gamma Irradiation on the Moisture Sorption Isotherm of *T. tetraptera* Fruit

Equilibrium relative humidity, expressed as a fraction, determines the water activity in a given sample. It has been used as a basis to understand microbial growth and to determine the extent of enzymatic reaction in food products [26,27,28]. The relationship between water activity and moisture content in a food is often expressed as a sorption isotherm. Food moisture isotherms are important in equipment design for drying, packaging and storage for prediction of shelf-life, and determination of critical moisture and water activity for acceptability of products that deteriorate mainly by gaining moisture [29]. It has also been observed that biological activity occurs only when moisture is present [30]. Therefore the moisture content of the product itself, as well as the moisture content of surrounding air, is important for safe storage [30].

Results obtained were not near the sigmoid curve reported [31] to be typical of foods (Figs. 1-3). In all three samples, the samples kept at 85% and 95% showed a curve characteristic of samples stored at those ERHs. After an initial gain in moisture, there was a sharp drop by day 10 and this continued through to the end of the storage period. The moisture gained favoured the growth of fungi which had taken over the entire sample by the end of the experiment. Several researchers have made similar observations in other foods. It has also been indicated that irradiated cowpea samples absorb more moisture than non-irradiated samples stored at 85% and 95% ERH [32,33]. This was because irradiation weakens the inter-molecular bonds between starch and water thereby enhancing increase in water uptake [34]. The growth of fungi from 85% ERH has been reported to be dangerous as harmful microorganisms like *Staphylococcus aureus* can grow at these relative humidities [27]. The net gain of moisture of samples stored at 55% to 75% equilibrium relative humidity was minimal and no growth of fungi was observed in these samples. This was consistent with the findings which stated that food water activities of less than 0.70aw (70% ERH) is unlikely to support spoilage by microorganisms [35]. It is thus anticipated that storage of these products at ambient relative humidity of 20 to 75% would keep the products safe and extend the shelf-life.

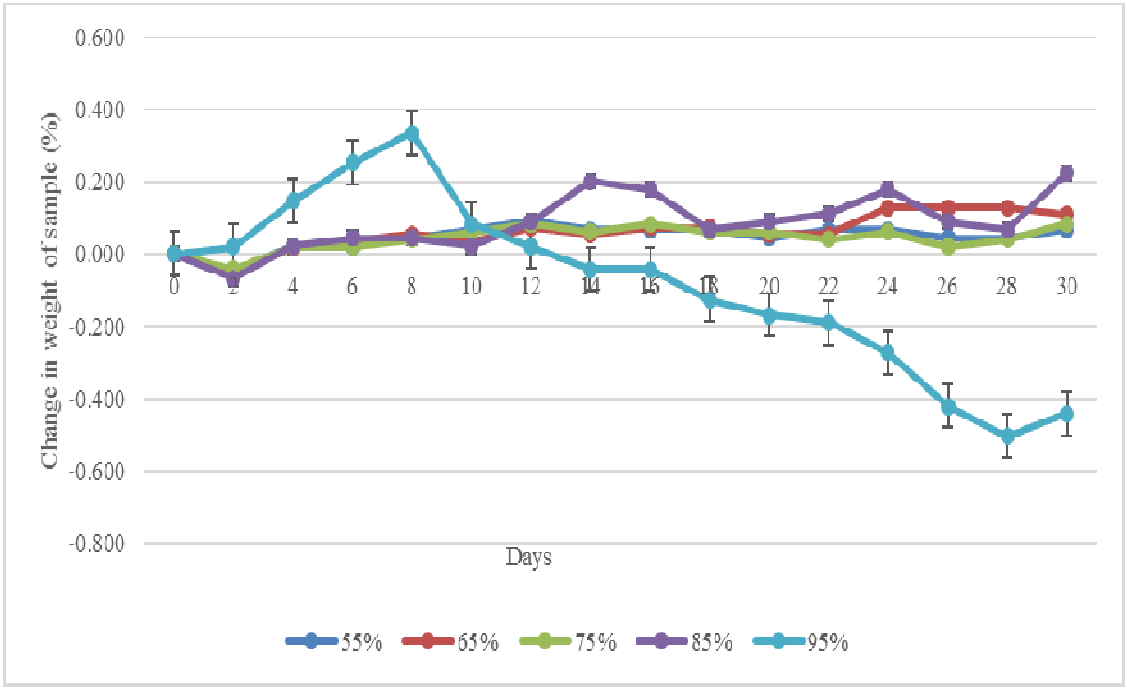

Fig. 1. Moisture sorption isotherm of the unirradiated *T. tetraptera fruit* at different equilibrium relative humidities at 29±1 ℃ for 30 days

