PRESERVATION OF SEMI-PERISHABLE FOOD AND DEVELOPMENT OF CONVENIENCE FOOD USING A COMBINATION OF IRRADIATION AND OTHER PHYSICOCHEMICAL TREATMENTS*

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Abstract

PRESERVATION OF SEMI-PERISHABLE FOOD AND DEVELOPMENT OF CONVENIENCE FOOD USING A COMBINATION OF IRRADIATION AND OTHER PHYSICOCHEMICAL TREATMENTS.

Studies were carried out on the development and irradiation preservation of semi-dried fish, e.g. *Labeo rohita* (Ruhi) and *Cirrhiuas mrigala* (Mrigel), the extension of shelf-life at ambient temperature, and the improvement in the microbiological quality of sealed, ready to eat, commercially prepared fish kebabs by a combination of gamma irradiation with spices and an acidulant such as ascorbic acid. In the processing of semi-dried fish, the combination treatment of a salt dip and irradiation at a dose of 4 kGy extended the shelf-life by more than 3 months. Kebabs prepared in the laboratory and irradiated at a dose of 5 kGy were found to have a shelf-life of up to 6 months at room temperature. With commercially prepared fish kebabs collected from ordinary and sophisticated food shops, the maximum shelf-life extension was 14 days for the 5 kGy treated samples stored at ambient temperature. The microbiological quality of such kebabs indicated that the fish used was of poor quality, resulting in a limited shelf-life, even after chemical and irradiation treatments. Inoculated pack studies of *Clostridium botulinum* spores showed that when oil fried, the kebab size had a definite effect on heat penetration, and consequent spore reduction. No spores were recovered from the 5 kGy irradiated fried kebabs.

1. INTRODUCTION

In recent years, with the urbanization of societies, the demand for ready to eat convenience food commodities has increased. These food items must be safe, visually

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attractive, organoleptically acceptable, nutritious, convenient to prepare and serve, with minimal use of preservatives (chemicals), and available throughout the year. Consumers have become increasingly aware of the microbiological risks associated with food and, in particular, with ready to eat food. As a result, increased regulatory controls have been introduced to ensure the safety of food.

Minimally processed, chilled foods have been marketed in developed countries to satisfy the demand for convenient and fresh food products. However, such food could lead to the introduction of new microbiological risks, in view of the emerging problems related to certain pathogenic microorganisms that can multiply at chilled temperatures, e.g. *Listeria* sp. and *Yersenia* sp. [1]. Combination treatments involving irradiation can ensure the safety of such products, while retaining the quality demanded by the consumer. Developing countries such as Bangladesh require wholesome foods with a prolonged shelf-life. Irradiation in combination with other preservation techniques can be of help in developing such convenient food items, thus reducing food losses and promoting distribution.

The objectives of the present study were: (1) the development and irradiation preservation of semi-dried fish, (2) the extension of shelf-life at ambient temperature, and (3) improvement in the microbiological quality of sealed, ready to eat, commercially prepared fish kebabs by a combination of gamma irradiation with spices and an acidulant such as ascorbic acid.

2. MATERIALS AND METHODS

2.1. Fish samples

Two varieties of carp, *Labeo rohita* (Ruhi) and *Cirrhiuas mrigala* (Mrigel) (medium sized, 12 in long[1]), in prime condition were collected from the distribution point in presterilized polyethylene pouches. These were used for preservation experiments using a combination treatment involving semi-drying and irradiation, and also for the development of shelf stable convenience food (fish kebabs).

2.1.1. Processing of fish for semi-drying

The fish were gutted, beheaded, washed thoroughly and then processed group wise for controlled drying after the following pretreatments: (1) blanching at 90°C for 1 min; (2) blanching at 80/90°C in 2/5% brine for 2 min; (3) soaking in a 20% (mass/vol.) salt solution overnight, and then washing away the salt; and (4) a control

[1] 1 in = 2.54×10^1 mm.

group, with no pretreatment. All the salt solutions were prepared on a mass/vol. basis.

2.1.1.1. Sun drying

The fish samples were dried in the sun in a solar tent, where the exposed temperature fluctuated between 24 and 29°C. The samples were dried for as long as several days, until the moisture content had dropped to 45–55%, and ultimately to 28–35%.

