

Validation of o-Tyrosine as a Marker for Detection and Dosimetry of Irradiated Chicken Meat

NOEMI CHUAQUI-OFFERMANN*, TOM E. MCDOUGALL, and ABRAHAM M. GUERRERO

Radiation Applications Research Branch, AECL Research, Whiteshell Laboratories, Pinawa, Manitoba, Canada ROE 1LO

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ABSTRACT

o-Tyrosine has been proposed as a marker for postirradiation identification of food that contains protein. In this study, the validity of using o-tyrosine for this purpose has been tested and established. The validation process involved examination of background levels of o-tyrosine in unirradiated chicken, radiation dose yield, postirradiation storage, dose rate, radiation type, temperature during irradiation, and oxygen concentration during irradiation. o-Tyrosine is present in unirradiated chicken meat at variable levels. However, these background levels are low enough that o-tyrosine can serve to determine whether chicken has been irradiated or not at the commercially approved doses (3 kGy). The radiation dose response curve for the formation of o-tyrosine is linear. The apparent yields may vary with the analytical method used; however, it is independent of the dose rate, radiation type, atmosphere, and temperature (above freezing) during irradiation. It is also independent of the storage time and temperature after irradiation. It is concluded that this marker can be used to determine the absorbed dose in chicken meat irradiated with either gamma rays or electrons under normal or modified atmosphere.

Irradiation of phenylalanine and of protein that contains phenylalanine gives rise to the formation of a mixture of three isomeric compounds: o-, m-, and p-tyrosine. Based on the assumption that o- and m-tyrosine are unique radiolytic products, several authors proposed their use as potential markers to detect irradiated food (5,8,13). Since o-tyrosine is easier to separate by chromatography (gas, high-pressure liquid) from p-tyrosine (3,7,8,12,13,17), it was chosen as a potential indicator of radiation processed foods.

A suitable marker to detect or quantify radiation treatment of food should fulfill the following requirements: it should be absent in unirradiated food or present in levels significantly smaller than those produced by irradiation at commercially applicable doses; it should be stable under common storage conditions; it should correlate in a simple manner with the dose applied; and it should be unaffected by dose rate, radiation type, and temperature or atmosphere during irradiation. Several studies have been conducted to assess the suitability of o-tyrosine as a marker to detect and/or to quantify radiation treatment of foods (4,7,8,11,16,17). However, the results from these studies are difficult to compare and they appear to be somewhat controversial. Some of the areas in which discrepancies are

reported include method reproducibility, background levels, and radiation dose yields.

Regarding the method, some authors (3,12) were unable to reproduce the GC/MS method (7,8) to measure o-tyrosine in chicken meat. Others were able to reproduce the method but unable to reproduce the results reported (13). Several authors have recommended a high-liquid chromatography (HPLC) method with fluorescence detection because it is simpler (no derivatization of o-tyrosine is needed) and faster than the GC-MS method (3,11,16). A linear increase of o-tyrosine with radiation dose has been reported in all the studies; however, the reported radiation yields are different, i.e., they fluctuate from 0.05 mg/(kg.kGy) at 20°C (11) to 1.1 mg/(kg.kGy) at 20°C (8), and 0.32 mg/(kg.kGy) at 0°C (12).

Similar discrepancies have been reported regarding the levels of o-tyrosine in unirradiated chicken meat. All the published data indicate that o-tyrosine is not a "unique radiolytic product" and is present in unirradiated food at variable levels. The reported levels of o-tyrosine in unirradiated chicken meat vary from <0.01 mg/kg (8,9,12) to 0.5 mg/kg (13). These levels are significantly less than those produced by irradiation at the commercially approved dose of 3 kGy.

The inconsistencies in the published results have raised questions about the suitability of o-tyrosine as a marker to detect radiation treatment of food. The discrepancies of the published data may have resulted from differences in the analytical method used in each study, conditions used during radiation processing (dose rate, temperature during irradiation, availability of oxygen, and others), and biological variability (1). Consequently, in order to determine the validity of o-tyrosine as a marker, a close examination of the effect of these variables on its radiation induced levels was needed (1).

The objective of this paper is to present evidence that validates o-tyrosine as a marker. The effect of the following variables on the radiation induction of o-tyrosine were studied and are reported here: background levels in unirradiated chicken, postirradiation storage time and temperature, dose rate, radiation type, temperature during irradiation, and oxygen concentration during irradiation. In this communication it should be noted that while some of the

results have been previously published, they are summarized here to provide a complete picture of evidence for the validity of o-tyrosine. In each section the new data are presented and discussed first, followed by previously reported data (which is clearly indicated as such and presented in a summarized form).

EXPERIMENTAL PROCEDURES

Sample preparation

(i) *Individual samples.* Whole chicken breasts were obtained from a local supplier about 24-36 h postslaughter. The breasts were skinned and boned, and the fat adhering to the meat was carefully scraped away with a knife. The clean whole breast was either freeze dried for approximately 72 h until it reached a constant weight (samples used in the determination of background levels), or it was packaged in a polyethylene pouch (oxygen transmission rate of 8000 cm³/m² 24 h at 23°C), irradiated, and then freeze dried as above. The freeze-dried samples were homogenized (powdered) in a blender, placed in tightly sealed polypropylene containers (moisture transmission rate <10g/m²/24 h at 38°C, 90% RH), and stored at -40°C until analysis.

