Identification of Irradiation of Boned Chicken by Determination of *o*-Tyrosine and Electron Spin Resonance Spectrometry

Makoto Miyahara,[*, a] **Taeko Nagasawa,**[b] **Tomomi Kamimura,**[b] **Hitoshi Ito,**[c] **Masatake Toyoda,**[a] **and Yukio Saito**[a]

[a]*National Institute of Health Sciences, 1–18–1 Kamiyoga Setagaya, Tokyo 158–8501, Japan,* [b]*School of Allied Health Sciences, 1–15–1 Kitasato Sagamihara, Kanagawa 228–8555, Japan, and* [c]*Japan Atomic Energy Research Institute Takasaki Establishment, 1233 Watanuki Takasaki, Gunma 370–1292, Japan*

Ortho-tyrosine detection method is used for the detection of irradiated protein-rich foods. A new procedure determining *o*-tyrosine content was examined in regard to the identification of food having undergone ionization treatment. A new fluorometric HPLC method allows the detection of irradiated foods at 10 kGy. This method was compared with an electron spin resonance (ESR) method recommended by European countries for the detection of irradiated foods. The method is applied only those food samples that contain bone or cellulose. The dose response of *o*-tyrosine production was linearly increased upto 30 kGy. Chicken bones were dried in a desiccator, after which ESR signals were recorded in the samples. The dose response of the ESR signal was observed two weeks after irradiation, and the equation for curve fitting was a quadratic polynominal. The ESR signal intensity correlated with the logarithm of *o*-tyrosine production, a close relationship observed.

Key words —— ESR, *o*-tyrosine detection, irradiated food, identification

INTRODUCTION

Several recent events have been reported that may affect the development of methods to identify irradiated foods. In 2001, the Codex committee dis-

cussed the removal of the upper limit of food irradiation doses, which had been effect since 1982.[1] In the United States, the Food and Drug Administration (FDA) has permitted fresh and frozen meat to be irradiated at below 4.5 and 7 kGy,[2] respectively, since 1997. The U.S. Department of Agriculture and Food Safety and Inspection Service issued new rules for irradiated food production that comply with the FDA regulations.[3] Several food-processing factories in the U.S.A. currently utilized electrical irradiating machines. This situation was not predicted during the Analytical Detection Methods for Irradiation Treatment of Foods (ADMIT)[4] committee's days.

In the European Union (EU),[5] irradiated foods can not be marketed without proper identification method. The British Standard Institute approved 5 detection methods, including two electron spin resonance (ESR) spectrometry, thermoluminescence (TL), hydrocarbon detection (HC), and cyclobutanone detection (CB), based on reports by the ADMIT committee.

In the course of developing these ESR methods, defects were recognized by the laboratories involved in the co-laboratory studies. A corrective action was taken in 2000,[6] and an old ESR method was replaced with new standards.

This method includes measurement of the hydroxyapatite or cellulose radicals in foods and determination the amount of spin in the food samples. Irradiation induces many radicals in foods, and some of these radicals trapped in hydroxyapatite of bone are believed to be stable, and can easily be detected several years after irradiation.[7] In dry vegetables, radicals induced by irradiation are stable and can be detected several months following irradiation.

In principal, ESR spectroscopy are very sensitive and reliable. For instance, ESR spectrometry[8] is used with alanine as the standard dosimertic method for correcting irradiation machine power (10 Gy to 100 kGy). The alanine dosimetric system is the method adopted to test irradiation equipment according to the standards established by the Japan Industrial Standards (JIS).[9] But practically, the detection limits in foods are higher than that expected (*i.e.*, 5 kGy for dried vegetables; 0.5 kGy for bone in meat).[10] These problem arise from the nature of radicals themselves: ESR signals are easily reduced if the foods are stored in conditions of high temperature and humidity; contaminants also produce radical signals that interfere with the analysis; the signal can easily disappear accidentally during pre-

*To whom correspondence should be addressed: National Institute of Health Sciences, 1–18–1 Kamiyoga Setagaya, Tokyo 158–8501, Japan. Tel.: +81-3-3700-1141; Fax: +81-3-3707-6950; E-mail: mmiyaha@nihs.go.jp

treatment for analysis. Practically speaking, the present method can identify only few types of irradiated foods and needs 2 or 3 days to produce results. For these reasons and others, the IAEA and BSI recognize the ESR methods as qualitative analytical testing methods.[5,6]

If foods is subject to testing for over-irradiated or multiple irradiation, then quantitative analysis is required at high doses. Several chemical methods can effectively detect many kinds of foods which have been treated with high doses of radiation.