2.1.1.2. Oven drying

Two temperature ranges were used that aimed at regulating the drying rates: (1) 60–80°C for rapid drying; and (2) 40–50°C for slow drying.

2.1.2. Laboratory processing of fish for convenience food

The fish were descaled, beheaded, gutted, cut into 2.5 × 12 cm pieces and washed in tap water. The fish pieces were blanched in boiling water for 5 min and excess water was allowed to drain off. The fish flesh, excluding the bones and skin, was then collected for subsequent kebab preparation.

The following ingredients were well mixed with the blanched fish flesh: flour = 7.5; ginger paste = 2.8; cumin powder = 1.5; clove powder = 0.15; and table salt = 0.50 g/100 g. Finally, 4–5 × 3–3.5 × 1.5 cm kebabs were prepared. All the preparations were carried out under hygienic conditions.

The kebabs were fried for 5 min in soyabean oil and cooled in a laminar air flow cabinet. They were then packed aseptically in previously sterilized (25 kGy) polyethylene pouches. One set of kebabs was maintained as the control (non-irradiated) and a second set was subjected to a 5 kGy gamma irradiation dose. The two sets were then stored, both at room temperature and at approximately 4°C in a refrigerator.

2.1.3. Treatment of commercial kebabs for shelf-life extension

A survey was conducted to assess the microbiological status of the different, indigenously prepared and processed, ready to eat food samples in various categories of shop and restaurant in Dhaka City. Meat kebab, fish kebab and vegetable roll samples were collected aseptically in presterilized polyethylene bags from ordinary (open footpath shops for low income groups) and sophisticated (prepared food kept under hygienic conditions and generally consumed by affluent people) shops.

The commercial fish kebabs were ordered and collected from a reputable restaurant. Two identical batches of fish kebab were supplied, of which one contained

1% ascorbic acid as the acidulant. The two batches of kebab were oil fried for 3 min. After cooling, they were placed in presterilized containers and transported to the laboratory. The kebabs were then individually sealed aseptically in presterilized polyethylene pouches on a laminar bench. The two batches, one with ascorbic acid and the other without, were again divided into three portions, thus forming six subgroups. One group was maintained as the control, the second irradiated at 2.5 kGy, and the third at 5 kGy at room temperature (32°C). All six subgroups were stored at ambient temperature on shelves for storage studies and quality assessment.

2.2. Packaging material

The presterilized (25 kGy), sealed, polyethylene pouches were used as the packaging material for all the experiments on semi-dried fish and for all the convenience food.

2.3. Irradiation

Irradiation was applied to all the samples from a ^{60}Co gamma source of 25 038 Ci (Atomic Energy of Canada Ltd Gammabeam 650).[2]

2.4. Quality assessment

Four major parameters, i.e. the moisture content and the microbiological, chemical and organoleptic scores, wherever applicable, were monitored periodically for quality assessment of the control and the treated and irradiated fish samples/kebabs stored at an ambient or at a refrigerated temperature.

2.4.1. Moisture content

The moisture content of all the samples was determined according to the method of Ramayana [2].

2.4.2. Microbiological parameters

The microbiological counts of all the samples were scored using the decimal dilution technique, followed by standard spread plate counts, as described by Sharp and Lyles [3]. Identification was carried out according to Ref. [4].

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[2] 1 Ci = 3.70×10^{10} Bq.

The media used for the microbiological study were nutrient agar (Difco, United States of America) for the total bacterial counts, MacConkey agar (Difco) for the total coliform counts, staphylococcus medium No. 11 (Difco) for the total staphylococcus counts, starch ampicillin agar for the *Aeromonas* counts, selenite broth (Difco) for the enrichment of *Salmonella*, SS agar for the detection of *Salmonella*, potato dextrose agar for the total fungal counts, and thioglycolate agar for the anaerobic microbial counts.

2.4.3. Chemical parameters

The total volatile nitrogen (TVN) value, the peroxide value (POV) and the free fatty acid (FFA) value of the control and treated fish kebab samples were estimated at different storage times. These three parameters were taken as the chemical indices of freshness for the fish kebabs.

The TVN value of the fish kebabs was determined by the method of Farber and Ferro [5], and the POV value according to the method of the Association of Official Analytical Chemists [6]. Determination of FFA was carried out using the method described in Ref. [7].

