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Microbiological quality assessment of gamma irradiated fresh and dried mushrooms (*Pleurotus ostreatus*) and determination of D_{10} values of Bacillus cereus in storage packs.

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Abstract
The microbiological food contamination in Ghana is alarming. Gamma radiation was used to decontaminate and preserve fresh and dried mushrooms (*Pleurotus ostreatus*). Fresh mushrooms were irradiated with doses of 0 kGy (control), 1 kGy and 2 kGy and stored in polythene and polypropylene storage packs at 20 ^{o}C for a period of 5 days. Dried mushrooms were also irradiated at doses of 0 kGy, 0.5 kGy, 1 kGy, 1.5 kGy and 2 kGy and stored in the same packs and temperature for 12 months. The samples were analysed for aerobic plate counts, total coliforms, *Bacillus cereus, Staphylococcus aureus*, *Salmonella spp*, yeasts and molds counts using standard microbiological methods at intervals of 0 and 5 days for fresh mushrooms while dried mushrooms were monitored at 0, 3, 6 and 12 months. The D_{10} values of *Bacillus cereus* were calculated for fresh and dried mushrooms using a linear regression model after gamma irradiation. Generally fresh mushrooms counts ranged $3x10^3$- $7.5x10^8$, $5x10^1$- $4x10^2$ and $8x10^1$- $9x10^4$ for aerobic mesophiles, *Bacillus cereus*, yeasts and molds respectively. Dried mushrooms recorded count ranges of $2.8x10^2$- $8.3x10^5$, $1x10^1$- $5x10^3$ and $1x10^1$- $3x10^3$ for same. *Salmonella spp, coliforms* and *Staphylococcus aureus* were not detected on both fresh and dried mushrooms. The mean D_{10} values for *Bacillus cereus* on fresh mushrooms were 3.21±0.81 kGy (polypropylene), 0.76±0.04 kGy (polythene) while dried mushrooms recorded 2.40±0.90kGy (polypropylene) and 1.80±0.85 kGy (polythene). Low dose radiations were effective in reducing the contaminants to acceptable standards.

Keywords: *Pleurotus ostreatus*, *Bacillus cereus*, microbiological safety, D_{10} value, irradiation

1. Introduction
Food is a basic need while its safety is a basic human right. Edible mushrooms have been part of human diet for centuries (Ayetayo and Oriyo, 2013) and are becoming so popular in Ghana (Obodai and Johnson, 2002; Apetorgbor *et al*, 2005) owing to their superior nutritional, medicinal and culinary attributes (Kalac, 2009; Pani, 2011; Ferreira *et al*, 2011; Singh *et al*, 2012). They are available in their fresh or preserved states in various packaging materials and are mostly sold at the supermarkets, local markets, city shopping malls, street vendors and the farm-gates.

The hygienic quality of these mushrooms has become an issue of great concern worldwide despite important developments in reducing the incidence of certain pathogens in foods through better farm practices and food regulations (Cuprasitrut *et al*, 2011). Foods in general have been identified as vehicles of microbial agents and generate food safety problems, especially gastroenteritis (Mosupye and Holy, 2000; Kubheka *et al*., 2001; Meng and Doyle, 2002; Adu-Gyamfi and Nketsia-Tabiri, 2007). Microorganisms implicated include bacteria (*Campylobacter*, *Salmonella*, *Yersinia enterocolitica*, *Clostridium per-fringens*, *Staphylococcus aureus*, *E. coli* O157:H7, *Listeria monocytogenes*) and some fungi (*Aspergillus spp, Mucor spp, Rhizopus spp*.) (Mead *et al*, 1999; Lavelli *et al*, 2006; Adjrah *et al*, 2013).

Bacillus cereus, a gram positive, facultative anaerobic and spore forming rod bacteria has been reported by several authors (Thayer and Boyd, 1994; Giwa and Ibrahim, 2012 ; El-Nour and Hammad, 2013) to be ubiquitous organism found in water, soil and air.

B. cereus is the aetiologic agent of two discrete types of food poisoning characterized by both diarrhea and abdominal pain or by nausea and vomiting after ingestion of contaminated foods due to its ability to form two types of enterotoxins: thermostable emetic enterotoxin or a thermosensitive diarrheal enterotoxin (Schneider *et al*. 2004). *B. cereus* form endospores which are resistant to inactivation agents such as heating, desiccation, UV, low doses of gamma radiation, high pressure and oxidizing agents (Van German *et al.* 1999 and Seltow, 2006). It has been isolated from a wide range of food products such as cooked and raw rice (Sarrias *et al*. 2003), seafood (Rahmati and Labbé, 2008), milk (Bartoszewicz *et al.* 2008), fresh vegetables and refrigerated-minimally processed foods (Valero *et al*. 2002). Thus, food safety issues are of major importance to world health (WHO, 2000).

