

Effects of γ -Irradiation and Subsequent Storage on Amino Acids and Ribonucleotides of Boiled-Dried Anchovy

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Abstract

Amino acids and ribonucleotides were measured for boiled-dried anchovy to determine the effect of 5 kGy γ -irradiation on its quality stability during storage at ambient and cooling (5~10°C) temperatures for 12 months in a laminated-film package (Polyethylene 10 μ m/nylon 15 μ m). The anchovy samples contained about 55%(d.b) of total amino acids and about 1%(d.b) of free amino acids. Although there found a significant change ($p < 0.01$) in the content of leucine and lysine immediately after irradiation, the overall content of total amino acids was little changed in stored anchovy even six months after irradiation at cooling conditions. γ -Irradiation caused a significant reduction ($p < 0.01$) in the total content of free amino acids, showing a similar tendency by storage conditions. However, the ribonucleotides, which were 12.00mg/g(d.b) in inosine-5'-monophosphate and 0.38mg/g(d.b) in guanosine-5'-monophosphate, were resistant to 5 kGy-irradiation. With the lapse of storage time, it was also shown that storage temperature was more influential than irradiation on the contents of amino acids and taste compounds of dried anchovy.

Key words: boiled-dried anchovy, γ -irradiation, amino acids, taste components

INTRODUCTION

Fresh anchovy (*Engraulis encrasicolus*) is largely processed as a boiled-dried product having below 30% of moisture content before it is used in food processing and cooking in Korea. Under the unusual changes of weather, we have recently met with a rare case in history to import dried anchovy from Asian countries. According to an official announcement, this is mainly due to insufficient production of raw fish.

Owing to the seasonal nature of the catch, boiled-dried anchovy should be preserved for a long term in order to ensure a stable supply throughout the year. The current preservation methods for boiled-dried anchovy include packaging in a corrugated-cardboard box of 3kg unit, followed by freezing at below -18°C (1). Present problems encountered, were not only with oxidative and microbial deteriorations and hygienic quality during distribution after freezing, but also with preservation cost, capacity and packaging methods(1,2).

In this respect, there have been many domestic studies with different factors, such as antioxidants(3), non-enzymatic browning(4), microbial contamination(5,6),

water activity(4), packaging efficacy(7,8) and storage atmosphere(2,9), etc. On the other hand, we have been doing a series of research associated with keeping the quality of boiled-dried anchovy. The work was intended to determine the efficacy of γ -irradiation combined with improved packaging for preserving and improving the quality of boiled-dried anchovy in consideration of its commercial practice(10,11).

Food processing by ionizing radiation is one of the emerging technologies in the food industries and now attracting the world-wide attention as a means of increasing the availability of foodstuffs, producing the hygienic food, and quarantine treatment in food trade (12). As of 1996, more than twenty items of γ -irradiated foods including fish and shellfish powders, are approved in Korea for human consumption, and an industrial irradiation facility is also domestically available(13).

The present work was designed to investigate the effect of γ -irradiation on the contents of amino acids and ribonucleotides of boiled-dried anchovy during storage for twelve months at ambient and cooling temperatures in terms of its quality changes.

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MATERIALS AND METHODS

Materials, irradiation and storage

Boiled-dried anchovy, purchased from a wholesale market in Seoul, was medium-sized and was approximately 63mm length and 960mg body weight. The proximate compositions were 21.5% moisture, 55.0% protein, 3.3 % fat, and 18.2% ash. Approximately 1kg of dried anchovy was packed in an air-tight laminated film (polyethylene, PE 100 μ m/nylon, NY 15 μ m : water permeability, 4.7g/m² · 24hrs ; oxygen permeability, 22.5 cc/m² · 24hrs) before post-irradiation preservation. Based on the previous works(1,10,11) on the radiosensitivity (D_{10} value : decimal reduction dose) of microorganisms contaminated in boiled-dried anchovy samples, pre-established optimal dose level of γ -irradiation was applied to the packaged samples at a dose of 5 kGy, that was absorbed by a ⁶⁰Co γ -irradiator in a dose rate of 0.2 kGy · hr⁻¹ at ambient conditions. The absorbed dose was assured with ceric-cerous and free radical dosimeter (14). Irradiated samples, together with non-irradiated control ones, were stored at both conditions, ambient (15~33°C) and cooling temperature(5~10°C) for twelve months. Stored samples were used for instrumental determinations of amino acids and taste components at the sixth and twelfth month of storage periods.

