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Full Length Research Paper

Effects of gamma irradiation on the microbial quality and the shelf life of anchovies

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The effect of irradiation on microbial load and shelf-life of anchovies (*Engraulis encrasicolus*) was assessed in this study. Irradiation doses used in the study were 2.5, 5.0, 7.5 and 10 kGy. The assessment was carried out at 3 weeks intervals for the period of 9 weeks. Samples were analyzed for microbial load (total viable count, total coliform count and *Staphylococcus aureus*). Smoked samples were more contaminated as compared to sun-dried samples from both locations. A dose dependent effect was observed in all the samples obtained from both Chorkor and Keta ($p<0.05$). Samples from Keta were less contaminated for both smoked and sundried samples as compared to the samples from Chorkor whether sun dried or smoked ($p<0.05$). At 2.5 kGy, microbial load levels (CFU/g) for total viable count, total coliform count and *S. aureus* were below the standard (CFU/g of microbial load) set by the Ghana Standards Authority for microbial load in fish. This dose is the most appropriate dose for the decontamination and shelf extension of anchovy from Ghana.

Key words: Microbial load, processing methods, irradiation dose, shelf-life.

INTRODUCTION

Fishing is an extremely important economic activity in Ghana. It has been estimated that the fish resources in Ghana's water bodies support the livelihoods of a total of about 2 million people which includes fishermen, fish processors (including fish canneries and cold stores), traders and boat builders. These people, together with their dependents, account for about 10% of the Ghanaian population (Onumah et al., 2010).

The FAO indicated that annually, there are about 10 to 12 million tons postharvest losses of fish caused by spoilage. It is also estimated that about 20 million tons of fish in a year become wasted at sea which could possibly lead to further losses (FAO, 2010). It has been estimated that in high temperatures of the tropics of which Ghana is a part, fish deteriorate within 12-20 h after being caught, depending on the kind and size of fish. In order to reduce fish deterioration in the tropics, a considerable proportion of the landed catch is processed to preserve most of their catch by artisanal methods (FAO, 2001) in order to reduce losses. Fish deterioration and storage may be due to autolysis, rancidity, mechanical damages due to handling methods and the presence of spoilage

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organisms (Huss, 1994).

The major challenge worldwide including Ghana, is the unhygienic environmental conditions in which fish finds itself before and after capture and before it comes to the table for consumption (Debrah et al., 2011).

Fish without any preservative or processing measures is exposed to a number of physiological and microbial deterioration and thereby degrading the fish (Davies and Davies, 2009). The common traditional preservative methods used in Ghana are depuration, freezing, smoking, sun-drying and salting (Obodai et al., 2011).

Traditionally, harvested anchovies are sun-dried on the roadside or at the sea shore and sometimes on raised racks by artisanal fishermen. Sun drying (also known as open air drying) is one of the methods of preserving anchovies in Ghana; however, the duration of drying depends on the weather vagaries. Apart from sun drying, anchovies are exposed to smoke from fire wood for the purpose of preservation (Abolagba and Uwagbai, 2011).

Gamma irradiation does not only ensure food safety due to its ability to decontaminate food by inactivating pathogenic organisms but also extends the shelf life of the commodity that has been exposed to it (Mahapatra et al., 2005).

Arvanitoyannis and Stratakos (2010) reported that irradiation doses of 2 to 7 kGy can reduce important food pathogens or microbes such as *Salmonella, Listeria* and *Vibrio* spp., as well as many fish specific spoilers such as *Pseudomonaceae* and *Enterobacteriaceae*. Ionizing radiation treatment of food is an effective means of slowing down growth of pathogenic bacteria such as *Escherichia coli* and *Salmonella* (Olson, 1998; Thayer, 1994).

This study aimed to determine the effect of gamma irradiation on the microbial quality and the shelf life of anchovies harvested in two main anchovies fishing communities in Ghana.

MATERIALS AND METHODS

Experimental designs

The experimental design for the study on processed anchovies was a 2 × 2 × 2 × 5 factorial in completely randomized design (CRD) (two locations: Chorkor and Keta; source: processors and marketers; processing methods: smoked and sun-dried; and irradiation doses: control, 2.5, 5.0, 7.5 and 10 kGy).

Sampling procedure

A total of 1600 g of anchovies were collected from both locations (Chorkor and Keta). Two hundred grams (200 g) of sun-dried and 200 g smoked anchovies were collected randomly from processors (400 g) and marketers (400 g) from both locations.

Gamma irradiation

Packaged samples in polyethylene zip lock bags were irradiated at doses of 2.5, 5.0, 7.5 and 10.0 kGy at a dose rate of 1.43 kGy/h using the category IV cobalt 60 (^{60}Co) wet storage gamma irradiation source at the Ghana Atomic Energy Commission. Non-irradiated samples were used as control.

