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Shelf Life Extension of Chicken Meat by γ-Irradiation and Microflora Changes

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The microbiological quality of chicken meat samples produced in several different areas in Japan was investigated. The total aerobic bacteria were between 8×10^4 to 6×10^6 per gram. Coliforms were 9×10^1 to 2×10^4 per gram for *Escherichia*, *Proteus* and *Klebsiella*. The dominant putrefactive bacteria under chilled conditions were determined to be lactic acid bacteria, *Pseudomonas* and *Flavobacterium*. Low dose γ-irradiation at 1 kGy resulted in disappearance of the dominant putrefactive bacteria, coliforms and *Staphylococcus* on plate agars. The shelf life of irradiated chicken meat at 1 kGy was prolonged 3 times compared with non-irradiated chicken meat and could be stored for 6 days at 10°C storage. Irradiation of chicken meat at 3 kGy reduced the aforementioned dominant flora to the yeasts and *Psychrobacter*. *Salmonella* was detected slightly in some samples and was reduced to a 10^{-4} survival by a 1 kGy irradiation dose.

Keywords: chicken meat, γ-irradiation, shelf life, hygienic quality, microflora

Chicken meat is one of the important animal protein sources for consumers. However, chicken meat has been contaminated with a high microbial load and pathogens such as *Salmonella*, *Escherichia* or *Staphylococcus* which cause disease or food poisoning in consumers (Katsube, 1990; Kudoh, 1990). Chicken meat products have been transported from slaughterhouses to markets and consumers under chilled conditions. Under the chilled conditions of storage or transportation, psychrotrophic bacteria should be increased with some kind of pathogenic bacteria even at lower growth compared with ambient temperature. For the purpose of extending the shelf life or to eliminate the number of foodborn pathogens, chicken meat can be irradiated with γ-rays or electron beams. A number of studies have been carried out in the world (Thayer *et al.*, 1992; Thayer, 1993) except in Japan. Many reports have required an optimum dose of 2.5 to 3 kGy for these purposes. However, a study on the microflora changes in irradiated chicken meat products is still necessary to confirm the microbiological safety under the chilled conditions of the Japanese standard.

This report presents data on the microbiological quality of chicken meat produced in various areas in Japan and changes in microflora after low dose irradiation of γ-rays and during storage under chilled conditions.

Materials and Methods

Sample preparation Ten different kinds of chicken meat produced in 4 different prefectures were obtained from a wholesale trader for evaluation of microbiological quality. Eighteen packs of the same kind of chicken meat were obtained directly from supermarkets in Takasaki city for the storage study of irradiated chicken meat.

For enumeration of microorganisms, each sample was cut into small pieces under sterile conditions. Five grams of each sample was added to 50 ml saline [0.75% (w/v) NaCl] and homogenized thoroughly in a plastic bag with a Colworth stomacher Lab-blender 400 for 1 min. Each suspension was diluted 10^2 or 10^4 times with salines, and then 0.2 ml aliquots were spread on agar plates.

Enumeration of microbiological quality Total aerobic bacteria were determined by the surface agar method with a medium containing 23 g nutrient agar (Difco, Detroit, Michigan, USA), 5 g glucose, 5 g yeast extract, and 2 g K_2HPO_4 per liter (pH 7.0). Coliforms were counted on MacConkey agar (Difco). Lactic acid bacteria were counted using a medium containing 20 g glucose, 10 g yeast extract, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.1 g KCl, 1 g K_2HPO_4, 5 g $CaCO_3$, and 20 g agar per liter. Yeasts were counted on MYG/chloramphenicol agar containing 10 g malt extract, 4 g yeast extract, 20 g agar, and 20 mg chloramphenicol per liter (pH 6.0). *Staphylococcus* were counted on mannitol salt agar containing 10 g D-mannitol, 10 g polypeptone, 1 g beef extract, 75 g NaCl, 15 g agar, and 25 mg phenol red per liter (pH 7.0). Total aerobic bacteria and yeasts were counted after 3 to 7 days' incubation at 30°C. Coliforms and *Staphylococcus* were counted after 18 or 24 h incubation at 37°C. Lactic acid bacteria were counted after 3 to 5 days' incubation at 25°C under anaerobic incubation (Ito & Sato, 1973).