Determination of total amino acids

Total amino acids were analyzed after HCl hydrolysis. One milliliter of 6N HCl was added to 1mg of sample protein in a Pyrex cap tube, then sealed *in vacuo*. It was heated at 110°C for 24hrs to allow for a complete hydrolysis. After cooling, the solution was filtered and evaporated to dryness under reduced pressure. Subsequently, oxidation of cysteine into cystine was carried out by adding 0.5ml of 0.01N NaOH solution to the sample, then allowing the sample to stand at room temperature for 4hrs. The volume was adjusted with 0.02N HCl solution and the final solution was injected into an amino acid autoanalyzer (Hitachi Model 835-50).

Determination of free amino acids

Free amino acids of the sample were extracted 3 times with 75% ethanol for 20min, then filtered on a Buchner funnel. The solid residue was washed with

ethanol solution. The combined extracts were evaporated under reduced pressure to obtain an aqueous solution to which an equal volume of diethyl ether was added and shaken to remove the residual lipids from the sample. Following evaporation of the aqueous layer, oxidation of cysteine into cystine was achieved by adding of a 0.01N NaOH solution, then standing at room temperature. A solution of 0.02 N HCl was added to make up the appropriate volume for injection. Tryptophan measurement was not performed for this sample.

Determination of ribonucleotides

A Waters Auto-Tag system liquid chromatograph, equipped with a UV detector at 254nm, was used to determine taste compounds, inosine-5'-monophosphate (IMP) and guanosine-5'-monophosphate (GMP) in the stored boiled-dried anchovy. The column used was μ -Bondapak C₁₈ (i.d. 3.9×300mm). The mobile phase was 0.01% hexane sulfonate Na-methanol(99 : 1, v/v). The flow rate was 1.2ml/min ; the temperature of the column was about 25°C. A 0.5ml of HCl solution was added to 100ml of the sample solution for hydrolysis at 100°C for 60min, which was prepared with approximately 4.1g of powdered samples in distilled water. The hydrolysate was filtered with filter paper (Whatman No.2), Sepak C₁₈ and millipore (0.45 μ m) before injection into a HPLC(15).

In order to preliminarily determine the radiostability of IMP and GMP, the 5'-ribonucleotides were dissolved in buffer(0.01M sodium acetate at pH 4.0) to make 0.01M solution, respectively. The solution in a sealed Pyrex tube was subjected to γ -irradiation at doses of 2.5, 5.0, 10.0, 20.0, and 30.0 kGy in the same manner as above. The irradiated solution was used for an injection sample into a HPLC.

Statistical analysis

The results obtained from triplicate determinations were analyzed statistically by the Tukey's test(16) at the 1% significant level.

RESULTS AND DISCUSSION

Effect on amino acids

The total content of total amino acids was about 55%, which were mainly composed of glutamic acid,

aspartic acid, lysine, leucine, proline and alanine, as shown in Table 1. The results showed that there is no apparent difference in the total content of total amino acids between the control and 5 kGy-irradiated samples, but immediately after γ -irradiation significant changes were observed in some amino acids, such as leucine and lysine($p < 0.01$).

At the sixth month of storage at ambient temperature, aspartic acid, alanine, isoleucine and phenylalanine were not changed irrespective of treatments, but threonine, glutamic acid, leucine and proline were significantly decreased($p < 0.01$). After 12 months of storage at ambient condition, the control samples were unacceptable due to rot, while irradiated samples still showed an acceptable quality from the organoleptic point of view(1), although each amino acid was apparently decreased in its contents as compared with those at the beginning time of storage($p < 0.01$).