Microbial load analysis

The microbiological analysis was performed at the Food Microbiology Laboratory, Biotechnology and Nuclear Agriculture Research Institute (BNARI) according to the standard procedure of APHA (2000) using methods of serial dilution and pour plate to determine total viable count, total coliform count and *Staphylococcus aureus.*

Enumeration of total viable count (TVC)

Ten (10) grams of anchovies sample was aseptically weighed into sterile Petri dish, macerated in a stomacher and transferred into a sterile 90 ml peptone water in a 250 ml conical flask to make a 1:10 dilution of the anchovy sample. Each dilution bottle was agitated to re-suspend material that may have settled out during preparation and serially diluted to 10^6. 1 ml of each diluent was aseptically transferred into sterile well-labeled Petri dishes and pour-plated on plate count agar (PCA) in duplicates and aerobically incubated in inverted positions at 37°C (APHA, 2000).

Enumeration of total coliform count (TCC)

About 10 g of anchovies sample was aseptically weighed into sterile Petri dish, macerated in a stomacher and transferred into a sterile 90 ml peptone water in a 250 ml conical flask to make a 1:10 dilution of the anchovy sample. Each dilution bottle was agitated to re-suspend material that may have settled out during preparation and serially diluted to 10^6. 1 ml of each diluent was aseptically transferred into sterile well-labeled Petri dishes and pour-plated using eosin methylene blue agar (EMBA) in duplicates and aerobically incubated at ±37°C (APHA, 2000).

Enumeration of *Staphylococcus aureus*

About 10 g of anchovies sample was aseptically weighed into sterile Petri dish, macerated in a stomacher and transferred into a sterile 90 ml peptone water in a 250 ml conical flask to make a 1:10 dilution of the anchovy sample. Each dilution bottle was agitated to re-suspend material that may have settled out during preparation and serially diluted to 10^6. 1 ml of each diluent was aseptically transferred into sterile well-labeled Petri dishes and pour-plated using bird parker agar (BP) in duplicated and aerobically incubated at 37°C. Colonies were counted promptly after the incubation period using the Stuart colony counter-SC6+. Plates with 30-300 colonies or nearest to the 30-300 range were counted (including pinpoint colonies). Colonies were counted as colony forming units per gram of fish sample (CFU/g) (APHA, 2000).

Shelf-life (storage) studies

Packaged samples of both unirradiated and irradiated sun-dried and smoked anchovies were stored and visually assessed every three weeks for nine weeks. This was done to determine the effect of the irradiation doses (2.5, 5.0, 7.5 and 10 kGy) on the anchovy samples. The samples were stored in an enclosed

mesh shelf under ambient conditions (average temperature of 22°C and RH of 50%).

The samples were assessed visually for color change, moldiness, insects and pest attacks. This monitoring was done every three (3) weeks before samples were taken to the laboratory for microbial analysis. Shelf life was taken as the number of days or period for a sample to be contaminated or infested. The shelf-life of a product is a critical factor in both quality and profitability, and is influenced by several factors, such as light, heat, gases intrinsic to the product and stresses on the material.

Statistical analysis

Sample analyses were conducted in triplicates for the study. Results were expressed as mean values and the differences among means of both unirradiated and irradiated samples of smoked and sun-dried Anchovies (*Engraulis encrasicolus*) obtained from Chorkor and Keta were calculated using analysis of variance (ANOVA) and statistically significant differences were reported at $p<0.05$. The least significant difference (LSD) was conducted for independent sample t-test as required between two treatments. Data analyses were done with the use of GenStat software version 18.0.

