Effect of Medium-dose Gamma Irradiation on Microbial Quality of Cut-cabbage (*Brassica oleracea*): A case study in Greater Accra Region of Ghana

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Research Article

Abstract: Consumption of fresh and fresh-cut fruits and vegetables has increased every year in the past decade because of their convenience and nutritional benefits. Unfortunately, the increasing consumption of fresh produce has been accompanied by an increase in the number of outbreaks and recalls due to contamination with human pathogens. Irradiation of this kind of ready-to-eat product is a feasible alternative treatment to be considered in order to improve the microbiological quality and to extend the shelf life. The objective of this study was to explore the effect of medium-dose gamma irradiation on the microbial quality of cut-cabbage. Cut-cabbage was irradiated and its microbial quality determined. The initial total viable count (TVC) for unirradiated or control cut cabbage samples was 9.11 $\log_{10}$ cfu/g and that of irradiated samples (1-3kGy) at 0 day ranged from 7.39 to 8.95 $\log_{10}$ cfu/g. However, total coliform count for cut-cabbage irradiated at 2kGy was not significantly different from the rest of irradiated samples. The effective irradiation dose of 2kGy was able to reduce the bioburden of by up to 4 log cycles in fresh pre-cut cabbage.

Key words*:* Cut- cabbage, irradiation dose, total viable count (TVC), total coliform count (TCC), contamination.

Introduction

Cabbage and lettuce are the two most consumed leafy vegetables in the world (Bender and Bender 2005). Cabbage contains several phytochemicals which are very useful for human health. However most preservation methods deplete or reduce the amount of these useful nutrients from cabbage. Unlike cereals and legumes, the water activity of most fruits and vegetables is very high. Therefore, most fruits and vegetables are highly perishable because they play host to several micro-organisms and insect pests supporting their growth and proliferation (Dris and Jain, 2004). For example microbiologically contaminated foods are estimated to cause approximately 76 million case of illness yearly in the United States (Mead *et al*., 1999). Highly publicized foodborne outbreaks in recent years have stimulated major efforts by regulatory authorities and the food industry to reduce the incidence and eliminate or control pathogens at all stages of the food chain. Due to direct contact with soil during production, vegetables could be highly contaminated by soil microbes which may contain faecal pathogens e.g. *Escherichia coli*. Under poor sanitary conditions very high load of faecal pathogens can be present in vegetables. Normally, leafy vegetables are reported to harbor 2 to 7$\log_{10}$ colony-forming units (CFU)/g of mesophilic bacteria including coliform or spoilage bacteria such as *Pseudomonas*, as their normal microflora (Nguyen- The and Carlin 1994). Microorganisms associated with food-borne diseases have been found to reside in ready-to-eat vegetables and fruits. These include *Escherichia coli O157:H7, Listeria monocytogenes, Shigella and Salmonella* (Solomon *et al*., 2002). Different types of vegetables and fruits could be contaminated with different kinds and numbers of microorganisms. The profile of initial microorganisms may indicate the quality of the final products. If the initial quantity of spoilage microorganisms in raw materials were high, the quality of vegetables and fruits would deteriorate and their shelf life shorter than usual (Sanusi *et al*., 2008). Unlike spoilage microorganisms which cause repulsive changes which facilitate consumer rejection of the food, the proliferation of pathogenic organisms in food may not give these indications and when consumed may endanger human health.

The aim of this research was to assess effect of gamma radiation on microbial load of resident bacteria in cut cabbage with the view of decontaminate and extending shelf life.

a) to determine microbial numbers on selected cut cabbage in some supermarkets in Accra.

b) to determine the effective radiation dose for improving microbiological quality and shelf life extension of cut-cabbage.

2.0 Methodology

Materials and Methods

Sample collection and Preparation

Freshly cut cabbage packaged in low density polyethylene was purchased from Fruits and Vegetables Processing Company on the Spintex Road, in Accra-Ghana. This company was identified as the main supplier of cut vegetables to most supermarkets in the Greater Accra Region. These samples were then transported to Gamma Irradiation Facility of the Biotechnology and Nuclear Agriculture Research Institute of the Ghana Atomic Energy Commission for irradiation.

