Radiation decontamination and disinfestation of salted dried tilapia fish *(koobi)*

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ABSTRACT

Salted dried tilapia (*Oreochromis niloticus*) fish locally called *koobi* was investigated with the view of establishing the effective radiation dose for controlling microbial and insect activity on the product. Total viable count (TVC) of market samples of *koobi* ranged between $\log_{10}$ 4.11 - 6.78 cfu/g, whilst mould and yeast count ranged between $\log_{10}$ 1.38-3.38 cfu/g. *Staphylococcus aureus* counts ranged between $\log_{10}$ 2.85 - 4.15 cfu/g. After 4 weeks' storage under ambient conditions, total viable count increased to $\log_{10}$ 7.5 ± 2.5 cfu/g. Significant reduction in total viable count was observed after treatment with gamma radiation. A least square regression fitted through the data points indicated that 1.3 kGy would be required to reduce the microbial population on the product by one log cycle. Insects and pink colonies of halophilic bacteria were observed on all the non-irradiated samples after 4 weeks' storage. Treatment with 3 kGy gamma radiation eliminated all insect forms, while microbial population was controlled with TVC ranging between $\log_{10}$ 1.9 ± 1.1 and $\log_{10}$ 2.7 ± 1.6 cfu/g throughout the 16 weeks' storage period. The proliferation of halophilic bacteria and subsequent appearance of pink colonies on irradiated *koobi* was suppressed until the 16th week. Irradiation, therefore, extended the shelf-life of *koobi* from 4 to 15 weeks.

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RÉSUMÉ

NKETSIA-TABIRI, J.: *Décontamination et dés infestation par radiation de poisson tilapia (*koobi*) salé et séché.* Le poisson tilapia *(Oreochromis niloticus)* salé et séché localement appelé *koobi* était étudié en vue d'établir la dose de radiation efficace pour le contrôle d'activités d'insecte et de microbe sur le produit. Le compte viable total d'échantillons du marché de *koobi* variait de 4.11 - 6.78 cfu/g alors que le compte de moisissure et levure était de $\log_{10}$ 1.38 - 3.38 cfu/g. Les comptes de *Staphylococcus aureus* variaient de $\log_{10}$ 2.85 - 4.15 cfu/g. Après 4 semaines de conservation sous les conditions ambiantes, le compte viable total augmentait à $\log_{10}$ 7.5 ± 2.5 cfu/g. Une réduction considérable en compte viable total était observée après le traitement avec les rayons gamma. Une moindre regression carrée ajustée à travers les points des données indiquaient que 1.3 kGy serait nécessaire pour réduire la population microbienne sur le produit par un cycle de log. Les insectes et les colonies roses de bactérie halophilique étaient observées sur tous les échantillons non-irradiés après 4 semaines de conservation. Traitement avec les rayons gamma 3 kGy éliminait tous les formes d'insectes alors que la population microbienne était contrôlée avec TVC variant entre $\log_{10}$ 1.9 ± 1.1 et $\log_{10}$ 2.7 ± 1.6 cfu/g pendant la période de 16 semaines de conservation. La prolifération de bactérie halophilique et l'apparition suivante des colonies roses sur *koobi* irradié était réprimée jusqu' à la 16^{me} semaine. Irradiation, par conséquent, prolongeait la durée de conservation avant vente de *koobi* de 4 à 15 semaines.

Introduction

Curing processes such as salting, fermentation, sun-drying, and smoking contribute immensely to the preservation of fish in most developing countries. The methods used are simple and have developed over the years according to local traditions. In Ghana, over 60 per cent of the total fish landed is cured before sale and consumption (FAO, 1981). Cured fish products are relatively more stable than fresh fish under ambient tropical conditions; nevertheless, they suffer considerable losses due to spoilage by insects, bacteria, and moulds (FAO, 1981; Poulter, Ames & Evans, 1988; James, 1984). Much of the losses has been attributed to inherent characteristics of traditional curing technologies. For example, while open sun-drying can effectively reduce moisture content and control spoilage, it also exposes the fish to microbial contamination and infestation. Another source of infestation is the solar salt used for salting fish.

Salt-tolerant bacteria, such as *Halobacterium* and *Halococcus* species in solar salt (Sefa-Dedeh & Youngs, 1976) persist on the dried fish and produce characteristic pink colonies during growth. Visible appearance of pink colonies and insects on salted dried fish products is undesirable and cause physical and economic losses. Moulds and yeasts are the major spoilage agents of such intermediate moisture foods as *koobi;* and for these, fungistatic agents such as potassium sorbate are recommended. Unfortunately, however, these agents are less effective against certain bacteria that produce putrid odour and discolouration of cured fish products.