RESULTS AND DISCUSSION

Microbial load analysis

All anchovy samples collected from both processors and marketers in Chorkor and Keta recorded total viable count, total coliform count and counts of *S. aureus*. Table 1 shows the results of analysis of microbial load in unirradiated smoked and sun-dried anchovies obtained from both Chorkor and Keta. Total viable count (5.660 CFU/g) of the samples were significantly ($p<0.05$) higher than total coliform count (3.621 CFU/g). *S. aureus* had least contamination (2.911 CFU/g) at week 0. Sun-dried samples obtained from Chorkor at week 0, had significantly ($p<0.05$) higher total viable count (5.633 CFU/g) as compared to sun-dried samples from Keta anchovies (4.490 CFU/g); as well as smoked samples from Chorkor (6.175 CFU/g) and Keta (6.042 CFU/g). There were no significant ($p>0.05$) differences in total coliform count between sun-dried (3.487 CFU/g) and smoked samples (3.645 CFU/g) obtained from Chorkor as compared to sun-dried (3.272 CFU/g) and smoked samples (3.487 CFU/g) from Keta at week 0 and also at 9th week. *S. aureus* had least contamination in sun-dried (2.575 CFU/g) and smoked anchovy samples (3.318 CFU/g) from Chorkor; and sun-dried (2.972 CFU/g) and smoked samples (2.778 CFU/g) from Keta respectively. There was a general decrease in microbial load with time. At the 9th week, the level of contamination had generally decreased in sun-dried samples obtained from Keta generally when compared when sun-dried samples obtained from Chorkor. Both locations had no significant ($p>0.05$) difference when anchovy samples were smoked even though some amount of contamination was recorded in the period of study (week 0, 3, 6 and 9). The quality and freshness of fish are known to rapidly deteriorate through microbial and biochemical mechanism and therefore, with thorough drying, microbes will reduce (Al-Jasser and Al-Jasass, 2014). In this study, samples that were sun dried had lower microbial contamination as compared to the smoked ones, which might be due to the effect of solar radiation on the microbes in the samples that were sun dried from both towns. The general decrease of TVC, TCC and *S. aureus* in unirradiated samples when stored may be attributed to the effective packaging of the anchovies which created an environment that was not conducive for the microbes to grow or multiply. The absence of oxygen would generally decrease multiplication of microbes even though initial oxidation may lead to rancid taste and off flavor and development of many different compounds from which some have even adverse effects on human health. After storing the unirradiated smoked fish for five (5) weeks, attack by insects was evidenced which is similar to the observation of Bari et al. (2000).

Effect of irradiation on microbial load of processed anchovies from Chorkor and Keta

Table 2 shows the effect of gamma irradiation on the microbial load of smoked and sun-dried anchovies obtained from both locations, respectively. There was a dose dependent effect on the microbial load as there was a general reduction in TVC, TCC and *S. aureus* in all the samples irradiated at 2.5, 5.0, 7.5 and 10.0 kGy as compared to the unirradiated samples (whether sun dried or smoked).

Total viable count was high in smoked samples (4.502 CFU/g) as compared to sun-dried samples (3.393 CFU/g) at 0 kGy and least in smoked samples (1.158 CFU/g) and in sun-dried samples (1.607 CFU/g) at 5.0 kGy respectively.

Microorganisms in samples irradiated at 7.5 and 10 kGy were below the detection limit for both processing methods. There was high CFU/g of TVC as compared to *S. aureus.* There was significant ($p<0.05$) difference between the microbial loads of irradiated samples from control to 10 kGy.

Lower microbial load was recorded in sun-dried samples as compared to smoked samples. Lower CFU/g of *S. aureus* were recorded in sun-dried samples (1.308 CFU/g) and smoked samples (1.453 CFU/g) at 2.5; at 5.0 kGy, 0.263 and 0.554 CFU/g in sun dried and smoked samples, respectively. There was significant ($p<0.05$) difference (in the contamination levels) in the processing methods used for the treatment and preservation of *E. encrasicolus.*

Microbial load of smoked anchovies reduced from 4.502 (control) to 1.158 CFU/g for TVC in the lower doses of at 5 kGy while the same trend was observed for the sun-dried samples which reduced from 3.393 to

Table 1. Microbial load of unirradiated sun-dried and smoked anchovies obtained from Keta and Chorkor.

Microbial load (CFU/g)	Processing Methods	Locations	Storage period (weeks)				
			0	3	6	9	Mean
Total viable count	Sun dried	Keta	4.490*	3.542	2.94	2.602	3.394
		Chorkor	5.633	5.597	3.668	2.428	4.332
	Smoked	Keta	6.042	5.117	4.285	2.265	4.430
		Chorkor	6.175	6.082	3.023	2.562	4.500
		Mean	5.66	5.085	3.479	2.464	
Total coliform count	Sun dried	Keta	3.272	2.62	2.59	2.047	2.632
		Chorkor	3.487	3.412	2.938	2.202	3.001
	Smoked	Keta	4.08	3.432	2.972	2.002	3.122
		Chorkor	3.645	3.645	3.212	2.163	3.166
		Mean	3.621	3.277	2.928	2.104	
Staphylococcus aureus	Sun dried	Keta	2.972	2.31	2.547	1.605	2.359
		Chorkor	2.575	1.985	1.767	1.62	1.987
	Smoked	Keta	2.778	2.243	2.313	1.873	2.302
		Chorkor	3.318	2.917	2.32	1.692	2.562
		Mean	2.911	2.364	2.237	1.698	

LSD (5%); Microbial load = 0.1939; processing methods = 0.1583; storage = 0.2239; microbial load x processing methods x storage = 0.2743; * = $\log_{10}$

Table 2. Effect of gamma irradiation on microbial load on smoked and sun-dried anchovy.