We reported relation of ESR signal intensity and hydrocarbon production in meat.[11]

Several reports have addressed the *o*-tyrosine evaluation method.[12,13] In the course of developing this procedure, the advantages of this method over other detection methods were discussed.[12] *o*-Tyrosine is a radiolytic product of phenylalanine that exists widely in food samples. Therefore, irradiated foods which contain proteins can be detected by this method; *i.e.*, the *o*-tyrosine detection method is to detect a much wider range of irradiated food than can the ESR methods.

We have investigated the procedure using LASER fluorometric HPLC. Unfortunately, a detector is not currently available for use: therefore, the HPLC procedure must be performed using a xenon exciting fluorometric detector.

This paper describes a new fluorometric procedure for *o*-tyrosine detection and compares quantitative ESR spectrometric detection and the new *o*-tyrosine fluorometric detection in order to confirm the performance of the new *o*-tyrosine detection procedure. For this purpose, we used a chicken sample which consists of bone and meat, irradiated it, detected *o*-tyrosine in the meat using HPLC, measured the radicals in bone using an ESR spectrometer, and then correlated the data.

MATERIALS AND METHODS

o-Tyrosine Determination ——

Sample: Chicken wings including bone were purchased at a retail store in the Setagaya area. The age of the samples were estimated as approximately 6 weeks. These samples were also used for ESR detection. Two samples were used.

Samples were prepared for HPLC according to the method previously reported.[12]

Irradiation Procedure: The skin was peeled off and was chopped into small pieces. The pieces were packed in a 10 ml-test tube. The tube was placed in an ice bath for 2 hr before irradiation. The bath temperature was maintained at 4ºC. Irradiation apparatus, dose rates, and other conditions were same as those described previously.[11]

Dosimetry: The absorbed dose was determined using Gammerchrom dosimeters and Radix dosimeters, emplying a Hitachi Spectrometer Model 301. Dosimeter correction was carried out using a Frick dosimeter provided by Nordion, Canada.

HPLC: A Shimadzu model of the HPLC system employing a Shimadzu model RF10A fluorometric detector was used. The pre-column derivatization system and procedure were the same as those previously reported.[12,13] The separation column for HPLC was ODS-3, 4.7 mm i.d. $\times$ 250 mm, 5 μm, GL Science, Tokyo, Jpan. Injection volume was 20 μl. The calibration curve was established at a range of 10 to 200 μg.

ESR Determination ——

Sample: Bones were removed from the samples prepared as described in section (*Sample*) and wrapped with Polyvinydene chloride (PVDC) film.

ESR System: A Nihon Denshi RE-2X was used for ESR measurement. The conditions used were as follows: microwave,1 mW X-band; magnetic field, 335 mT central field; scan range, 7.5 mT; modulation wave length, 100 kHz; modulation amplitude, 0.32 mT; scan rate, 3.5 mT/min; time constant, 30 msec; temperature, room temperature; external standard for *g*-value, 3rd and 4th signals of manganese ion.

Sample Preparation: An irradiated sample was dried for 12 hr in the desiccator in the presence of phosphorus pentoxide after all attached meats and bore marrow were removed. The dried bone was crushed using a hammer, and sieved using a 1–2 mm screen. One hundred mg of sieved bone particles were packed in a tube with 4 mm i.d. for ESR measurement. The ESR measurements were carried out two weeks after irradiation.

ESR Data Treatment: ESR signal was precisely measured at peak to peak.

RESULTS AND DISCUSSION

HPLC Analysis Using Xenon Exciting Fluorometric Detector

The analytical procedure was modified to employ a Xenon exciting fluorometric detector whose low detection limit is below that of LASER
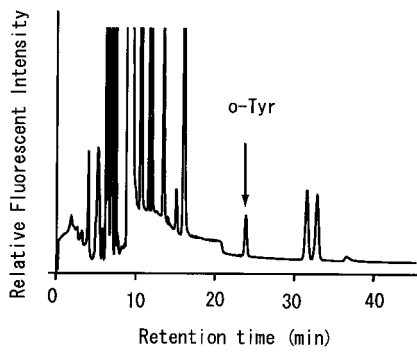
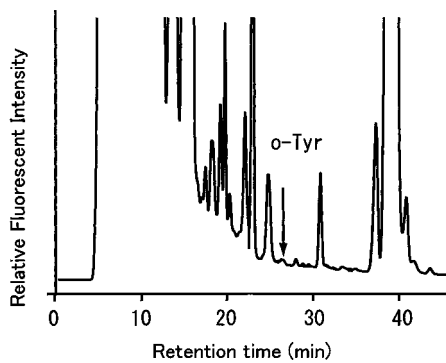**Fig. 1.** HPLC Chromatogram of 20 ng of *o*-Tyrosine Standard