Gamma irradiation as a physical treatment effectively eliminates spoilage and pathogenic microorganisms in foods (Neimera, 2003; Sommers, 2003) and has been utilized for the reduction and elimination of pathogens in foods (Farkas, 2001; ICGFI, 1999). However in order to utilize irradiation as a food processing technology, it is imperative to study the radiation sensitivity of contaminating microorganisms since this provides a basis for accurate estimation of inactivation doses (Thayer, 2000; Adu-Gyamfi *et al*, 2009). Sensitivity to irradiation varies among microbial and fungal species and is affected by the components of foods and temperature during irradiation and subsequent storage (Neimira, 2007; Adu-Gyamfi *et al*, 2012).

The D_{10}-value (decimal reduction dose) is the radiation dose required to inactivate 90% of a viable bacterial population or reduce the population by a factor of 10 (Smith and Pillai, 2004). Published data on D_{10}-values for some foods range from 0.022 kGy for *Vibrio parahaemolyticus* in freshwater fish homogenate at 24 °C (Gamage *et al*, 1998) to 0.78 kGy for *Salmonella enteritidis* in ground beef at 3 °C (Molins, 2001). *E. coli* O157:H7 inoculated onto fresh sprouts of radish, alfalfa, or broccoli seeds, showed a D_{10} value range of 0.27 to 0.34 kGy (Rajkowski and Thayer, 2000). There is a comparatively great range of D10-values and therefore differences in resistance to gamma radiation by various microorganisms of public health significance. Estimation of D_{10}-values may be incorporated into risk assessments for designing processes for reduction of microbial populations in food (Cheroutre-Vialette and Lebert, 2000).

The objectives of the present study were: 1) To investigate the microbiological quality of fresh and dried mushrooms in polythene and polypropylene. 2) To determine the D_{10}-value (decimal reduction dose) of *Bacillus cereus* on fresh and dried mushrooms.

2. Materials and Methods

2.1 Sample collection and Drying

A total of 16 samples comprising of 6 fresh mushrooms and 10 dried mushrooms were obtained from Mushroom Unit, CSIR- Food Research Institute in Ghana. Growth and harvesting of mushrooms was from the period of September to December, 2013. The collected mushroom material was solar-dried at temperature range of (40-60 ^oC) to a moisture content of about 12±1%. Dried mushroom parts were cut up and stored in tight-seal polythene and polypropylene containers at room temperature until needed for microbiological analysis within one hour of collection.

2.2 Determination of Moisture content

The moisture content was determined by the gravimetric method of (AOAC, 1995).

2.3 Irradiation of mushroom samples

Forty (40) grams of dried mushrooms (*Pleurotus ostreatus)* were packed into polythene and polypropylene containers and irradiated at doses of 0 kGy, 0.5 kGy, 1 kGy, 1.5 kGy and 2 kGy at a dose rate of 1.7 kGy per hour in air from a cobalt- 60 source Radiations absorbed were confirmed using the ethanol-chlorobenzene (ECB) dosimetry system at the Radiation Technology Centre of the Ghana Atomic Energy Commission, Accra, Ghana.

Sixty (60) grams of fresh mushrooms (*P. ostreatus*) were packed into same packaging materials and irradiated at doses of 0 kGy, 1 kGy and 2 kGy at the same conditions as stated above.

2.4 Microbiological analysis

Ten (10) grams of each sample was mixed with 9ml peptone water and serial dilutions of each mushroom sample homogenate were made to 10^{-3} dilutions. Approximate 1 ml aliquot portions of the dilutions were spread onto duplicate sterile plates of Plate Count Agar (Oxoid, England), Violet Red Bile Agar (Oxoid, England), Baird Parker medium (Oxoid, England), Bacillus cereus agar (Oxoid, England) and Dichloran Rose Bengal Chloramphenicol (Oxoid, England) for total mesophilic bacteria, total aerobic plate count, coliform count, *Staphylococcus aureus, Bacillus cereus* and moulds and yeasts respectively,. Isolation of Salmonellae spp. was done on Rapaport Soy Broth (Oxoid, England) and streaked on Xylose Lysine Deoxycholate Agar (Oxoid, England) and Brilliant Green Agar (Oxoid, England).