At cooling temperatures(5~10°C), threonine, serine, alanine and phenylalanine were not changed in both groups during the whole periods, whereas glycine, leucine and histidine could be preserved up to 12 months

without significant changes by applying irradiation processing for prepackaged samples(Table 2).

On the other hand, most free amino acids were shown resistant to 5 kGy γ -irradiation immediately after treatment, although a significant decrease was observed in total contents of free amino acids as given in Table 3. During the whole periods of cooling storage, most free amino acids were not changed in their amounts in all stored samples(Table 4), while ambient temperature and storage periods brought about a significant change in the content of most of the free amino acids, such as glutamic acid, glycine, alanine, cystine, isoleucine, leucine, tyrosine, lysine, histidine and arginine, as shown in Table 3.

Chemical changes resulting from irradiation have been extensively investigated for food protein(17). The primary effects when higher doses of irradiation are applied, involve deamination, decarboxylation and denaturation of irradiated protein, and the secondary effects are associated with the decomposition and/or recombination of free radicals that originate from the primary reactions. Thiol or disulfide moieties found in amino

Table 1. Changes in total amino acid contents of boiled-dried anchovy as affected by γ -irradiation and subsequent storage at ambient temperature¹⁾

Amino acids (%, dry basis)	Storage period(months)					
	0		6		12	
	Control	5kGy	Control	5kGy	Control	5kGy
Aspartic acid	5.61 ^a	5.60 ^a	5.63 ^a	5.62 ^a	- ²⁾	5.47 ^b
Threonine	2.43 ^a	2.44 ^d	2.26 ^b	2.40 ^d	-	2.12 ^e
Serine	2.42 ^a	2.41 ^a	2.41 ^a	2.34 ^b	-	2.20 ^e
Glutamic acid	8.17 ^a	8.17 ^a	8.05 ^b	8.14 ^a	-	7.52 ^e
Glycine	2.75 ^a	2.74 ^a	2.65 ^b	2.64 ^b	-	2.60 ^b
Alanine	3.65 ^a	3.66 ^a	3.66 ^a	3.64 ^a	-	3.43 ^b
Cystine	1.27 ^a	1.25 ^{ab}	1.23 ^a	1.00 ^b	-	0.30 ^c
Valine	2.36 ^a	2.38 ^a	2.30 ^b	2.31 ^b	-	2.00 ^e
Methionine	1.79 ^{ab}	1.81 ^b	1.80 ^b	1.72 ^c	-	1.75 ^{bc}
Isoleucine	1.96 ^a	2.00 ^a	2.00 ^a	2.00 ^d	-	1.67 ^b
Leucine	4.35 ^d	4.43 ^b	3.26 ^c	4.37 ^a	-	4.15 ^d
Tyrosine	1.68 ^a	1.66 ^a	1.60 ^{bc}	1.62 ^b	-	1.57 ^c
Phenylalanine	2.53 ^a	2.51 ^a	2.50 ^a	2.49 ^a	-	2.40 ^b
Lysine	4.86 ^a	4.79 ^b	4.79 ^b	4.74 ^c	-	4.62 ^d
Histidine	1.63 ^a	1.64 ^a	1.54 ^b	1.50 ^c	-	1.46 ^d
Arginine	3.25 ^a	3.25 ^a	3.23 ^b	3.13 ^c	-	3.20 ^b
Proline	3.94 ^{ab}	3.96 ^a	3.90 ^b	3.98 ^a	-	3.62 ^e
Total	54.65 ^a	54.70 ^a	52.81 ^c	53.64 ^b	-	50.08 ^d

¹⁾ Sample was packaged in a laminated film(PE 100 μ m/NY 15 μ m) and each value was the average of triplicate determinations

²⁾ Sample was decayed

^{a-d} Different letters within the same row are significantly different($p < 0.01$)

Table 2. Changes in total amino acid contents of boiled-dried anchovy as affected by γ -irradiation and subsequent storage at cooling(5~10°C) temperature¹⁾