Fig. 2. HPLC Chromatogram of *o*-Tyrosine in 10 kGy-Irradiated Chicken

Fig. 3. Corelation Curve of *o*-Tyrosine Production in Irradiated Chicken *vs*. Absorbed Dose

Fig. 4. ESR Spectra of Non-Irradiated (a), 1 kGy-Irradiated (b), and 5 kGy-Irradiated Chicken (c)

Fig. 5. Corelation Curve of ESR Signal Intensity in Irradiated Chicken Bone *vs*. Absorbed Dose

fluorometory. To compensate for the lower detectability, the injection volume of the HPLC system and concentration ratio were increased 2 and 10 times, respectively. Typical chromatograms are shown in Figs. 1 and 2. The modification of the original procedure is limited by the HPLC column capacity. Over-modification resulted in the reduction of the separation of peaks in the chromatograph. The detection limit (10 μg/ml) was 10 times higher than that of LASER detection (1 μg/ml).

Dose Response

Using the new detection procedure, dose response was examined. The results are shown in Fig. 3. The mean detection limit for irradiated chicken was approximately 10 kGy, because of the high background. In some cases, it could detect a sample irradiated at 1 kGy.

ESR Spectra

Typical ESR spectra are shown in Fig. 4. Spectrum a) of an un-irradiated sample shows a signal at $g_1 = 2.005$, estimated to originate from collagen. b) and c) are spectra of 1 and 5-kGy irradiated samples, respectively. These show free radical signals at
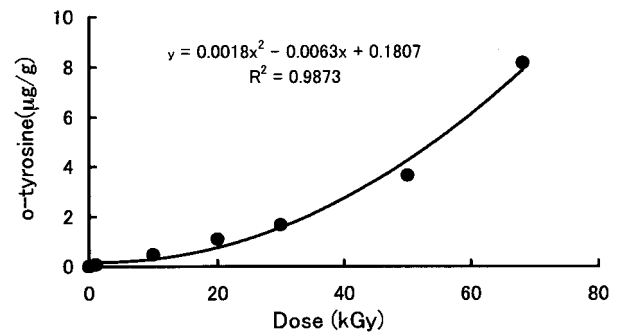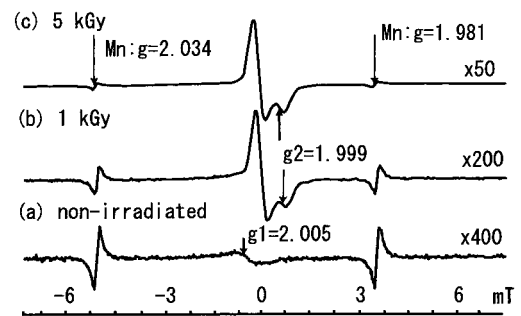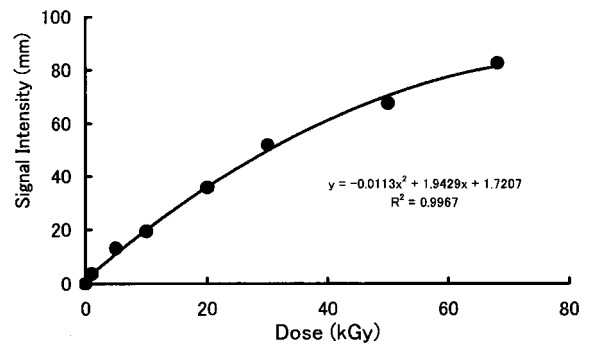
$g_2 = 1.999$ and are clearly distinguished from non-irradiated bone. These results are in agreement with data published by the BSI.[5]