2.4.4. Organoleptic tests

Sensory evaluation of the shelf-life of fish kebabs was done by organoleptic tests scored on a 9 point hedonic scale, as described by Peryam and Pilgrim [8].

2.5. Inoculated pack study with botulinum spores

An inoculated pack study was conducted with *Clostridium botulinum* spores on two sets of kebab: one large (25 g), and the other small (5 g); different methods of preparation were used.

The fish paste used for the preparation of the kebabs was first sterilized by irradiation at 30 kGy. Three methods were applied to test the efficacy of killing of the microbes during oil frying: (1) the normal method; (2) the spongy method, by adding baking powder to make the kebab spongy; and (3) the porous method, by piercing the kebab with a needle during the oil frying. Each kebab was then injected with 0.2 mL of a 10^6 spore suspension of type E *C. botulinum*. The kebabs were then sampled as: (a) non-irradiated, unfried; (b) irradiated, unfried; (c) non-irradiated, fried; and (d) irradiated, fried. Irradiation in all cases was 5 kGy. All the samples were analysed for spore recovery on the day of treatment. Spore production and recovery were determined in thioglycolate broth and thioglycolate agar media, respectively.

TABLE I. THE MOISTURE CONTENT, TOTAL BACTERIAL COUNTS AND TOTAL COLIFORM COUNTS OF FISH SUBJECTED TO A COMBINATION TREATMENT OF BLANCHING IN NaCl, OVEN DRYING AND IRRADIATION

Treatment	Moisture content (%)	Bacterial counts with storage time (d)						Organoleptic scores (d)	
		Total bacterial counts (CFU/g)			Total coliform counts (CFU/g)				
		0	7	28[a]	0	7	28	7	28
Fresh fish	79	2.2×10^3	4.2×10^8	UC $\times 10^8$	4.1×10^1	< 10	0	4	1
Blanched in 2% NaCl for 2 min	75	1.7×10^2	3.4×10^8	UC $\times 10^8$	< 10	< 10	0	4	1
Blanched in 2% NaCl for 2 min and dried at 60°C for 8 h	70	6.8×10^1	6.0×10^7	UC $\times 10^8$	< 10	< 10	0	5	4
Blanched in 2% NaCl for 2 min, dried at 60°C for 8 h and irradiated at 5 kGy	70	< 10	2.5×10^5	5.7×10^6	< 10	0	0	7	6
Blanched in 5% NaCl for 2 min	70	2.7×10^1	2.3×10^3	UC $\times 10^8$	< 10	< 10	0	4	3
Blanched in 5% NaCl for 2 min and dried at 80°C for 5 h	48	< 10	1.7×10^1	1.4×10^6	0	0	0	6	5
Blanched in 5% NaCl for 2 min, dried at 80°C for 5 h and irradiated at 5 kGy	45	0	0	0	0	0	0	7	7

[a] UC = unknown counts.

Scoring: 9 = like extremely; 8 = like very much; 7 = like; 6 = like slightly; 5 = neither like nor dislike; 4 = dislike slightly; 3 = dislike; 2 = dislike very much; 1 = dislike extremely.

3. RESULTS AND DISCUSSION

3.1. Effects of salt concentration, drying time and temperature on the moisture content and shelf-life of fresh fish

The moisture content of fresh fish was 79%, as shown in Table I. The total bacterial counts and the organoleptic scores indicated that fresh fish spoiled within 7 days. Blanching in 2% NaCl for 2 min and drying at 60°C for 8 h followed by irradiation at 5 kGy helped to extend the shelf-life to up to 7 days, both microbiologically and organoleptically, with the moisture content reduced to 70%.

On the basis of the microbiological and organoleptic findings (Table I) it was observed that a combination treatment of blanching in 5% NaCl for 2 min, drying at 80°C for 5 h and then irradiation with a 5 kGy dose extended the shelf-life of the fish to beyond 4 weeks. The moisture content was reduced to within the desired range (45–55%).

The shelf-life of the semi-dried fish depended on the pretreatment used, which exerted a profound controlling effect on the growth and development of the spoilage microflora. The shelf-life of the salt dipped fish containing 7–8% salt in the dry product was longer, more than 1 month (Table II), than that of the samples without a salt dip. Irradiation (4 kGy) of the salt dipped product increased the shelf-life twofold (Table II). In comparison, the sun dried products had a shorter shelf-life, probably because of the poor hygienic conditions to which the fish were exposed during drying.