Doses (kGy)	Microbial Load (CFU/g)	Processing method	
		Smoked	Sun dried
Control	Total viable count	4.502*	3.393
	Total coliform count	3.121	2.632
	Staphylococcus aureus	2.302	2.358
2.5	Total viable count	2.333	2.305
	Total coliform count	1.902	1.794
	Staphylococcus aureus	1.453	1.308
5.0	Total viable count	1.158	1.607
	Total coliform count	1.017	0.271
	Staphylococcus aureus	0.554	0.263
7.5	Total viable count	ND	ND
	Total coliform count	ND	ND
	Staphylococcus aureus	ND	ND
10	Total viable count	ND	ND
	Total coliform count	ND	ND
	Staphylococcus aureus	ND	ND

LSD (5%): Dose = 0.184; microbial load = 0.184; processing methods = 0.150; dose x microbial load x processing methods = 0.319; *log 10; ND = no detection.

1.607 CFU/g when a dose of 5.0 kGy was applied. CFU/g of the organisms investigated in this study, were below detection limits when doses greater than 5 kGy was applied to the samples in general.

During the present study, the results showed that contamination levels of samples that were irradiated at

Table 3. Effect of gamma irradiation on the shelf life of smoked and sun dried anchovy obtained from Chorkor and Keta.

Dose (kGy)	Chorkor		Keta		Mean
	Smoked	Sun dried	Smoked	Sun dried	
Control	3	5	5	7	5
2.5	9	9	9	9	9
5	9	9	9	9	9
7.5	9	9	9	9	9
10	9	9	9	9	9
Means	9.75	10.25	10.25	10.75	

2.5 kGy was found to be below the guidelines set by the Ghana Standard Authority: (Total heterotrophic bacteria count: 1 x 10^6 CFU/g; Total coliform count: 1 x 10^4 CFU/g; *Bacillus cereus* count: 1 x 10^4 CFU/g), which implies that irradiation to a dose of 2.5 kGy is enough to decontaminate the processed anchovies to meet standards set by the GSA. Mahin et al. (2011) stated that high radiation doses of 2.5 and 5.0 kGy reduced TVC by 3 logarithmic cycles for mola (*Amblypharyngodon mola*) at -20°C for 6 months.

Effect of gamma irradiation on the shelf-life of processed Anchovies

Table 3 shows the shelf-life of irradiated smoked and sun-dried anchovies obtained from both locations when stored for a period of 9 weeks at a 3-week interval studies. Smoked but unirradiated samples obtained from Chorkor showed signs of pest destruction at the 3rd week as well as sun-dried samples in week 5. Samples obtained from Keta at the 5th and 7th week for smoked and sun-dried samples respectively, had signs of insect pest damage. Samples exposed to doses of 2.5, 5.0, 7.5 and 10 kGy had no records of insect destruction within the 9 weeks of storage.

Microbial load in all the samples (both sun dried and smoked samples) reduced as the doses of gamma irradiation increased (apart from unirradiated samples that had higher microbial load). It was observed that samples obtained from Keta stored better than those from Chorkor. Unirradiated, sun-dried samples remained for up to a period of 7 weeks without pest damage Irradiation doses of 2.5, 5.0, 7.5 and 10 kGy when stored for 9 weeks showed no sign of pest infestation. This indicated that, even at a dose of 2.5 kGy, the samples could be stored at a period of 9 weeks and even beyond without insects or pest damage. Both irradiated smoked and sun-dried samples could possibly be stored beyond the 9 weeks duration. It could also be concluded that, 2.5 kGy dose applied on smoked and sun-dried anchovies is enough to eliminate microbes and extend the shelf-life of the product. From the study, generally, both smoked and sun-dried anchovies obtained from processors and marketers in Keta are more hygienic than samples from Chorkor. This confirms the assertion of Kombat et al. (2013) that unhygienic environment in which fish are caught and processed have influence on the contamination level in the fish. Thus, the differences observed in the contamination levels of the samples from Keta and Chorkor might be due to the differences in the contamination of the environment in which the fishes were obtained from.

Conclusion

Unirradiated *E. encrasicolus* from both locations had some level of contamination which was above the threshold of the Ghana Standards Authority and therefore not wholesome for human consumption. The application of gamma irradiation reduced the level of contamination in the anchovies thus, making it safe for consumption and also could be preserved for a longer period of time for future use. At a dose rate of 2.5 kGy, effectiveness of the irradiation was able to eliminate harmful microbes in the fish. The use of irradiation was also able to keep the fish samples for the duration of study. After the 9 weeks of storage period of *E. encrasicolus*, sun-dried samples were less contaminated than smoked samples. Irradiation is a safe and effective method of food preservation used in many countries all over the world.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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