Detection of *Salmonella* was performed by the same method as used for fish meal (Ito *et al.*, 1986) and the FDA method (1976).

Identification of microorganisms The isolated bacteria were transferred mainly to nutrient agar slants. Taxonomic characteristics of these isolates were determined mainly by the method of Cowan & Steel (1974). Confirmation of the

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identification of microflora in total aerobic bacteria was made according to standard procedures (Buchanan & Gibbons, 1974) and other published description (Ito & Iizuka, 1983).

Radiation effect on chicken meat for inactivation of microorganisms Three kinds of chicken meat contaminated with a higher microbial load were used for the determination of microflora changes after irradiation. Each 10 g of cut chicken meat was packed into a polyethylene bag. The samples were then irradiated at a dose rate of 6 kGy/h using a 150 kCi (5.55×10^{15} Bq) slab-type ^{60}Co γ irradiator. The dose rate was determined with a Fricke dosimeter. For the study of the radiation sensitivities of *Salmonella* and *Staphylococcus*, pure cultures of each isolate were grown for 16 h in 100 ml of nutrient broth under constant aeration at 30°C. Cells of the stationary phase were harvested, washed with 0.067 M phosphate buffer, and were resuspended in the same buffer. These suspensions of about 10^9 cells/ml were irradiated at ca. 25°C in equilibrium with atmospheric air. For the study of the radiation sensitivity of *Salmonella enteritidis* in chicken meat, samples of 0.5 ml of 16-h cultures in nutrient broth (ca. 10^8/ml) were transferred to 5 g chicken meat samples which had been pasteurized by exposure to 10 kGy of γ-rays. The inoculated bags were incubated for 1 h at 37°C and irradiated at doses ranging from 0.5 to 4 kGy.

For the study of the irradiation effect on storage, each package containing about 200 g of chicken meat obtained directly from a supermarket was irradiated at doses of 0, 1 and 3 kGy. All of these samples were stored at 10 or 5°C to evaluate the growth of the microorganisms.

Results

Microbiological quality of chicken meat The total aerobic bacteria in various samples of chicken meat were between 8.0×10^4 to 5.7×10^6 per gram (Table 1). The microflora on the chicken meat depended on the area of production, and the predominant bacteria were *Pseudomonas*, *Flavobacterium*, *Arthrobacter* and lactic acid bacteria including *Lactobacillus* and *Streptococcus*, with lesser amounts of *Micrococcus*, *Acinetobacter* and *Psychrobacter* (Tables 2 and 3). Coliforms were detected in the range of 9.0×10^1 to 2.2×10^4 per gram. Predominant coliforms were *Escherichia*, *Proteus* and *Klebsiella*. *Staphylococcus* were

Table 1. Distribution of microorganisms in chicken meat samples which were produced in various prefectures (per gram).

Sample No.	Total aerobic bacteria	Coliforms	Lactic acid bacteria	Yeasts	*Staphylococcus*
I-1[a]	2.1×10^5	1.0×10^2	6.3×10^3	2.1×10^3	2.4×10^2
I-2	3.3×10^5	1.5×10^2	1.5×10^4	2.2×10^3	1.2×10^2
I-3	3.8×10^5	8.3×10^2	1.4×10^4	3.0×10^3	4.8×10^2
I-4	8.0×10^4	9.0×10^1	3.0×10^3	1.0×10^3	1.8×10^2
M-1	5.7×10^6	1.8×10^4	4.2×10^6	3.0×10^4	1.4×10^2
M-2	4.2×10^6	2.2×10^4	2.3×10^6	5.6×10^4	7.5×10^1
K-1	2.0×10^6	1.8×10^4	4.0×10^5	2.2×10^4	2.1×10^2
K-2	2.5×10^6	2.2×10^4	1.2×10^5	3.8×10^4	2.9×10^2
G-1	1.0×10^6	7.5×10^3	2.3×10^4	1.3×10^4	3.1×10^2
G-2	1.1×10^6	6.7×10^3	2.0×10^4	1.8×10^4	1.5×10^2

[a] I, M, K, G: Lot from different prefectures in Japan.