The semi-dried fish were stored under ambient temperature for the storage studies and analysed periodically for their organoleptic properties. The organoleptic

TABLE II. EFFECT OF DRYING METHODS ON THE SHELF-LIFE OF IRRADIATED, SEMI-DRIED FISH STORED UNDER AMBIENT CONDITIONS (25–30°C)

Drying method	Shelf-life (weeks)			
	Treatment		Salt dip	
	0 kGy	4 kGy	0 kGy	4 kGy
Oven dried at 40–50°C	1–2	3	7	12
Oven dried at 60–80°C	2	3	>5	7
Sun dried	1	2	4	7

TABLE III. ORGANOLEPTIC SCORES OF IRRADIATED, SEMI-DRIED FISH STORED UNDER AMBIENT CONDITIONS (25–30°C)

Time (weeks)	Sample/dose	Organoleptic scores			
		Flavour	Texture	Odour	Taste
1	WSD: 0 kGy	7.5	7.0	7.6	8.0
	WSD: 4 kGy	7.0	6.6	6.5	7.4
	SD: 0 kGy	8.0	7.6	7.0	7.0
	SD: 4 kGy	7.5	7.6	6.5	7.0
2	WSD: 0 kGy	6.0	5.5	4.0	4.4
	WSD: 4 kGy	6.5	6.0	5.0	6.5
	SD: 0 kGy	7.5	7.4	6.5	6.5
	SD: 4 kGy	7.5	7.5	7.0	6.6
4	WSD: 0 kGy	Spoiled	Spoiled	Spoiled	Spoiled
	WSD: 4 kGy	6.0	5.5	5.0	5.5
	SD: 0 kGy	7.0	6.5	6.5	5.6
	SD: 4 kGy	6.6	7.0	6.5	6.5
6	WSD: 0 kGy	Spoiled	Spoiled	Spoiled	Spoiled
	WSD: 4 kGy	5.0	4.5	4.0	Spoiled
	SD: 0 kGy	6.4	6.0	5.5	5.5
	SD: 4 kGy	6.5	7.0	6.0	6.4
8	WSD: 0 kGy	Spoiled	Spoiled	Spoiled	Spoiled
	WSD: 4 kGy	Spoiled	Spoiled	Spoiled	Spoiled
	SD: 0 kGy	5.0	4.5	5.5	5.0
	SD: 4 kGy	6.5	6.6	5.5	6.0

Notes: WSD = without salt dip; SD = salt dip.

Organoleptic values on a 9 point hedonic scale:

9 = like extremely;
8 = like very much;
7 = like moderately;
6 = like slightly;
5 = neither like nor dislike;
4 = dislike slighly;
3 = dislike;
2 = dislike very much;
1 = dislike extremely.

scores recorded on a 9 point hedonic scale by a panel of judges, placing more stress on the texture and flavour parameters, are given in Table III. The different irradiated products, although they initially fared unfavourably on the odour and taste scores, showed a marked improvement in rating after 1 week of storage. The salt dipped products showed longer shelf stability: about 7 weeks for the 0 kGy samples and more

than 12 weeks for the 4 kGy samples. However, these products had a slightly salty taste; this can be removed by soaking the product in warm water for several hours and then rinsing under tap water prior to cooking. This process also helps to improve the texture of the product.

Further studies conducted with fish semi-dried under a solar tent showed that without salt treatment they could not be stored for more than 3–4 days, even after applying a dose of 4 kGy (Table IV). Soaking in 8% salt solution with or without irradiation did not greatly extend the shelf-life: 5–6 days. The combination of an 8% salt treatment and a dose of 4 kGy extended the shelf-life to only 10–12 days. With a 12% salt treatment combined with a dose of 4 kGy, a 4 week extension in shelf-life was achieved. With a 16% salt treatment and a 2 or 4 kGy dose, the extension in shelf-life was 5 and 8 weeks, respectively. A 20% salt treatment without irradiation gave a microbiological shelf-life extension of 6–7 months. The same salt concentration combined with a dose of 2 or 4 kGy gave an extended shelf-life of 10–11 months. Soaking the fish in salt solution helps to lower the microbial load because of osmolysis of the microbial cells. Irradiation after semi-drying of the salt treated fish