Irradiation of Samples

The freshly packaged cut cabbage was irradiated at various doses (0, 1.0, 2.0, and 3.0 kGy). Irradiation of the samples was carried out in air at a dose rate of 2.42 kGy/h using a Cobalt-60 source. The absorbed dose was determined by using Lithium fluoride photo-fluorescent film dosimeter (SUNNA Dosimeter System, UK).

Microbiological analysis

The enumeration of total viable and coliform counts was done at Food and Medical Laboratory of the Radiation Technology Centre. Isolation and identification of pathogenic organisms was carried out in Bacteriology Department of Noguchi Memorial Institute for Medical Research, Ghana.

All the three batches of freshly cut cabbage were microbiologically analyzed to determine the population of indicator and pathogenic microorganisms. Total viable cells, total coliform counts, counts of *Salmonella sp*., *Staphylococcus aureus* and *Escherichia coli* were determined for all the three batches of samples. For each sample, 10 g was weighed into 90 ml Peptone water diluent (0.1% peptone and 0.5 NaCl) and stirred on a mechanical shaker (Junior Obit Shaker, Lab-Line Instrument, USA) for 30 minutes and serially diluted up to 10^6. One milliliter aliquots from each dilution were dispensed into Petri dishes and about 15 ml of the appropriate media was added. Total viable cells were determined on Plate Count Agar (Oxoid, England). Total Coliform counts were determined on Violet Red Bile Agar (Oxiod, England) and *Staphylococcus aureus* was estimated on Baird- Parker (BP) agar (Oxoid, England). *E. coli* was determined on Eosin methylene blue (EMB). All determinations were undertaken in duplicate and three separate experiments were conducted. All samples were incubated at 37^{o}C for 24 hours and observed for colonies. Plates that had between 20-200 colonies were selected for the determination of colony forming units per gram (cfu/g) using a colony counter (Stuart Scientific, UK). The number of cfu/g was calculated by multiplying the number of bacteria by the dilution factor. The pathogenic organisms were identified using Analytical Profile Index (API 20E). BioMerieux SA, France

Experimental design and data analysis

A 3x4x4 factorial design was used with the main factors and respective levels being: 3replicate, treatment (Dose): 0, 1kGy, 2kGy, and 3kGy, storage period: 0, 5, 10 and 15 days. GenStat 12 edition was used for statistical analysis and mean separation. The mean count of total viable cells were calculated and transformed into logarithms. The mean log10(x) values and standard deviations (SD) were calculated on the assumption of a log normal distribution.

Results and Discussion

4.1 Microbiological quality

The initial total viable count (TVC) for unirradiated or control cut cabbage samples was 9.11 $\log_{10}$ cfu/g and that of 1-3kGy irradiated samples at 0 day ranged from 7.39 to 8.95 $\log_{10}$ cfu/g (Figure 1). The magnitude of the reduction of TVC was dependent on the applied radiation dose. For example, TVC of 10.022 $\log_{10}$ cfu/g (control) was statistically different from 7.574, 6.258 and 5.319$\log_{10}$ cfu/g for 3 irradiated cut cabbage. Again TVC of 8.407 and 8.365$\log_{10}$ cfu/g for 0 and 15day were significantly different ($p \leq 0.05$) from 6.178 and 6.224$\log_{10}$ cfu/g for day 5 and 10 respectively. However, the effect of irradiation at 0 day on TVC was not significantly different from the unirradiated sample. Table 1 shows that the TVC on unirradiated consistently increased during storage. However the TVC of all the irradiated samples after 5 days storage were significantly lower than the initial count (Day 0). This supports reports that free radicals generated during irradiation have long term effect than immediate effect as reported by Fan *et al*., (2008) and Diehl, (1995).

Table 1: Total viable count ($\log_{10}$cfu g^{-1}) of non-irradiated and irradiated cut-cabbage during 15 days storage period at $8^0C \pm 2^0C$.