Table 2. Some characteristics of typical bacteria in chicken meat samples.

Genus	Morphology	Gram reaction	Motility	Oxidase	Catalase	Glucose
Pseudomonas	Rods	−	+	+	+	Oxidized or −
Flavobacterium	Rods	−	−	+	+	Oxidized or −
Acinetobacter	Plump rods	−	−	−	+	Oxidized or −
Psychrobacter	Plump rods	−	−	+	+	Oxidized or −
Lactobacillus	Rods	+	−	−	−	Fermented
Arthrobacter	Rods	+	−	−	+	−
Micrococcus	Coccl	+	−	−	+	Oxidized or −
Staphylococcus	Coccl	+	−	−	+	Fermented

Table 3. The conformation of microflora in chicken meat samples produced in various prefectures (in % of total).

Microorganism	I-1	I-2	I-3	I-4	G-1	G-2	M-1	M-2	K-1	K-2
Pseudomonas	26.5	−	15.5	13.0	1.3	−	92.2	69.8	6.2	40.1
Flavobacterium	6.1	25.0	4.3	3.5	36.1	2.9	−	3.8	46.9	1.6
Acinetobacter	−	−	−	−	−	30.6	−	−	−	−
Psychrobacter	−	−	−	−	−	7.1	−	−	−	−
Lactobacillus	53.1	49.0	78.5	31.4	−	−	26.4	26.4	46.2	47.7
Arthrobacter	−	11.4	−	0.6	55.2	59.4	−	−	−	10.6
Micrococcus	14.3	14.6	1.7	51.5	4.4	−	−	−	0.7	−
Yeast	−	−	−	−	3.0	−	−	−	−	−

Table 4. The conformation of surviving flora in irradiated chicken meat sample M2 (in % of total).

Microorganism	Dose (kGy)						
	0	0.5	1.0	1.5	2.0	3.0	4.0
Pseudomonas	69.8	–	–	–	–	–	–
Flavobacterium	3.8	–	–	–	–	–	–
Acinetobacter	–	5.9	15.2	29.0	–	–	–
Psychrobacter	–	–	–	10.0	74.6	42.9	33.3
Lactobacillus	26.4	94.1	84.0	35.0	–	–	–
Yeast	–	–	0.4	20.0	25.4	57.1	66.7

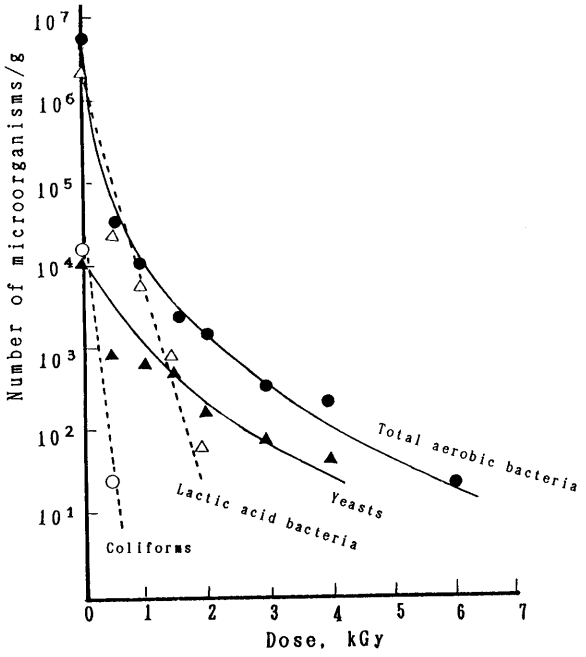

Fig. 1. Change in main residual microorganisms in chicken meat sample M2.

observed in all of the samples from 7.5×10^1 to 4.8×10^2 per gram. *Salmonella* was detected only at 8 and 16 per 100 g in M1 and M2 samples.