TABLE IV. EXTENSION OF SHELF-LIFE OF IRRADIATED, SEMI-DRIED FISH STORED UNDER AMBIENT CONDITIONS (25–30°C)

Treatment (salt + dose)	Shelf-life extension	
	Microbiological analysis	Sensory evaluation
0% + 0 kGy	3–4 days	3–4 days
0% + 4 kGy	3–4 days	3–4 days
8% + 0 kGy	5–6 days	5–6 days
8% + 2 kGy	5–6 days	5–6 days
8% + 4 kGy	10–12 days	10–12 days
12% + 0 kGy	2 weeks	2 weeks
12% + 2 kGy	2 weeks	2 weeks
12% + 4 kGy	4 weeks	4 weeks
16% + 0 kGy	2 weeks	2 weeks
16% + 2 kGy	5 weeks	5 weeks
16% + 4 kGy	8 weeks	8 weeks
20% + 0 kGy	6–7 months	6 months
20% + 2 kGy	10–11 months	6 months
20% + 4 kGy	10–11 months	6 months

TABLE V. THE TOTAL MICROBIAL COUNTS OF THE MEAT KEBABS, FISH KEBABS AND VEGETABLE ROLLS FROM ORDINARY AND SOPHISTICATED SHOPS

Media	Ordinary shops (CFU/g)			Sophisticated shops (CFU/g)		
	Meat kebabs	Fish kebabs	Vegetable rolls	Meat kebabs	Fish kebabs	Vegetable rolls
Nutrient agar (mesophilic)	3.11×10^6	1.13×10^4	3.20×10^7	1.66×10^4	2.95×10^3	3.07×10^7
Nutrient agar (thermophilic)	4.16×10^5	3.75×10^3	2.80×10^6	3.25×10^3	2.85×10^3	5.20×10^5
MacConkey agar (coliform)	1.25×10^3	0	3.20×10^4	0	0	3×10^3
Phenol red (*Aeromonas*)	1.70×10^3	0	3.20×10^3	0	0	5.20×10^3
Staphylococcus medium No. 11	3.05×10^3	0	3×10^4	1.25×10^4	3.50×10^3	1.50×10^4
SS agar	0	0	0	0	0	0
Potato dextrose agar	3.20×10^1	<10	9.20×10^1	0	0	<10

Organisms present in the nutrient agar plates of the meat kebabs, fish kebabs and vegetable rolls

Shops	Gram positive rod			Gram positive cocci			Gram negative rod/small rod		
	Meat kebabs	Fish kebabs	Vegetable rolls	Meat kebabs	Fish kebabs	Vegetable rolls	Meat kebabs	Fish kebabs	Vegetable rolls
Ordinary	4	1	5	2	3	1	6	1	2
Sophisticated	6	7	4	2	0	1	1	0	2

further lowers the remaining microbial load, which helps to extend the shelf-life. A critical value may exist for salt treatment after which an increase in dose does not result in a further increase in shelf-life; in this case it was 20%. Although microbiologically the 20% salt treated fish samples retained their quality even up to 6–7 months and 10–11 months for non-irradiated and irradiated samples, respectively, textural deterioration was observed after 6 months of storage. This may have been due to the high salt concentration which, in the course of time, affected the physico-chemical structure of the fish. This particular group of combination treated fish samples had a water activity of 0.774 after 6 months of storage, which again implies that the process showed microbiological suitability.

3.2. Survey of the convenience food sold in local retail markets

The total bacterial counts of the meat kebabs and fish kebabs collected from ordinary shops were higher (10^4–10^6 CFU/g) than those from sophisticated shops (10^3–10^4 CFU/g) (Table V). The contamination levels in the vegetable rolls collected from ordinary and sophisticated shops were similar ($2–3 \times 10^7$ CFU/g) with respect to the total bacterial loads (Table V). The total coliform counts of the meat kebabs and vegetable rolls collected from sophisticated shops were lower than those from ordinary shops, whereas the coliform counts of the fish kebabs collected from ordinary and sophisticated shops were zero. *Aeromonas* were present only in the meat kebabs collected from ordinary shops, whereas none were detected in the fish kebabs from ordinary and sophisticated shops. The *Aeromonas* counts ($3–5 \times 10^3$ CFU/g) of vegetable rolls were almost the same in both types of shop. The staphylococcal counts were always higher in samples from sophisticated shops than in those from ordinary shops. *Salmonella* and fungi were absent in all the samples (Table V).