Although some wide-spectrum chemicals and fumigants are effective against fungi and bacteria, they have negative health and environmental implications; some have already been banned (Halliday, 1986; IAEA, 1993). This has necessitated the search for alternative control measures. The use of high temperatures to eliminate and control microbial proliferation is incompatible with products which need to retain their uncooked status. Fortunately, radiation processing, which is basically a cold process, can kill microorganisms and insects, reduce post-harvest losses, and enhance food safety and quality (Loaharanu & Thomas, 2001). Consequently, irradiation is increasingly being applied to disinfest and decontaminate foods and other products, while gaining recognition as an effective quarantine treatment for agricultural produce (IAEA, 1989; IAEA, 1993; IAEA, 2002).

This study determined the effective radiation dose for controlling microbial and insect spoilage of salted dried tilapia fish (*koobi*), a delicacy in Ghana.

Materials and methods

Sampling

Eight batches of tilapia, *Oreochromis niloticus (koobi),* were bought from two markets in Accra (Madina and Agbogbloshie markets). All the samples were randomly mixed and packaged (one fish/pack) in low-density, sterile, polyethylene pouches (150 μ gauge).

Establishment of effective radiation dose

An 8 × 4 experimental design, representing eight replicates and four doses (0, 1, 2, 3 kGy) of gamma radiation, was used to determine the effective radiation dose for controlling microbial and insect activity on *koobi*. Thirty-two of the packaged *koobi* were randomly grouped into four batches of eight packages. Each batch was randomly assigned one of the four doses and irradiated accordingly, using gamma radiation from a Cobalt-60 source at a dose rate of 531 Gy/h. All the four batches were transported to the irradiation facility, but only those to be irradiated were treated. The control and irradiated samples were then returned to the laboratory for microbiological and chemical analyses.

Storage tests

An 8 × 2 × 6 randomized complete block design, representing eight replicates, two radiation doses (0, 3 kGy) and six storage times (0, 4, 8, 12, 16, 20 weeks), was used for the study. Ninety-six samples of packaged *koobi* were prepared and randomly assigned treatments indicated by the design. All the samples were transported to the irradiation facility and treated. The control and irradiated samples were then transported to the storage room (under ambient conditions) and arranged on shelves. At the end of the storage times, the control and irradiated samples were removed from the shelves and visually examined for insects and pink colonies; they were also subjected to microbiological analysis.

Microbiological analysis

Ten grams of the salted dried fish was homogenized in 90 ml of 0.1 per cent sterile peptone solution. From this initial 1 in 10 dilution, serial dilutions up to 10^{-7} were prepared. Using the pour plate method, total viable count was determined on Plate Count Agar at 37 °C/24 h. *Staphylococcus aureus* count was determined on Baird-Parker Agar incubated at 36 °C/48 h, while mould/yeast count was determined on Oxytetracycline Glucose Yeast Extract Agar incubated at 28 °C/48 h (1978).

Chemical analyses

Moisture content was determined by the oven-drying method, and salt content by the precipitation of salt as silver chloride (AOAC, 1975). Water activity of the *koobi* samples was calculated based on moisture and salt contents, using the equation (Lupin, Boeri & Moschair, 1981):

$$Aw = 1.002 - 0.042\, m$$

(where 'm' is the molality of salt in the fish)

Extent of pinking and insect infestation

The number of packages (out of the eight replicates) containing *koobi* with visible pinking and/or insects were counted and expressed as a percentage of the total number of packages.

Results and discussion

Moisture, salt and water activity

Table 1 shows the moisture and salt contents of the market samples of the salted dried fish to be 45.9 ± 4.1 and 17.7 ± 3.4, respectively. Using the moisture and salt data, the water activity of the salted dried fish was calculated, ranging between 0.69 and 0.83, with a mean value of 0.77 ± 0.05. Water activity has direct bearing on microbial activity in that the growth of microorganisms is inhibited as water activity falls below 0.90. However, some fungi and halophilic bacteria can grow at water activity levels below 0.8 (Olley, Doe & Herwati, 1988). The low water activity of the market samples of *koobi* can be attributed to the high salt and low moisture levels. This results from the practice of regular sun-drying of *koobi* by processors and handlers to extend its shelf-life. Although the low water activity of *koobi* suppresses the growth of a wide range of spoilage organisms, some persist and over time degrade the product quality and reduce economic value. Hence, the need for additional treatment for effective control of surviving microbes and insects.