DAYS/DOSES	Day 0	Day 5	Day 10	Day 15
0 kGy	9.11 ± 2.55 Ba	9.66 ± 2.05 Ba	9.79 ± 3.19 Ba	11.54 ± 2.97 Aa
1 kGy	8.95 ± 1.56 Aa	5.84 ± 2.28 Bb	6.59 ± 1.99 Bb	8.91 ± 2.26 Ab
2 kGy	8.17 ± 0.77 Aa	4.78 ± 1.86 Bc	4.43 ± 1.94 Bc	7.65 ± 0.35 Ac
3 kGy	7.39 ± 1.05 Aa	4.44 ± 2.31 Bc	4.09 ± 2.14 Bc	5.36 ± 1.53 Bd

Different uppercase letters within column represent significant difference between irradiation doses ($p<0.05$). Different lowercase letters in a row represent significant difference between storage days ($p<0.05$). Mean ± standard error.

There was also no significant ($p<0.05$) interaction between radiation dose and storage time. This means that

the effect of dose on TVC was independent on storage time as shown in figure 1; between 0-5 days, unirradiated sample increased while 1-3kGy treated samples decreased in TVC; this is due to the fact that free radicals generated by the irradiation was lethal to the microorganisms. Furthermore between days 5-10, TVC of 0 and 1kGy samples increased while TVC of 2kGy and 3 kGy decreased. Table 1 also indicated that during the last 5 days, TVC of 0 – 2 kGy samples increased at a relatively faster rate than the 3 kGy samples.

Table 2: Total coliform count (log_{10}cfu g^{-1}) of non-irradiated and irradiated cut-cabbage during 15 days storage period at 8 ± 2^0C.

DAYS/DOSES	Day 0	Day 5	Day 10	Day 15
0 kGy	8.03 ± 2.93 Ba	8.17 ± 2.16 Ba	8.28 ± 1.68 Ba	11.54 ± 2.97 Aa
1 kGy	7.23 ± 2.15 Aa	5.37 ± 3.26 Bb	6.55 ± 3.61 Ab	8.91 ± 2.26 Ab
2 kGy	6.70 ± 1.54 Aa	4.78 ± 2.00 Bb	5.31 ± 2.45 Bc	7.65 ± 0.35 Ac
3 kGy	6.90 ± 1.77 Aa	3.03 ± 2.20 Bc	3.62 ± 1.84 Bd	5.36 ± 1.53 Bd

Different uppercase letters within column represent significant difference between irradiation dose ($p<0.05$). Different lowercase letters in a row represent significant difference in storage period. Mean ± standard error.

Irradiation caused significant log cycle reduction in initial total coliform count (TCC) of cut cabbage. The total coliform count for the unirradiated cabbage followed the same growth pattern for the aerobic plate count as shown in table 2. The TCC for unirradiated cut cabbage samples ranged between 8.03 – 10.34 log_{10} cfu/g. For the irradiated samples initial TCC ranged between 7.03 - 3.03 log_{10} cfu/g. Samples treated with 3 kGy had the lowest coliform counts during storage compared to those treated with 1-2 kGy doses. The lowest were 3.03 and 8.95 log_{10} cfu/g for irradiated 3kGy and the control respectively. According to the ANOVA, the effects of radiation dose on coliform counts were highly significant ($p<0.001$) i.e. TCC of 8.705log_{10} cfu/g (control) was significantly different 6.431, 5.550, 4.410log_{10} cfu/g for all irradiated samples. Contrary to the effect of doses on cut cabbage, storage period had no significant effect ($p>0.05$) on the sample. The reductions in total coliform counts caused by irradiation doses were independent of storage time. According to Ghana Standard Board (GSB) legislation GS 995:2009 on minimally processed vegetables, acceptable range for *E. coli* should be less than or equal to 3 log_{10} cfu/g. This result also indicated that the correlation between total viable counts and coliform counts was highly significant (Table 3).