3.2.1. Effects of the combination treatment on the shelf-life of kebabs

The moisture content of the unfried kebabs was 61% and that of the fried (5 min) kebabs before irradiation, 39.63% (Table VI). The total bacterial counts of the unfried kebabs were 3×10^3 CFU/g, and the total coliform counts were zero. The total bacterial counts of the fried kebabs were reduced to 1.2×10^2 CFU/g, and coliforms were absent.

Table VI shows that the total bacterial counts of the non-irradiated fried kebabs increased at a greater rate, and within 7 days the kebabs were judged unacceptable for consumption. However, no bacteria, coliforms or fungi were detected in the irradiated (5 kGy) kebab samples after up to 6 months of storage at room temperature. The anaerobic culture of the non-irradiated kebab samples showed some diplococcal growth, but anaerobic rods were absent from the samples. Anaerobic growth was totally absent from the irradiated kebab samples.

TABLE VI. THE TOTAL BACTERIAL COUNTS (TBC), TOTAL FUNGAL COUNTS (TFC) AND TOTAL COLIFORM COUNTS (TCC) OF NON-IRRADIATED AND IRRADIATED FISH KEBABS AT DIFFERENT DAYS OF STORAGE AT ROOM TEMPERATURE

Storage (d)	Raw fish			Unfried kebabs (moisture content of 61%)			Fried kebabs (moisture content of 39.63%)					
							Non-irradiated (0 kGy)			Irradiated (5 kGy)		
	TBC/g	TFC/g	TCC/g	TBC/g	TFC/g	TCC/g	TBC/g	TFC/g	TCC/g	TBC/g	TFC/g	TCC/g
0	5×10^4	2.8×10^2	9×10^1	3×10^3	5×10^1	0	1.2×10^2	0	0	0	0	0
7	Spoiled	Spoiled	Spoiled	Spoiled	Spoiled	Spoiled	2.1×10^6	0	0	0	0	0
21							6.7×10^8	0	0	0	0	0
75							3.9×10^9	0	0	0	0	0
120								0	0	0	0	0
180								0	0	0	0	0

3.2.2. *Shelf-life extension of the commercial kebabs by the combination treatment*

Microbiological, chemical and organoleptic evaluations were made.

3.2.2.1. Microbiological evaluation

The total initial bacterial, coliform and fungal counts of the control fish kebabs were 1×10^6, 1.67×10^3 and 6×10^2 CFU/g, respectively (Table VII). The initial microbiological counts showed that the commercial factory had not followed good manufacturing practices. The initial total bacterial and coliform counts were found to decrease by 1 log as a result of the addition of ascorbic acid. On the other hand, the total fungal counts increased by 2 logs in the ascorbic acid incorporated kebabs. For the combined ascorbic acid and irradiation treatment it was observed that at both 2.5 and 5 kGy the total bacterial counts were lower than those in the samples without ascorbic acid. A dose of 5 kGy was effective in eliminating both fungi and coliforms. On the basis of microbiological analysis it was found that a single treatment of either ascorbic acid or irradiation was not effective for the shelf-life extension of fish kebabs, but combined ascorbic acid with a 5 kGy dose extended the shelf-life for more than 1 week.

3.2.2.2. Chemical evaluation

The TVN value, which is regarded as one of the standard chemical indices of freshness of fish, was assessed because the kebabs contained fish as the major component. The TVN values found in all the treated kebabs, including the control samples at different storage times, were well within the acceptable limit for up to 14 days of storage (Table VIII). Irradiation results in a definite improvement in the keeping quality of the kebabs, as evidenced by their low TVN values compared with those of the control samples. The TVN values increased with storage, but still remained well within the acceptable limit.

For the kebabs, the POV and FFA values (indicating the rancidity of the samples) may be a better index of freshness. Therefore, both values were assessed in the treated and control kebab samples at different storage times. Table VIII shows the POV and FFA values of the treated and control kebabs at different storage times. The values indicate that these were also within the acceptable limit.