Cultures were incubated at 37 ^oC for 24 to 48 hrs. After the incubation, the different culture plates were examined for microbial growth. Colonies were counted using the colony counter (Gallenkamp, England), counts were expressed as colony forming unit per gram of sample homogenate (cfu/g).

2.5 D_{10} values Determination

The D_{10} value is the reciprocal of the slope of the exponential part of a survival curve. This value may also be obtained from equation (1). Microbial counts (cfu/g) obtained after subjecting fresh and dried mushrooms to radiation doses of 0, 1, 2, kGy were transformed into ($\log_{10}$ cfu/g) and the data was subjected to regression analysis. The surviving fractions, $\log_{10}$ (N/N_0) of microorganisms, was calculated and used as relative changes of their actual viable cell counts. The D_{10} values were calculated by plotting $\log_{10}$ (N/N_0) against dose (D) according to the equation

$$D_{10} = \frac{\text{Radiation Dose (D)}}{\log_{10}(No - N)} \quad \ldots\ldots 1)$$

Where No is the initial viable count; N is the viable count after irradiation with dose D; D is the radiation dose

(Mohan *et al*, 2011; Adu-Gyamfi *et al*, 2012). The linear correlation coefficient (r^2) and the regression equations were also calculated.

2.6 Statistical analysis: The values obtained for total aerobic plate count, *Bacillus cereus* and fungal counts were subjected to analysis of variance.

3. Results and Discussion

The results of analyzed microbial counts of irradiated fresh and dried mushrooms are showed in Tables 1- 4. The total aerobic mesophile count, *B. cereus* and fungal counts for fresh mushrooms stored in polypropylene pack ranged $1.5x10^4$ – $8.6x10^7$, $1x10^2$- $4x10^2$ and $2.6x10^1$- $9x10^4$ cfu/g (Table 1) respectively. There was an average log reduction of 5.5, 0.8 and 1.2 respectively after exposure to gamma radiations. Total aerobic mesophile count, *B. cereus* and fungal counts for fresh mushrooms stored in polythene pack ranged $2x10^4$- $7.5x10^6$, $3x10^2$- $4x10^2$ and $1x10^2$- $1x10^4$ cfu/g (Table 2). Gamma radiation reduced these counts to 3.7, 0.22 and 1.4 log cycles respectively.

Microbial counts showed an increase after 5 days storage. High aerobic mesophilic counts found in samples according to Najafi and Bahreini, (2012) may reflect poor handling, inappropriate processing or a general lack of hygiene. The results obtained for total aerobic mesophile count were in agreement with results of Kamal *et al*, (2011) who recorded average counts of 10^6 on fresh oyster mushrooms collected from Sutrapur Dakar city. *Staphylococcus aureus*, *Salmonella spp, E.coli* and coliforms were not recorded. This was in disagreement with work of Beraha *et al*, (1961) who recorded 27, 13, 13 and 7% respectively in the case of fresh cut mushrooms. Non- irradiated (0 kGy) fresh mushroom samples recorded lower fungal counts of range 1.4- 3.8 log cfu/g (Table 1 and 2) than results reported by researcher such as Abadias *et al*, 2008; Seo *et al*, 2010 and Najafi and Bahreini, 2012 who all worked on fresh cut vegetables. The role of yeasts and molds in the spoilage of mushrooms is not well documented and their growth on foods can cause major problems. Some of molds may produce mycotoxins which could be carcinogenic, mutagenic, teratogenic and allergic (Eaton and Groopman, 1994; Guengerich *et al*, 1996; Tournas, 2005; Adu-Gyamfi *et al*, 2011).

Dried mushrooms stored in polypropylene recorded mean counts of $1.6x10^3$- $7.7x10^3$, $1x10^2$- $7x10^2$ and $3x10^1$- $2x10^3$, polythene also had mean counts of $1.67x10^3$- $6.3x10^4$, $2x10^2$-$5x10^3$ and $1x10^1$- $8x10^2$ for total aerobic mesophiles, *B. cereus* and fungal counts respectively. Our results indicate a general increase in microbial counts over storage period of 12 months. The increase in microbial load content was apparent in 6^{th} and 12^{th} months. These results may be attributed to the fact that they are spore formers (Oranusi *et al*, 2010). These dormant spores were resistant to gamma radiation and other processing so might have germinated and multiplied with time. Also, physical environmental factors such as moisture, pH and temperature in the packs became conducive to support growth of microorganisms (Food Safety, 2003). Dried mushroom samples recorded lower microbial counts than fresh mushrooms. This might be due to the processing activities such as solar drying. Statistically, there was no difference ($P<0.05$).