Table 3: Correlation between TVC and TCC of unirradiated and irradiated cut cabbage

	TVC (cfu/ml)	TCC (cfu/g)
TVC (cfu/ml)		0.8829
		(48)
		0.0000
TCC (cfu/g)	0.8829	
	(48)	
	0.0000	

Correlation
(Sample Size).

Out of the numerous pathogens associated with foodborne diseases, the two most prevalent pathogens of diseases outbreak found to be involved in consumption of fresh fruit and vegetables are *E. coli* and *Salmonella* (Fan *et al*., 2008). Of these two pathogens only *E. coli* was detected from the cut cabbage samples but *Salmonella* was not detected. The levels of *Escherichia coli* detection is presented in Table 4.

Table 4: *Escherichia coli* population (cfu g^{-1}) of non-irradiated and irradiated cut cabbage during 15 days storage period at $8^0C \pm 2^0C$

DAYS/DOSES	Day 0	Day 5	Day 10	Day 15
0 kGy	$\leq 10^4$	$\leq 10^6$	$\leq 10^6$	$\leq 10^6$
1 kGy	$\leq 10^2$	$\leq 10^3$	$\leq 10^5$	$\leq 10^4$
2 kGy	$\leq 10^2$	$\leq 10^2$	$\leq 10^3$	$\leq 10^3$
3 kGy	$\leq 10^2$	ND	$\leq 10^2$	≤ 10

ND= not detected. 10^6= *E. coli* was detected in the first six 10^6 dilution.

E. coli for example is a specific indicator of faecal contamination when present in foods and is an indication of the extent of Good Agriculture Practice (GAP) and Good Hygiene Practices (GHP) observed by food producers and handlers. *E. coli* was detected in almost all the irradiated and non-irradiated cut cabbage was commensurate with the level of irradiation dose applied. According to Ghana Standard Board (GSB) legislation GS 995:2009 on minimally processed vegetables, acceptable range for *E. coli* should be less than or equal to 3 log_{10} cfu/g. Unirradiated cut cabbage had the highest TCC among all the samples examined. These high counts