Radiation inactivation of microorganisms Microbiological reduction in sample M2 by γ-irradiation is shown in Fig. 1. In the dosage region of 0 to 2 kGy, total aerobic bacteria were reduced rapidly with increasing dosage. As shown in Table 4, *Pseudomonas* and *Flavobacterium* disappeared below 0.5 kGy irradiation when counted on plate agars, and coliforms and *Staphylococcus* were also not detected at 1 kGy irradiation. At doses of more than 2 kGy, the main microflora consisted of *Psychrobacter* (Juny & Heym, 1986) and yeasts similar to a previous report (Ito & Sato, 1973). *Acinetobacter* and lactic acid bacteria were more resistant to γ-rays compared with coliforms. Similar results were also obtained on the irradiation effects of chicken samples in M1 and K1.

The radiation sensitivities of *Salmonella* and *Staphylococcus* in 0.067 M phosphate buffer under air-equilibrium and on chicken meat were also observed. The D_{10} values of *E. coli*, *S. enteritidis*, 2 strains of isolated *Salmonella* and three strains of *Staphylococcus* ranged from 0.09 to 0.16 kGy as shown in Table 5. The D_{10} value of *S. enteritidis* in chicken meat was determined to be 0.5 kGy. Based on this result, *Salmonella*

Table 5. Radiation sensitivities of *Salmonella* and *Staphylococcus* in 0.067 M phosphate buffer.

Strain	D_{10} value (kGy)
Salmonella sp. M2-1	0.14
Salmonella sp. M2-2	0.15
Salmonella enteritidis YK-2[a]	0.13
Salmonella typhimurium YK-1[a]	0.16
Staphylococcus sp. K1	0.15
Staphylococcus sp. G1	0.16
Staphylococcus sp. I1	0.09
Staphylococcus aureus H12[a]	0.13
Escherichia coli S2[a]	0.11

[a] Stock cultures.

should be inactivated below a 10^{-4} survival at 1 kGy irradiation.

A slight pinkish color and an unpleasant odor were observed at doses higher than 3 kGy.

Storage effect of γ-irradiation on microorganisms in chicken meat The species of microorganisms which could grow in non-irradiated chicken meat at 10°C storage were mainly lactic acid bacteria, *Pseudomonas* and *Flavobacterium* with lesser amounts of coliforms and yeasts. During a few days of storage, lactic acid bacteria became predominant and reached a count of approximately 1×10^{10} per gram by the 5th day of storage (Fig. 2). A delay in the growth of microorganisms in irradiated chicken meat was clearly observed at 1 kGy irradiation (Fig. 3), and the shelf life was extended for 6 days or more compared with 1 or 2 days for non-irradiated sample. At a dose of 1 kGy irradiation, growth of lactic acid bacteria, *Acinetobacter* and yeasts was observed during 4 to 6 days of storage. Growth of coliforms was sometimes observed after the 6th day of storage. With irradiation at a dose of 3 kGy, only the growth of yeasts and *Psychrobacter* was observed, and shelf life was extended more than 10 days. The shelf life of irradiated chicken meat at 1 kGy was prolonged 3 times or more compared with non-irradiated chicken meat. Similar results were also obtained when a different sample was used for the storage effect of irradiated chicken meat. In the case of the storage study at 5°C, growth of microorganisms occurred very slowly compared with 10°C even on non-irradiated chicken meat, and the shelf life of irradiated samples at 1 kGy was extended more than 7 days compared with 2 or 3 days for non-irradiated samples.

Discussion

From the study on the microbiological quality of chicken meat produced from different areas in Japan, some samples