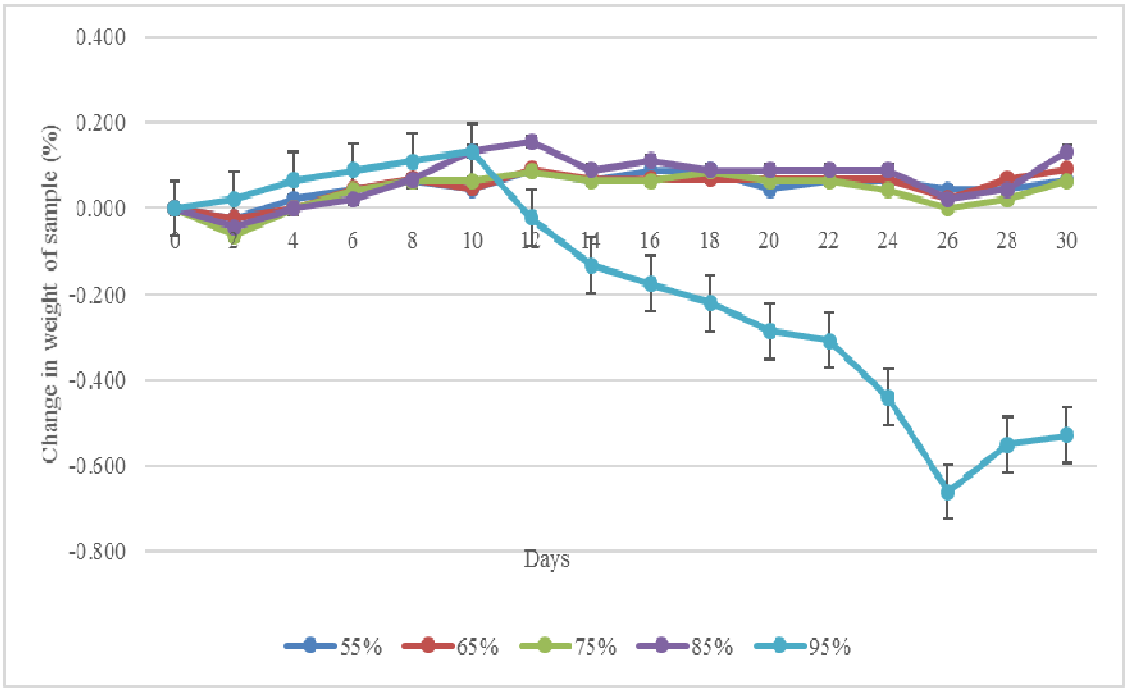

Fig. 2. Moisture sorption isotherm of *T. tetraptera fruit* irradiated at 5 kGy at different equilibrium relative humidities at 29±1 ℃ for 30 days

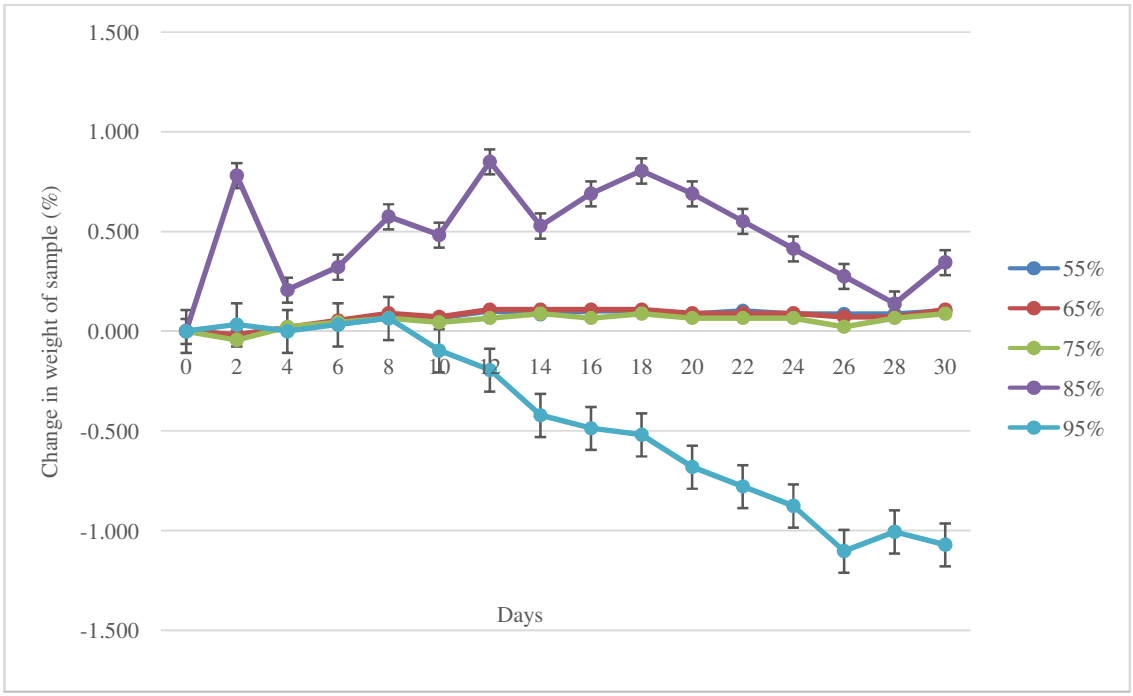

Fig 3. Moisture sorption isotherm of *T. tetraptera fruit* irradiated at 10 kGy at different equilibrium relative humidities at 29±1℃ for 30 days

4. CONCLUSION

Irradiating the samples reduced the microbial load significantly and all the microflora were completely eliminated from the products at 10 kGy. The irradiation substantially reduced the antibacterial ability of *T. tetraptera* fruit against the bacteria strains studied. Since the net gain of moisture of fruits stored between 55% and 75% equilibrium relative humidity was minimal, both the irradiated and unirradiated *T. tetraptera* were better stored up to 75% equilibrium relative humidity.

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CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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