The three chemical parameters, TVN, POV and FFA, which were chosen as the indices of freshness, were all well within the acceptable limit for up to a minimum of 2 weeks of storage at ambient temperature.

TABLE VII. DISTRIBUTION OF MICROORGANISMS IN DIFFERENT FISH KEBAB SAMPLES DURING STORAGE

Sample	TBC (CFU/g)			TCC (CFU/g)			TFC (CFU/g)		
	0 d	7 d	14 d	0 d	7 d	14 d	0 d	7 d	14 d
UK	1×10^6	4.2×10^8	1.03×10^8	1.67×10^3	2.01×10^5	4×10^5	6×10^2	7.5×10^2	7.8×10^3
TK	3.9×10^5	6.8×10^6	2.78×10^7	4.10×10^3	3.12×10^5	6×10^5	3.5×10^4	3.8×10^4	4×10^5
UK + 2.5 kGy	1.69×10^4	2×10^6	2.45×10^7	0	0	0	0	0	0
TK + 2.5 kGy	2×10^2	1×10^5	1.4×10^7	0	0	0	0	0	0
UK + 5 kGy	8.2×10^2	9.7×10^5	5.8×10^7	0	0	0	0	0	0
TK + 5 kGy	1×10^2	1.49×10^2	7.5×10^4	0	0	0	0	0	0

Notes: TBC = total bacterial counts; TCC = total coliform counts; TFC = total fungal counts; UK = untreated kebabs; TK = ascorbic acid treated kebabs.

TABLE VIII. THE TOTAL VOLATILE NITROGEN (TVN: mg N/100 g), PEROXIDE VALUE (POV: meq/100 g of oil), FREE FATTY ACIDS (FFA: %) AND ORGANOLEPTIC SCORES (OS) IN DIFFERENTLY TREATED FISH KEBAB SAMPLES STORED UNDER AMBIENT CONDITIONS (25–30°C)

Sample	0 d				7 d				14 d			
	TVN	POV	FFA	OS	TVN	POV	FFA	OS	TVN	POV	FFA	OS
UK	1.4	0.385	1.127	8.0	3.2	1.309	2.641	4.0	10.3	1.396	3.025	Spoiled
TK	1.0	0.348	1.347	7.0	3.0	1.238	2.618	3.5	9.5	1.339	3.235	Spoiled
UK + 2.5 kGy	0.7	1.039	1.690	6.5	1.8	1.373	2.774	5.5	5.6	1.429	3.562	4.0
TK + 2.5 kGy	0.5	0.998	2.205	6.0	1.4	1.257	2.778	5.0	5.4	1.387	3.663	3.0
UK + 5 kGy	0.2	1.169	2.167	7.0	1.4	1.579	2.797	6.5	5.0	1.659	3.647	5.0
TK + 5 kGy	0.2	1.057	2.244	6.0	1.2	1.455	2.807	6.0	4.8	1.526	3.851	4.5

Notes: UK = untreated kebabs; TK = ascorbic acid treated kebabs.

Organoleptic values on a 9 point hedonic scale:

9 = like extremely;
8 = like very much;
7 = like moderately;
6 = like slightly;
5 = neither like nor dislike;
4 = dislike slightly;
3 = dislike;
2 = dislike very much;
1 = dislike extremely.

3.2.2.3. Organoleptic evaluation

Microbiological and chemical analyses of the fish kebabs were performed to evaluate the quality and acceptability of the control, treated and irradiated kebabs stored for different times. However, in the final analysis organoleptic evaluation of the odour, texture, flavour, taste, etc. was important to determine the true acceptability of the preserved kebab samples. The kebab samples subjected to diverse treatments were presented to a group of judges on different days of storage so that various sensory parameters could be scored. Table VIII shows that the kebabs treated with a dose of 5 kGy were preserved for up to 2 weeks before they reached borderline acceptability. The shorter period of storage for these kebabs may have been due to the poor hygienic conditions prevalent at the commercial shop or to the selection of inferior quality fish. Addition of ascorbic acid improved the microbiological quality of the kebabs, but because of the sour taste their organoleptic evaluation was not entirely acceptable.