TABLE 1

Moisture and Salt Contents of Salted Dried Tilapia Fish

Batch no.	*% moisture*	*% salt (dmb)*	*Water activity*
1	41.7	25.5	0.69
2	52.8	17.4	0.74
3	52.0	18.7	0.72
4	44.6	15.0	0.81
5	44.5	21.6	0.72
6	43.1	15.5	0.81
7	44.1	13.4	0.83
8	44.7	15.8	0.80
Mean ± sd	45.9 ± 4.1	17.7 ± 3.4	0.77 ± 0.05

*Radiation treatment and microbiological quality of salted dried fish (*koobi*)*

Total viable count (TVC) of non-irradiated samples ranged from $\log_{10}$4.11 - 6.78 cfu/g (Table 2). Mould and yeast were present on all the eight batches of *koobi*, with counts ranging between $\log_{10}$ 1.38 - 3.38 cfu/g. *Staphylococcus aureus* count ranged between $\log_{10}$ 2.85 - 4.15 cfu/g. Although *S. aureus* is heat sensitive, certain strains produce heat-tolerant toxins; hence, the elimination of this potential pathogen would improve the microbiological safety of *koobi*.

Fig. 1 shows the reduction in microbial population on the salted fish when treated with 1-3 kGy gamma radiation. Analysis of variance test performed on the data indicated no significant difference ($P \geq 0.05$) between the TVC of the non-irradiated and those treated with 1 kGy gamma radiation. A least square regression fitted through the

TABLE 2

Microbiological Quality of Salted Dried Tilapia Fish

	Counts in $\log_{10}$ cfu/g		
Batch no.	*TVC*	*Moulds/Yeasts*	S. aureus
1	4.11	2.32	2.85
2	6.78	3.38	3.97
3	5.49	2.08	3.62
4	5.08	2.78	3.48
5	4.91	2.85	3.68
6	4.54	1.38	3.91
7	5.36	2.62	4.15
8	5.30	2.51	3.96

TVC = Total viable count

data points (Fig. 1) indicated that:

$$Y = 5.6 - 1.3\,X$$
$$R^2 = 0.93$$

(where Y = log N/No and X = dose)

Thus, 1.3 kGy would be required to reduce the microbial population on *koobi* samples by 1 log cycle. Maha *et al.* (1989) reported that the use of a combination of 0.1 per cent potassium sorbate and 2-4 kGy gamma radiation resulted in 2 - 4 log cycle reduction in total bacterial count on salted fish; in the absence of sorbate, however, higher radiation dose was required (Maha *et al.,* 1989). Thus, the impact of preservatives such as sorbate, acetic acid, and sodium chloride caused a reduction in the effective radiation dose.

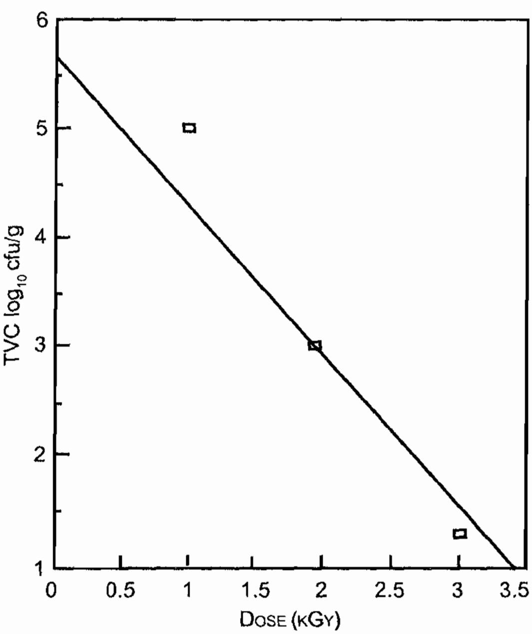

Fig. 1. Effect of radiation dose on microbial population of salted dried fish.