of cut cabbage ready to be sold in the supermarkets far exceeded the permissible microbiological quality standard of vegetables ($<10^6$) for human consumption. Unfortunately these already contaminated cut cabbages are further stored for additional 9 days which compounded the situation. Even the irradiated cut cabbage samples could not remain wholesome throughout the 15days storage period with respect to TCC. Moulds found were *Mucor sp, Penicillium digitatum, and Rhizopus sp*. These fungi were found in unirradiated samples throughout the storage period. All these fungi play important roles in the shelf life of these cut cabbage samples since they are mostly known to be saprophytic organisms and would therefore feed on the damaged cut cabbage cells.These results agree with the report of Lopez *et al. (*2008) that with minimally processed cabbage and celery (endive packed), the increase of the surviving flora was lower in the irradiated samples than that obtained in the non-irradiated samples during storage. The ability of ionizing radiation to kill living organisms is mediated by water, thus water is the principal target of ionizing radiation. The radiolysis of water generates free radicals, and these radicals, in turn, attack other components such as deoxyribonucleic acid (DNA) in microorganisms. The immediate effect of irradiation on organisms is cell damage which is gradually repaired if possible by the organism after which they begin to multiply again (Fan *et al*., 2008). The present result indicated that all the irradiation doses reduced *E. coli* detection level from 4 to 2 $\log_{10}$ cfu/g in irradiated samples which was within the acceptable range for human consumption. However, it is worth noting that only the 3kGy treated samples could be considered safe throughout 15 days storage period (Table 1). Mukherjee *et al.* (2004) showed the presence of *E. coli* in number of vegetables; 22.4% in lettuce, 10.2% in cabbage and 13.3% in bokchoi. *E. coli* was detected and counts were typically ≤ 2 $\log_{10}$ cfu/g. Exposure to *E. coli* contamination is linked to higher rates of faecal contamination which presupposes high risk of foodborne diseases. For example, leafy crops fertilized with inadequately composted manure and those fertilized with animal manure were found to have a higher risk of *E. coli* contamination (Mukherjee *et al.,* 2004). Edmonds and Hawke, (2004) reported the presence of *E. coli* on watercress irrigated with contaminated water. *Salmonella spp* was not detected in any of the cut cabbage samples in this present study. Although *Salmonella spp*. was not detected, considering that this is minimally processed cabbage, the observed initial contamination for TVC and TCC is high enough to render it unsafe for human consumption by the GSB legislation GS 995:2009. According to the GSB legislation GS 995:2009 TVC $\leq 10^5$ on fresh minimally processed vegetable is acceptable. Total viable counts over 6.0 log cfu/g observed in almost all the cut cabbage samples on the 0 day is a concern which must be addressed. More so similar results have been reported by different researchers. Prakash *et al*., (2000) reported aerobic counts of 6.57 $\log_{10}$ cfu/g in diced celery while Farkas *et al*., (1997) found value of 6.0 $\log_{10}$ cfu/g for total viable counts in pre-cut carrots. The Enterobacteriaceae counts were higher than 5.0 log cfu/g in 58% and 17% of celery and cabbage respectively (Farkas *et al*., 1997). These reports on bacterial pathogens in fresh carrots and cabbage agree with the results of the present study. It is necessary to consider that in the cited references, some of these findings were not obtained from freshly cut vegetables. (that is, neither with a previous treatment nor minimally processed cabbage like the ones used in this work). The label on the products used for the study states: "ready–to-eat" within 9 days from the date of processing. This means that the consumption of these vegetables could be a potential risk for sensitive people such as the young, the old, the pregnant and the immune-compromised consumers. The observed microbial levels were detected in samples purchased immediately after cutting and packaging. However microbial levels are likely to increase under the refrigeration storage within the 9 days as recommended expiration date on package at the supermarket. Considering these minimally processed vegetables as ready-to-eat products, the microbiological (TVC) limits specified by the GBS legislation (GS 995:2009) should be less than 6 $\log_{10}$ cfu/g. The initial total mould and yeast detection in freshly cut cabbage samples for control and irradiated (1kGy) were significantly different from 2 and 3 kGy samples as there was no growth on the 2 and 3 kGy treated samples. Irradiation at 1 kGy eliminated two (*Mucor sp and Rhizopus sp*) out of the three fungi found in the unirradiated cut cabbage samples. However, irradiation doses of 2 and 3 kGy eliminated all the fungi from the cut cabbage samples i.e. mould and yeasts in fresh cut cabbage samples exposed to 2 and 3 kGy were below detectable level (< 1 $\log_{10}$ cfu/g) throughout the storage period. Mould and yeast counts on non-irradiated samples stored for 15 days did not show any additional fungi than those that were found initially. This is also in agreement with the report by Narvaiz *et al*., (2001) that in control salad samples, total aerobic mesophiles, coliforms, moulds and yeasts were well above the limits permitted in a “clean diet”. This bioburden was sufficiently reduced by 2 kGy irradiation dose to the safe levels.

5.1 Conclusions

It is worth noting that the high levels of total viable and coliform counts with the exception *E. coli* detected in this study using minimally processed cabbage from the

Supermarket renders unirradiated cut cabbage unsafe for human consumption according to both national and international food standards. Irradiation significantly reduced microbial population by 3-6 log cycles in the cut cabbage stored under refrigeration compared to the control. Generally, there was no significant differences ($p>0.05$) in microbial population obtained from samples treated with 2 and 3 kGy. Those treated 1 kGy were inferior in microbial quality. Therefore, it can be concluded from this study that the minimum dose for achieving microbial safety in cut cabbage was 2kGy. Irradiation doses up to 2 kGy could reduce microbial pathogens by up to 4 log cycles on fresh pre-cut cabbage. Taking into account effects of storage on irradiated cut cabbage such as increase microbial numbers and antioxidant activity, softening and browning, the appropriate shelf life for 2 kGy irradiated cabbage must not exceed ten (10) days at 8 ± 2^0C.

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