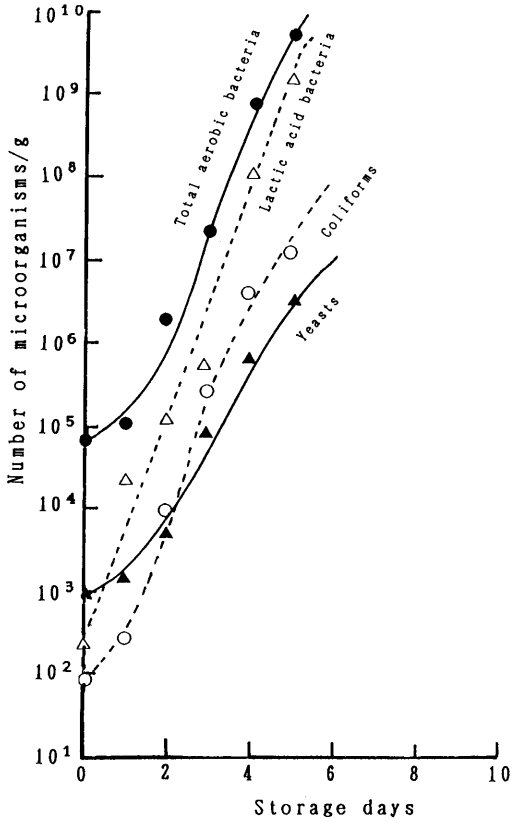

Fig. 2. Main microbial growth pattern of non-irradiated chicken meat at 10°C storage.

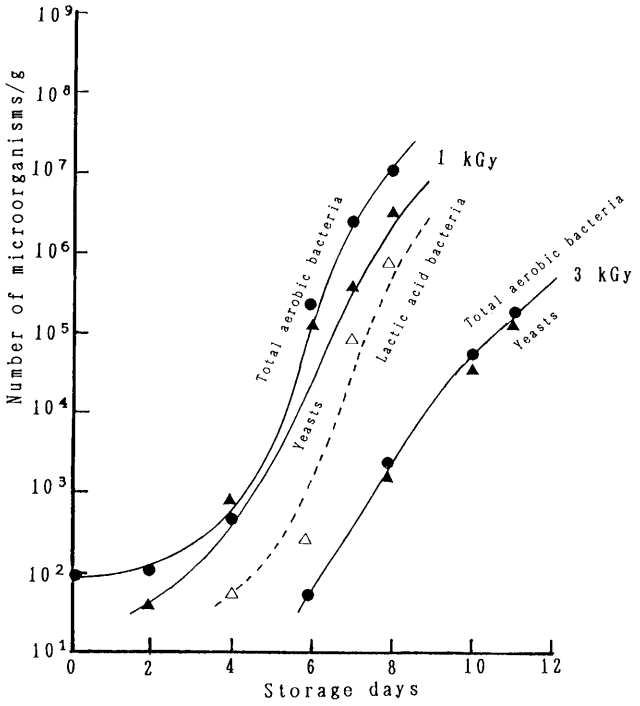

Fig. 3. Main microbial growth pattern of irradiated chicken meat of 1 kGy and 3 kGy at 10°C storage.

should have already undergone slight putrefaction during transportation or storage at a wholesale trader. Usually, chicken meat is consumed within 1 or 2 days by consumers after being purchased from a market, and the necessary shelf life should be 3 or 4 days after the processing in slaughterhouse. A higher count of coliforms also indicates contamination during handling, transportation or storage even under chilled conditions. Irradiation treatment can improve the shelf life and hygienic quality of chicken meat. Many putrefactive microorganisms and coliforms including *Salmonella* in chicken meat are sensitive to radiation and the amount of microorganisms should be reduced by low dose irradiation at 1 kGy which is substantially different from the necessary dose of 3 to 5 kGy for Vienna sausages or fish-paste products (Ito & Sato, 1973; Ito & Iizuka, 1978). In the study of microflora changes, irradiation of 3 kGy resulted in the disappearance of lactic acid bacteria, *Pseudomonas*, *Flavobacterium* and coliforms. The dominant microorganisms in chicken meat at more than 3 kGy irradiation were *Psychrobacter* and yeasts similar to the result with Vienna sausages (Ito & Sato, 1973; Ito & Iizuka, 1983). However, these microorganisms can grow slowly at refrigerated temperature and are not responsible for food poisoning.

The shelf life of chicken meat can be extended to 6 or 10 days by a dose of 1 kGy or 3 kGy. However, a slight pinkish color and an unpleasant odor can be detected at a dose of 3 kGy. This unpleasant odor should disappear after cooking. However, 1 kGy irradiation should be sufficient for the purpose of extending the shelf life and improving the hygienic quality of chicken meat under chilled conditions below 10°C. Further, it is substantiated that irradiation of chicken meat does not permit any hazardous microorganisms based on the observation of microflora changes after irradiation.

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