The presence of microorganisms in food is not necessarily an indicator of hazard to the consumers (Kamal *et al*, 2010). *Bacillus cereus* can be detected in many raw foods of plant origin and in raw milk. According to authors Zahran *et al*, 2008; NSW-FA (2009), their spores will survive cooking, and poor temperature control after cooking may result in germination of the spores and subsequent growth. *B. cereus* is of greatest concern in plant or cereal based ready-to-eat foods and cream based sauces. Ready-to-eat foods containing raw components may contain low levels of *B. cereus.* The International Commission for Microbiological Specification for Foods (ICMSF, 1996) states that ready-to-eat foods with plate counts between $0\text{-}10^3$ is acceptable; between 10^4- $\leq 10^5$ is tolerable and 10^6 cfu/g and above is unacceptable.

Radiation sensitivity (the killing effect of radiation) in microorganisms is generally expressed by the decimal reduction dose or D_{10} value (Mohan *et al*, 2010). Radiation sensitivity of *Bacillus cereus* on fresh oyster mushrooms stored in polypropylene and polythene packs were 3.21±0.81 and 0.76±0.04 kGy respectively (Table 5). Also, dried oyster mushrooms stored in polypropylene and polythene packs were 2.40±0.90 and 1.80±0.85 kGy respectively. The mean D_{10} values of *Bacillus cereus* on both fresh and dried mushrooms were 1.98 and 2.10 kGy, showed no significant difference ($P>0.05$). The observed difference ($P<0.05$) in D_{10} values for *Bacillus cereus* on mushrooms stored in polypropylene and polythene were probably due to the densities of materials constituting the walls of the packaging materials and how they affected the penetration of the gamma radiation to the target microorganisms (da Silva Aquino, 2012). Likewise, the radiosensitivity of bacteria varies depending on the packaging atmosphere used and are also very sensitive to irradiation in the presence of oxygen (IAEA, 2005).

The D_{10} values of *B. cereus* obtained, agreed with data from Zahran *et al*, (2008) who reported D_{10} values of 1.9 kGy and 0.4 kGy for *B.cereus* and *L. monocytogenes* on some chicken products. Also, in a study done by Abd El-Hady (1993), reported D_{10} values of 3 strains of *Bacillus cereus* were 2.3, 2.2 and 2.0 kGy on beef. D_{10} values are notable because it leads to an estimation of the dose required to inactivate any microorganism (Zahran *et al*, 2008).

4. Conclusion

Our results indicate that low doses of gamma irradiation was effective in reducing the *Bacillus cereus* populations of oyster mushrooms stored in polypropylene and polythene packs sufficiently to achieve the recommended levels of The International Commission for Microbiological Specification for Foods (ICMSF, 1996). Better understanding of the mechanisms involved in bacterial resistance to radiation exposure need to be explored.

5. Acknowledgement

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Table 1: Effect of irradiation on the microbial load of fresh mushroom fruit bodies of polypropylene pack (P2) stored for a period of 5 days.

Time	Dose	Aerobic	Coliforms	*B. cereus*	*S. aureus*	Molds	Yeasts
	(kGy)	Mesophiles	cfu/g	cfu/g	cfu/g	cfu/g	cfu/g
0 Day	0	$8.6x10^7$	0	$4x10^2$	0	$2.6x10^1$	$9x10^4$
	1	$2.7x10^3$	0	$1x10^2$	0	0	$6x10^3$
	2	$1.5x10^4$	0	$1.5x10^2$	0	0	$8x10^1$
5 Day	0	$7.5x10^8$	0	$4x10^2$	0	$2.6x10^1$	$9x10^4$
	1	$3.0x10^3$	0	$3x10^2$	0	0	$6x10^3$
	2	$2.0x10^4$	0	$5x10^1$	0	0	$8x10^1$

Table 2: Effect of irradiation on the microbial load of fresh mushroom fruit bodies of polythene pack (P1) stored for a period of 5 days.