Amino acids (%, dry basis)	Storage period(months)					
	0		6		12	
	Control	5kGy	Control	5kGy	Control	5kGy
Aspartic acid	5.61 ^b	5.60 ^b	5.62 ^b	5.68 ^a	5.46 ^c	5.60 ^b
Threonine	2.43 ^a	2.44 ^a	2.40 ^a	2.40 ^a	2.39 ^a	2.38 ^a
Serine	2.42 ^a	2.41 ^a	2.43 ^a	2.43 ^a	2.40 ^a	2.41 ^a
Glutamic acid	8.17 ^a	8.17 ^a	8.10 ^{bc}	8.14 ^{ab}	8.06 ^c	8.10 ^{bc}
Glycine	2.75 ^a	2.74 ^{ab}	2.70 ^c	2.73 ^{abc}	2.71 ^{bc}	2.72 ^{abc}
Alanine	3.65 ^a	3.66 ^a	3.64 ^c	3.66 ^a	3.66 ^d	3.66 ^a
Cystine	1.27 ^a	1.25 ^a	1.24 ^{ab}	1.20 ^{bc}	1.20 ^{bc}	1.16 ^c
Valine	2.36 ^{ab}	2.38 ^a	2.35 ^{ab}	2.34 ^b	2.32 ^b	2.33 ^b
Methionine	1.79 ^a	1.81 ^a	1.80 ^d	1.81 ^a	1.68 ^b	1.70 ^b
Isoleucine	1.96 ^a	2.00 ^a	1.91 ^b	1.92 ^b	1.90 ^b	1.91 ^b
Leucine	4.35 ^d	4.43 ^a	4.36 ^{bc}	4.40 ^a	4.34 ^c	4.39 ^{ab}
Tyrosine	1.68 ^d	1.66 ^a	1.68 ^a	1.62 ^b	1.67 ^a	1.64 ^{ab}
Phenylalanine	2.53 ^a	2.51 ^a	2.52 ^a	2.50 ^a	2.51 ^a	2.51 ^a
Lysine	4.86 ^d	4.79 ^b	4.68 ^c	4.70 ^c	4.71 ^c	4.72 ^c
Histidine	1.63 ^{ab}	1.64 ^a	1.64 ^a	1.65 ^d	1.60 ^b	1.62 ^{ab}
Arginine	3.25 ^{ab}	3.25 ^a	3.20 ^c	3.26 ^{ab}	3.22 ^{bc}	3.20 ^c
Proline	3.94 ^{ab}	3.96 ^d	3.86 ^c	3.90 ^{bc}	3.81 ^d	3.80 ^d
Total	54.65 ^a	54.70 ^a	54.13 ^{ab}	54.34 ^{ac}	53.64 ^b	53.85 ^{bc}

¹⁾Sample was packaged in a laminated film(PE 100 μ m/NY 15 μ m) and each value was the average of triplicate determinations
^{a-d}Different letters within the same row are significantly different($p < 0.01$)

Table 3. Changes in free amino acid contents of boiled-dried anchovy as affected by γ -irradiation and subsequent storage at ambient temperature¹⁾