With respect to the presence of *Staphylococcus aureus,* which is a potential pathogen, the radiation dose applied to *koobi* should be adequate to substantially reduce its population. The radiation sensitivity of *S. aureus,* expressed in terms of D_{10} value (or decimal reduction dose), is 0.10 kGy when the organisms are suspended in nutrient broth, and 0.60 kGy when in dry medium (IAEA, 1982). Considering the population ($\log_{10}$ 2.85 - 4.15 cfu/g) of *S. aureus* on *koobi* in this study (Table 2), the application of 3 kGy was considered adequate to substantially reduce spoilage and potential microbial pathogens on *koobi*. In a preliminary study (Nketsia-Tabiri, 2000), it was found that *S. aureus* occurred not only on all the batches of *koobi* bought from the market, but also in greater numbers than *E. coli*. The reported D_{10} value for *Escherichia coli,* when suspended in nutrient broth, is between 0.10 and 0.20 kGy (IAEA, 1982).

Quality of irradiated koobi *during storage*

Fig. 2 shows the total viable count on irradiated *koobi* during the 20 weeks' storage under ambient conditions. After 4 weeks' storage, the mean TVC for the non-irradiated samples was $\log_{10}$ 7.5 ± 2.5 cfu/g. The samples had deteriorated and were discarded. For the irradiated (3 kGy) samples, however, the mean TVC ranged between $\log_{10}$ 1.9 ± 1.1 and $\log_{10}$ 2.7 ± 1.6 cfu/g throughout the 16 weeks' storage period. Discolouration of the salted fish, resulting from growth of pink colonies of halophilic bacteria, was not apparent on any of the irradiated samples until the 16th week when one of the eight packs of the batch of

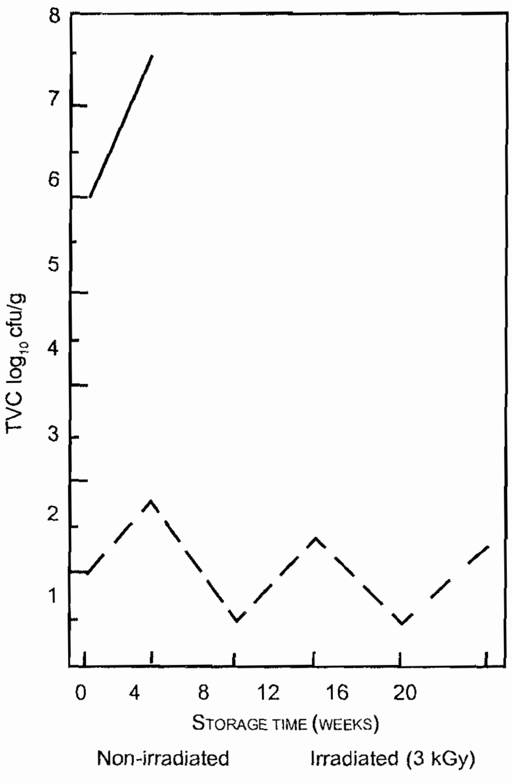

Fig. 2. Changes in microbial population of non-irradiated and irradiated (3 kGy) salted dried fish during storage under ambient conditions.

irradiated samples or 12.5 per cent of that batch showed pinking.

Halophilic bacteria are inherent to solar salt used for salting *koobi*. As subsequent sun-drying, which salted tilapia is subjected to, does not eliminate halophiles, other treatments are necessary to control the proliferation of halophiles and discolouration of *koobi* during storage. The results of this study suggested that 3 kGy gamma radiation controlled the growth of halophiles and pinking of *koobi* for 15 weeks. Maha *et al.* (1989) observed that salted fish samples irradiated with 4 kGy were mould-free after 15 weeks' storage under tropical ambient conditions, but the non-irradiated samples as well as those treated with 2 kGy gamma radiation were mouldy within 10 and 17 days, respectively (Maha *et al.*, 1989). Ahmed *et al.* (1989) also reported that 4 kGy was needed to control bacterial and fungal growth on sun-dried fish.

Live insects were found on the non-irradiated samples within 4 weeks' storage, but absent from all the irradiated samples throughout the 20 weeks' storage period. This observation was expected because insects are more susceptible to irradiation. Ahmed *et al.* (1989) reported that 1 kGy gamma radiation was sufficient to eliminate all insect forms on sun-dried fish (Ahmed *et al.*, 1989).

Conclusion

Treatment of *koobi* with 3 kGy gamma radiation was sufficient to control microbial spoilage and associated discolouration (pinking/reddening) for 15 weeks. Insect disinfestation required lower doses (1 kGy). Effective packaging in combination with 3 kGy gamma radiation could increase the income of *koobi* processors and handlers by reducing losses and enhancing quality during long-term storage.

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