Absorbed Dose *vs*. ESR Signal Intensity

As shown in Fig. 5, the relationship between absorbed dose and signal intensity is not linear at any range. The equation for the curve fitting is also shown in the Fig. 5. But below 10 kGy, the dose response curve can be approximated by the linear equation ($y = 1.87 + 0.830$, $R^2 = 0.9928$). Signals over 20 kGy were not proportional to absorbed dose.
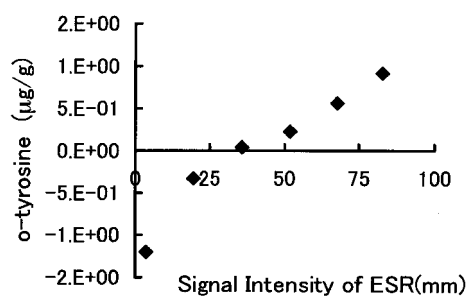
Fig. 6. Corelation Curve of ESR Signal Intensity in Irradiated Chicken Bone *vs.* *o*-Tyrosine Production in Irradiated Chicken Meat

Some of radicals formed in high dose range may quenched, due to recombination of highly concentrated radicals in sample. The low detection limit is estimated as 0.5 kGy.

Comparison of ESR Method and *o*-Tyrosine Determination Method

Response of the ESR signal and the amount of *o*-tyrosine at the same absorbed dose level are shown in Fig. 6. The result indicates that the ESR method is much more sensitive than the *o*-tyrosine determination method. The low detection limits are 0.5 kGy and 10 kGy for the ESR method and *o*-tyrosine determination method, respectively. However, the upper limits are 40 kGy and 60 kGy, respectively. The ESR signal saturated at lower doses than did that of the *o*-tyrosine method.

The time needed for analysis is 25–30 hr for both methods. Among the advantages of the *o*-tyrosine method described in the previous section, is its ability to detect a wider range of irradiated foods than can the ESR method. In addition to this, the new fluorometic HPLC procedure can detect over-irradiated samples which are prohibited from shipment to market.

REFERENCES

1) FAO/IAEA/WHO High-dose irradiation (1999) *Wholesomeness of food irradiated with doses above 10kGy*, WHO, Geneva.
2) FDA (1997) Irradiation in the production, processing and handling, *Fed. Reg.*, **62**, 64107.
3) FSIS (2000) Irradiation of meats and meat products, *FSIS Directive*, 7700.1.
4) Joint FAO/IAEA division of Nuclear techniques in food and agriculture (1994) *Final Research Co-ordination Meeting of the FAO/IAEA Co-ordinated Research Programme on Analytical Detection Methods for Irradiation Treatment of Foods*, IAEA/FAO, Belfast, United Kingdom.
5) The European parliament and of the Council (1999) Food and food ingredients treated with ionizing radiation, *Off. J.*, **L006**, 16–23.
6) British Standard (2000) Detection of irradiated food containing cellulose, by ESR spectroscopy, BS EN 1787, *the British Standard Institute*, London.
7) Desrosiers, M. F., McLaughlin, W. L., Sheahen, L. A., Doodd, N. J. F., Lea, J. S., late Evans, J. C., Rowlands, C. C., Raffi, J. J. and Agnel, P. L. (1990) Co-trial on ESR identification and estimates of gamma-ray and electron absorbed doses given to meat and bones, *International J. Food and Technology*, **25**, 682–691.
8) British Standard (1997) *Detection of irradiated food containing bone, Method by ESR spectroscopy*, BS EN 1786, *the British Standard Institute*, London.
9) JIS (2001) *The alanine dosimetry system*, JIS, z-4571, *the Japan Standards Association*.
10) Toyoda, M. and Miyahara, M. (1998) Recent Advance in Detection Methods for Irradiated Food, *J. Food Hyg. Soc. Japan*, **39**, J-372–J-378.
11) Goto, M., Tanabe, H. and Miyahara, M. (2000) Detection of Irradiated chicken by hydrocarbon method and ESR method, *Food irradiation*, **35**, 23–34.
12) Miyahara, M., Ito, H., Nagasawa, T., Kamimura, T., Saito, A., Kariya, M., Izumi, K., Kitamura, M., Toyoda, M. and Saito, Y. (2000) Determination of *o*-Tyrosine Production in Aqueous Solutions of Phenylalanine Irradiated with Gamma Ray, Using High Performance Liquid Chromatography with Automated Pre-column Derivatization and LASER Fluorometric Detection, *J. Health Sci.*, **46**, 192–199.
13) Miyahara, M., Ito, H., Saito, A., Nagasawa, T., Kariya, M., Toyoda, M. and Saito, Y. (2000) Detection of Irradiation of Meats by HPLC determination for *o*-Tyrosine Usinf Novel LASER Fluormotric Detection with Automatic Pre-column Reaction, *J. Health Sci.*, **46**, 304–309.