Amino acids (mg · g ⁻¹ , dry basis)	Storage period(months)					
	0		6		12	
	Control	5kGy	Control	5kGy	Control	5kGy
Aspartic acid	0.12 ^a	0.11 ^a	0.08 ^a	0.09 ^a	- ²⁾	0.08 ^a
Threonine	0.24 ^a	0.27 ^a	0.20 ^b	0.24 ^a	-	0.20 ^b
Serine	0.16 ^a	0.16 ^a	0.14 ^a	0.14 ^a	-	0.15 ^a
Glutamic acid	0.59 ^a	0.56 ^a	0.48 ^c	0.52 ^b	-	0.48 ^c
Glycine	0.34 ^{ab}	0.35 ^a	0.30 ^{bc}	0.30 ^{bc}	-	0.29 ^c
Alanine	0.71 ^a	0.70 ^a	0.64 ^b	0.65 ^b	-	0.62 ^b
Cystine	0.64 ^a	0.60 ^b	0.50 ^c	0.56 ^b	-	0.50 ^c
Valine	0.46 ^d	0.44 ^{ab}	0.39 ^{bc}	0.40 ^{bc}	-	0.36 ^c
Isoleucine	0.26 ^a	0.23 ^a	0.18 ^b	0.17 ^b	-	0.15 ^b
Leucine	0.30 ^a	0.29 ^{ab}	0.26 ^{abc}	0.25 ^{bc}	-	0.24 ^c
Tyrosine	0.29 ^a	0.29 ^a	0.18 ^b	0.20 ^b	-	0.19 ^b
Phenylalanine	0.39 ^a	0.39 ^a	0.30 ^b	0.36 ^a	-	0.28 ^b
Lysine	0.38 ^{ab}	0.40 ^a	0.32 ^c	0.36 ^b	-	0.32 ^c
Histidine	3.64 ^a	3.29 ^b	2.78 ^d	3.00 ^c	-	2.64 ^e
Arginine	0.24 ^{ab}	0.26 ^a	0.20 ^c	0.20 ^c	-	0.21 ^{bc}
Proline	0.29 ^a	0.32 ^a	0.24 ^a	0.30 ^a	-	0.29 ^a
Total	9.05 ^d	8.66 ^b	7.19 ^d	7.74 ^c	-	7.00 ^d

¹⁾Sample was packaged in a laminated film(PE 100 μ m/NY 15 μ m) and each value was the average of triplicate determinations

²⁾Sample was decayed

^{a-e}Different letters within the same row are significantly different($p < 0.01$)

acids are known to be particularly sensitive to ionizing energy as shown in this study, although no consistent tendency has been demonstrated to account for the tolerance of each amino acid that is exposed to irradiation in relation to the food and to the physical state of the proteins and the amino acids.

The above results were in partial agreement with the findings of Srinivas et al.(18) and Kwon et al.(19), who reported both no significant difference in total composition of wheat and ginseng proteins between the control and 5 kGy-irradiated samples, and significant reduction of sulfur-containing amino acids. The variation in response of the amino acids to ionizing energy treatment can be attributed to the compositional difference and to the physical status of the samples as well as to the irradiation conditions used. It has been also reported that dry amino acids or dry products were very resistant to irradiation effects as compared to the products in a moist state(17).

Effect on ribonucleotides

Since inosine 5'-monophosphate(IMP) and guanosine 5'-monophosphate(GMP) extracted respectively from dried fish and dried mushroom were found to be sub-

stances having the umami taste(20,21), they have been considered as a fifth taste, "umami taste" in addition to the four basic tastes such as sweet, bitter, salt and sour(22). Along with monosodium glutamate (MSG), they have become important enhancers in improving the palatability(or flavor) of broth cooked at home and in commercial foods.

The boiled-dried anchovy contained about 12.0mg/g of IMP and 0.38mg/g of GMP, respectively as ribonucleotides. They were relatively resistant to 5 kGy γ -irradiation, showing stable amounts during six months of storage at both temperatures as given in Table 5. After 12 months of storage, however, an appreciable decrease was found in IMP($p < 0.01$), whereas negligible changes were observed in GMP. The results obtained in this study showed that there was no significant influence of storage temperature on the contents of IMP and GMP during storage, even though irradiated groups showed an extended shelf-life as compared to the non-irradiated control.

To exclude the combined effects resulting from other components while irradiation and storage on these taste compounds, IMP and GMP solutions were exposed to the given doses of γ -irradiation before HPLC analysis.