Time	Dose	Aerobic	Coliforms	*B. cereus*	*S. aureus*	Molds	Yeasts
	(kGy)	Plate Count	cfu/g	cfu/g	cfu/g	cfu/g	cfu/g
0 Day	0	$7.5x10^6$	0	$4.0x10^2$	0	$2.1x10^1$	$5x10^2$
	1	$3.0x10^4$	0	$3.0x10^2$	0	0	$1.0x10^4$
.	2	$2.0x10^4$	0	$3.2x10^2$	0	0	$1.0x10^2$
5 Day	0	$8.6x10^7$	0	$4.0x10^2$	0	$2.1x10^1$	$9x10^4$
	1	$2.7x10^5$	0	$1.0x10^2$	0	0	$6x10^3$
	2	$1.5x10^4$	0	$1.5x10^2$	0	0	$8x10^1$

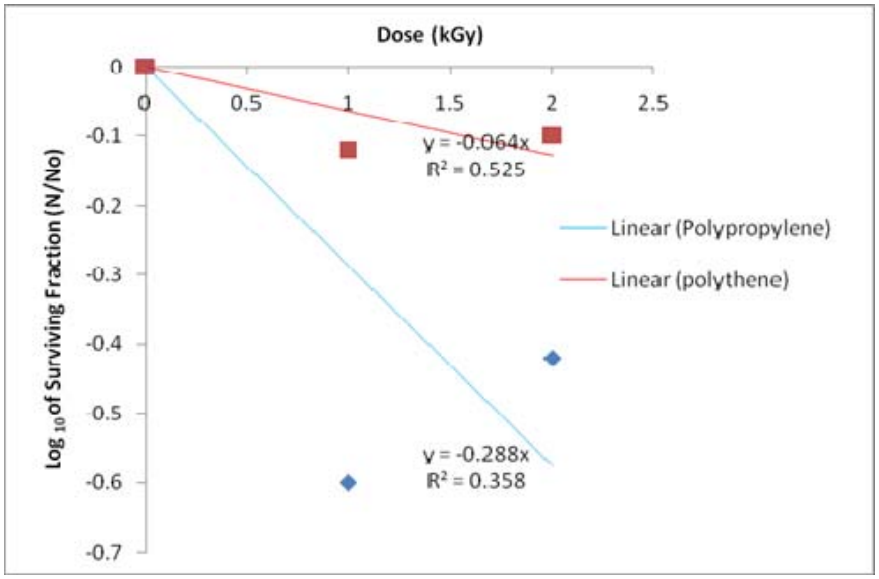

Fig 1: Radiation sensitivity curves for *Bacillus cereus* on fresh mushrooms

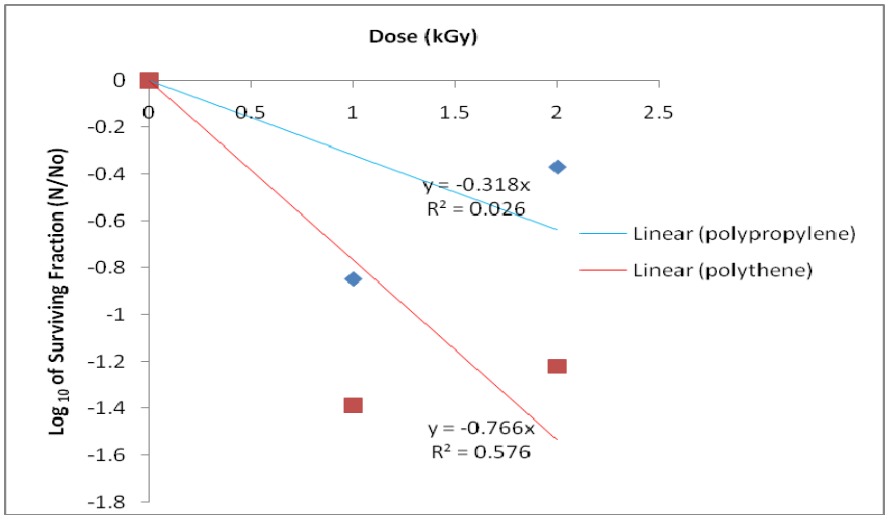

Fig 2: Radiation sensitivity curves for *Bacillus cereus* on dried mushrooms

Table 3: Effect of irradiation on the microbial load of dried mushroom fruit bodies of polypropylene pack (P2) stored for a period of 12 months.