3.3. Effects of the combination treatment and kebab size on the reduction of botulinum spores

The results, as tabulated in Table IX, showed that the different methods of frying reduced the number of spores by 1–3 logs; at a dose of 5 kGy there was a further 2 log reduction in the number of spores in the large kebabs. The effect of normal frying on spore kill was less (1 log) than the other two methods, which produced 3 log spore destruction. The greater effect on spore reduction in the latter cases was caused by the penetration deep into the kebabs of hot oil (240°C). These findings showed that the bacterial spores in the kebabs can be made more susceptible to heat destruction by even penetration of oil during frying. In the small kebabs, after frying, the destruction of spores varied between 4 and 5 logs; this was again dependent on the method of kebab preparation. Less than 10 spores survived in normally prepared kebabs when they were treated with a combined treatment of 5 kGy of irradiation and oil frying. No spores were recovered from the irradiated (5 kGy), fried kebabs prepared by the other two methods.

The results showed that, as the kebabs were small and thin, heat penetration during oil frying was more efficient, resulting in efficacious killing of the spores. Irradiation resulted in the further killing of the few remaining spores.

The water activity of the raw and fried kebabs was found to be 0.978 and 0.974, respectively. The control and the botulinum inoculated, unfried, raw kebabs were dried at 65°C for 10 h. The water activity in both types of kebab decreased to 0.91, but they became quite hard. Oil frying of these kebabs resulted in a further lowering of the water activity, to around 0.76, but they became very hard and tough. It is known that a water activity of ≤ 0.92 inhibits the growth of microorganisms, including

TABLE IX. EFFECTS OF FRYING AND IRRADIATION ON THE DESTRUCTION OF TYPE E *C. botulinum* SPORES IN TWO KEBAB SIZES PREPARED BY DIFFERENT METHODS

Preparation method	No. of spores in the different samples (counts/g)							
	Non-irradiated, unfried		5 kGy, unfried		Non-irradiated, fried		5 kGy, fried	
	Large size	Small size	Large size	Small size	Large size	Small size	Large size	Small size
Normal	8.3×10^6	8.6×10^6	2.5×10^5	1.7×10^5	1×10^5	1.2×10^2	8.6×10^2	< 10
Spongy	9.4×10^6	9×10^6	2.5×10^4	2.9×10^4	1.8×10^3	2.4×10^1	< 10	0
Porous	9.2×10^6	8.8×10^6	2.5×10^4	3.8×10^4	4×10^3	7.2×10^1	6.3×10^1	0

C. botulinum [9]. However, the organoleptic attributes of a product are sometimes impaired by lowering its water activity to this level, as observed in this study.

4. CONCLUSIONS

Although processing of semi-dried fish at a higher temperature maintained the freshness of the product, it was not as acceptable to the consumer because of the burnt odour imparted. Consumer appeal of slow dried products was better, and the reconstitution properties were also very good. Use of brining as a pretreatment before drying had a better effect on the colour and texture than blanching.

In semi-dried fish it was observed that pretreatment with salt extended the shelf-life by more than 1 month. By combining this treatment with a dose of 4 kGy, the shelf-life was extended by more than 3 months. Open sun drying of fish impaired the appearance because of the insect infestation scars remaining on the product.

Kebabs processed in the laboratory using our formulation and irradiated with 5 kGy resulted in a shelf-life of up to 6 months at room temperature.

On the basis of combined microbiological, chemical and organoleptic evaluation of the kebabs, the maximum shelf-life extension was 14 days for 5 kGy treated samples stored at ambient temperature. A correlation between microbiological and organoleptic evaluations was observed in relation to spoilage of the kebabs, but the chemical parameters did not correspond with the other two indices of spoilage, where the values were always well within the acceptable limit. This may have been due to the presence of other ingredients in the kebabs whose chemical indices did not reach the spoilage borderline in the kebabs as a whole.

The microbiological quality of the commercially prepared fish kebabs indicates that the fish used was of poor quality. As a result, the chemical and irradiation treatments were of little help in extending the shelf-life of the kebabs in our storage study. An improvement in production hygiene, combined with chemical and irradiation treatments, may further extend the shelf-life of fish kebabs. The kebab size has a definite effect on heat penetration, and consequent spore reduction, during oil frying, irrespective of the initial botulinum load. For the large kebabs, the reduction in spores was around 1 log, whereas that in the small kebabs was around 3 logs. The larger the size, the lesser the heat penetration deep into the kebab and the spore reduction. The smaller the size, the higher the heat penetration and the spore reduction.

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