Table 4. Changes in free amino acid contents of boiled-dried anchovy as affected by γ -irradiation and subsequent storage at cooling(5~10°C) temperature¹⁾

Amino acids (mg · g ⁻¹ , dry basis)	Storage period(months)					
	0		6		12	
	Control	5kGy	Control	5kGy	Control	5kGy
Aspartic acid	0.12 ^a	0.11 ^a	0.11 ^a	0.11 ^a	0.10 ^a	0.10 ^a
Threonine	0.24 ^{ab}	0.27 ^a	0.24 ^{ab}	0.23 ^{ab}	0.22 ^{ab}	0.21 ^b
Serine	0.16 ^a	0.16 ^a	0.16 ^a	0.16 ^a	0.15 ^a	0.14 ^a
Glutamic acid	0.59 ^a	0.56 ^{ab}	0.58 ^{ab}	0.57 ^{ab}	0.54 ^b	0.55 ^{ab}
Glycine	0.34 ^a	0.35 ^a	0.33 ^a	0.33 ^a	0.31 ^a	0.30 ^a
Alanine	0.71 ^a	0.70 ^a	0.70 ^a	0.70 ^a	0.68 ^a	0.68 ^a
Cystine	0.64 ^a	0.60 ^{abc}	0.62 ^{ab}	0.56 ^{bc}	0.59 ^{bc}	0.57 ^c
Valine	0.46 ^a	0.44 ^a	0.46 ^a	0.46 ^a	0.44 ^a	0.45 ^a
Isoleucine	0.26 ^a	0.23 ^{ab}	0.24 ^{ab}	0.23 ^{ab}	0.22 ^{ab}	0.21 ^b
Leucine	0.30 ^a	0.29 ^a	0.29 ^a	0.29 ^a	0.27 ^a	0.26 ^a
Tyrosine	0.29 ^a	0.28 ^a	0.27 ^a	0.28 ^a	0.26 ^a	0.24 ^a
Phenylalanine	0.39 ^a	0.39 ^a	0.38 ^a	0.37 ^a	0.35 ^a	0.36 ^a
Lysine	0.38 ^a	0.40 ^a	0.38 ^a	0.39 ^a	0.36 ^a	0.37 ^a
Histidine	3.64 ^a	3.29 ^d	3.52 ^b	3.30 ^d	3.40 ^c	3.26 ^d
Arginine	0.24 ^a	0.26 ^a	0.24 ^a	0.24 ^a	0.22 ^a	0.23 ^a
Proline	0.29 ^{ab}	0.32 ^a	0.28 ^{ab}	0.29 ^{ab}	0.27 ^b	0.28 ^{ab}
Total	9.05 ^a	8.66 ^{bc}	8.80 ^b	8.54 ^c	8.38 ^d	8.21 ^c

¹⁾Sample was packaged in a laminated film(PE 100 μ m/NY 15 μ m) and each value was the average of triplicate determinations

^a Different letters within the same row are significantly different($p < 0.01$)

Table 5. Changes in ribonucleotide content of boiled-dried anchovy as affected by γ -irradiation and subsequent storage¹⁾

Storage conditions	Storage months	GMP		IMP	
		Control	5 kGy	Control	5 kGy
Ambient temp.	0	0.38 ^{ah}	0.38 ^{ah}	12.00 ^{by}	12.10 ^{ay}
	6	0.39 ^{ah}	0.37 ^{ah}	12.10 ^{aby}	12.15 ^{by}
	12	- ²⁾	0.26 ^b	-	8.66 ^d
Cooling temp. (5~10°C)	0	0.38 ^{ah}	0.38 ^{ah}	12.00 ^{by}	12.10 ^{ay}
	6	0.35 ^{ah}	0.35 ^{ah}	12.21 ^{ay}	12.28 ^{ay}
	12	0.33 ^{ah}	0.33 ^{ah}	10.70 ^{cy}	10.66 ^{cy}

¹⁾Sample was packaged in a laminated film(PE 100 μ m/NY 15 μ m) and each value for GMP(guanosine-5'-monophosphate) and IMP(inosine-5'-monophosphate) was the average of triplicate determinations in a unit of mg \cdot g⁻¹ on a dry basis

²⁾Sample was decayed

^{a-d}Different letters within the same column are significantly different($p < 0.01$)

^hSame letters within the same row(GMP) are not significantly different($p > 0.01$)

^yDifferent letters within the same row(IMP) are significantly different($p < 0.01$)