zTime	Dose	Aerobic	Coliforms	*B. cereus*	*S. aureus*	Molds	Yeasts
	(kGy)	Mesophiles	cfu/g	cfu/g	cfu/g	cfu/g	cfu/g
0 Month	0	$7.7x10^3$	0	$7x10^2$	0	$1.7x10^1$	$1x10^2$
	0.5	$9.9x10^2$	0	$1x10^2$	0	0	$2x10^3$
	1.0	$4.9x10^2$	0	0	0	0	$3x10^2$
	1.5	$1.6x10^3$	0	$3x10^2$	0	0	$8x10^1$
	2.0	$3.8x10^2$	0	0	0	0	$3x10^1$
3 Month	0	$8.3x10^5$	0	$7x10^2$	0	$2.2x10^1$	$1x10^1$
	0.5	$9.9x10^2$	0	$1.0x10^2$	0	0	$2x10^3$
	1.0	$5.2x10^2$	0	0	0	0	$3x10^3$
	1.5	$3.1x10^3$	0	$3x10^2$	0	0	$8x10^1$
	2.0	$2.8x10^3$	0	0	0	0	$3x10^1$
6 Month	0	$7.3x10^5$	0	$5.0x10^3$	0	$2.2x10^1$	$1.0x10^1$
	0.5	$9.6x10^2$	0	$3.8x10^2$	0	0	$2x10^3$
	1.0	$4.9x10^2$	0	0	0	0	$3x10^1$
	1.5	$1.62x10^3$	0	$3x10^2$	0	0	$8x10^1$
	2.0	$3.8x10^2$	0	0	0	0	$3x10^2$
12Month	0	$7.7x10^4$	0	0	0	$1.7x10^1$	$1x10^1$
	0.5	$1.97x10^3$	0	$2x10^2$	0	0	$8x10^1$
	1.0	$1.77x10^3$	0	0	0	0	$5x10^1$
	1.5	$1.67x10^3$	$3x10^1$	$3x10^2$	0	0	$1x10^1$
	2.0	$2.08x10^3$	0	0	0	0	$7x10^1$

Table 4: Effect of irradiation on the microbial load of dried mushroom fruit bodies of polythene pack (P1) stored for a period of 12 months.

Time	Dose	Aerobic	Coliforms	*B. cereus*	*S. aureus*	Molds	Yeasts
	(kGy)	M. cfu/g	cfu/g	cfu/g	cfu/g	cfu/g	cfu/g
0 Month	0	$6.3x10^4$	0	$5x10^3$	0	0	$1.7x10^2$
	0.5	$1.97x10^3$	0	$2x10^2$	0	0	$8x10^2$
	1.0	$2.05x10^3$	0	0	0	0	$5x10^2$
	1.5	$1.67x10^3$	$3x10^1$	$3x10^2$	0	0	$1x10^1$
	2.0	$2.08x10^3$	0	0	0	0	$7x10^1$
3 Month	0	$7.8x10^4$	0	0	0	$1.7x10^1$	$1x10^1$
	0.5	$3.9x10^3$	0	$2x10^2$	0	0	$8x10^1$
	1.0	$4.27x10^2$	0	0	0	0	$5x10^1$
	1.5	$1.67x10^3$	$1.2x10^3$	$3x10^2$	0	0	$1x10^1$
	2.0	$2.08x10^3$	0	0	0	0	$7x10^1$
6 month	0	$7.7x10^5$	0	0	0	$4.2x10^3$	$1x10^1$
	0.5	$1.97x10^3$	0	$2x10^2$	0	0	$8x10^1$
	1.0	$3.8x10^2$	0	0	0	0	$5x10^1$
	1.5	$1.67x10^3$	$1.7x10^3$	$3x10^2$	0	0	$1x10^1$
	2.0	$2.1x10^3$	0	0	0	0	$7x10^1$
12 Month	0	$4.4x10^5$	0	0	0	$1.7x10^1$	$1x10^1$
	0.5	$1.97x10^3$	0	$2x10^2$	0	0	$8x10^1$
	1.0	$1.77x10^2$	$6x10^2$	0	0	0	$5x10^1$
	1.5	$3.5x10^3$	$3x10^1$	$3x10^2$	0	0	$1x10^1$
	2.0	$1.9x10^4$	0	0	0	0	$7x10^1$

Table 5: Mean D_{10} values of *Bacillus cereus* on fresh and dried oyster mushrooms in storage packages

Substrate	Regression equation	r^2	D_{10} value (kGy)
Fresh oyster mushrooms			
Polypropylene	y= -0.288x	0.358	3.21±0.81
Polythene	y= -0.064x	0.525	0.76±0.04
Dried oyster mushrooms			
Polypropylene	y= -0.318x	0.026	2.40±0.90
Polythene	y= -0.766x	0.576	1.80±0.85

D_{10} values are means of 2 replicates ± S.E

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