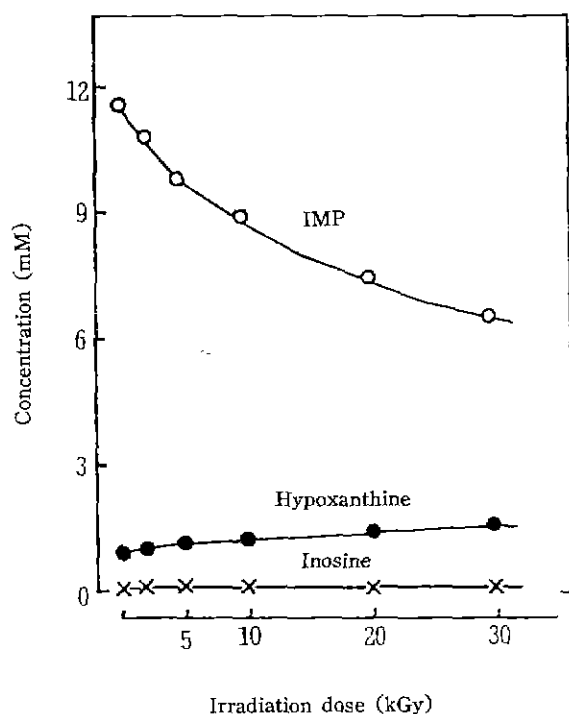


Fig. 1. Radiolysis of inosine 5'-monophosphate(IMP) in aqueous solution.

Under the HPLC conditions, the retention time(min), of 5'-ribonucleotides, nucleosides and bases were found to be 2.47 and 2.31 in IMP and GMP, 9.14 and 9.62 in inosine and guanosine, and 4.8 and 4.77 in hypoxanthine and guanine, respectively in both IMP and GMP solutions irradiated with higher doses of irradiation. Fig. 1 and 2 showed the changes in concentration of IMP and GMP and their degradation products(nucleoside and base) depending on irradiation doses at

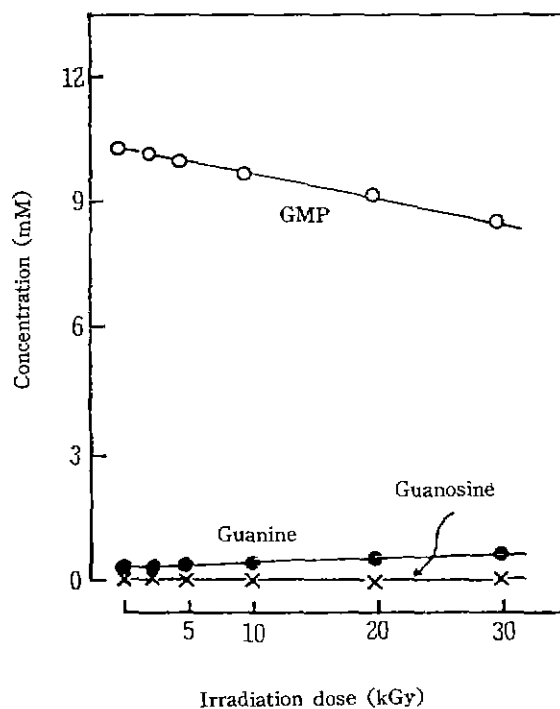


Fig. 2. Radiolysis of guanosine 5'-monophosphate in aqueous solution.

pH 4.0. The decrease of IMP concentration was observed on irradiation, with increase of hypoxanthine concentration as one of degradation products. In addition, the changes in GMP, guanosine and guanine on irradiation were similar to those IMP although there was a quantitative difference between GMP and IMP(Fig. 1 and 2).

The thermal degradation of IMP and GMP was demonstrated to be followed the first order kinetics in

aqueous solutions and their rate constants were considerably affected by pH and temperature(15). The preliminary study on radiolysis of IMP and GMP indicated that both components were less stable on irradiation treatment in a moist state, while they seemed to be more stable in dry products(1), as shown in Table 5. However, more efforts are needed to elucidate the mechanism of radiolysis of these flavor enhancers under various conditions(17,19).

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