(ii) *Pooled samples.* Ten chicken breasts were skinned, boned and cut into small (<2.5 cm) pieces. The pieces were mixed thoroughly to make a homogeneous sample and were then either freeze dried directly or packaged, irradiated and freeze dried as described above. These samples were also homogenized and stored at -40°C until analysis. Pooled samples were used throughout the study (except where indicated) to reduce the background variability.

In those studies where a control of the concentration of oxygen inside the pouch was required prior to irradiation, the samples were packaged in pouches made of an oxygen barrier film. The composition of this film is 20µ Saran™ coated nylon/50µ polyethylene 5% ethylene vinyl acetate (oxygen transmission rate 12 cm³/m² 24 h, 23°C).

Irradiation

Individual or pooled samples of chicken breast in plastic pouches were placed on ice and irradiated either in a ⁶⁰Co Gamma Cell 220 (dose rate 0.157 kGy/min, or 0.039 kGy/min when shielded with a 2 cm lead shield), or in a AECL I-10/1 (10 MeV, 1 kW) electron accelerator (dose rate 18.7 kGy/min). The studies on the effect of temperature during irradiation, where a strict control of the temperature was required, were done with a temperature control system that controlled the temperature within ± 1°C. Radiochromic dye dosimeters (GAF for doses <4kG and FWT for doses >4 kGy) were attached to each sample (2).

Method

o-Tyrosine was determined by HPLC with fluorescence detection, using a procedure developed in our laboratories and reported earlier (3,4). The method involves acid hydrolysis of freeze-dried samples, solid-phase extraction, fractionation by HPLC, collection of the fraction containing the o-tyrosine, and a second chromatographic separation using HPLC of the collected fraction.

The quantitation was done by measuring the area of the o-tyrosine peak from the second chromatography, where a baseline separation is obtained. Each sample was analyzed in triplicate.

RESULTS AND DISCUSSION

Dose rate and type of radiation

The results shown in Table 1 indicate that the dose rate (gammas versus shielded gammas) and radiation type (gam-

TABLE 1. *Effects of dose rate and radiation type on the radiation-induced level of o-tyrosine in chicken breast meat irradiated to 5 and 10 kGy.*

Radiation type	Dose rate (kGy/min)	o-Tyrosine (ppm, dry weight)*	
		5 kGy	10 kGy
Co-60	0.039	1.80 ± 0.10	3.80 ± 0.27
Co-60	0.157	1.83 ± 0.07	3.80 ± 0.33
10 MeV electrons	18.50	1.83 ± 0.07	3.82 ± 0.23

*Each tabulated value represents the mean ± the standard deviation (n = 3) of o-tyrosine in parts per million, dry weight.

mas versus electrons) do not affect the radiation induced levels of o-tyrosine.

To confirm these results, we compared the slopes of the dose response curves for samples irradiated with gammas (dose rate 0.157 kGy/min) and with electrons (dose rate 18.7 kGy/min). These values were 0.37 ppm (dry weight)/kGy (gammas) and 0.38 ppm (dry weight)/kGy (electrons), respectively, indicating the absence of a dose rate and radiation-type dependency.

The levels of o-tyrosine shown in Table 1 are smaller than those predicted by the equation of the dose response curve of o-tyrosine previously reported (4). The change in yield coincided with a change in the type of column used in our HPLC procedure. Previously we used Selectosil™ (Phenomenex), but poor reproducibility of resolution from column to column led us to switch to Hypersil™ columns (same supplier, different manufacturer), which eliminated the problem. Measured yields of o-tyrosine were consistently lower with Hypersil™ than with Selectosil™ columns. The reason for the lower apparent yields with Hypersil™ columns is not known. These inconsistencies reiterate the need for doing all comparative measurements on a single column type, with adequate standards and control included.

Temperature during irradiation

The results shown in Table 2 indicate that in samples irradiated at temperatures below freezing, the levels of radiation-induced o-tyrosine are significantly lower than those in samples irradiated at temperatures above freezing.

TABLE 2. *Effect of temperature during irradiation on the radiation-induced levels of o-tyrosine in chicken breast meat irradiated (gammas and electrons) at 5 kGy.*

Temperature °C	o-Tyrosine (ppm, dry weight)*
-7.5 ¹	0.80 ± 0.03
-4.5 ¹	0.70 ± 0.07
0.2 ¹	1.70 ± 0.20
4.5 ²	1.80 ± 0.13
10.0 ²	1.70 ± 0.17
15.0 ²	1.80 ± 0.13
30.0 ¹	1.80 ± 0.13

* Each tabulated value represents the mean ± standard deviation (n = 3) of o-tyrosine in parts per million, dry weight.

^{1,2} Denote samples from different lots of chicken meat.

This observation can be attributed to the fact that the mobility of the radiolytically generated free radicals is reduced in ice; the yield of free radicals ($\bullet\text{OH}$, $\text{H}\bullet$, e) resulting from radiolysis of water depends on the phase and temperature of the water. In ice the yield of $\bullet\text{OH}$ radicals has a G value of approximately 1.0, whereas in water at 25°C the G value is 2.87 (10). Consequently, the availability of $\bullet\text{OH}$ to react with phenylalanine to form o-tyrosine is decreased in samples irradiated in the frozen state, resulting in lower levels of radiation-induced o-tyrosine.

It can also be concluded from the data shown in Table 2 that when samples are irradiated above freezing (0.2 to 30°C), the levels of radiation-induced o-tyrosine are not affected by the temperature during irradiation.

Oxygen effect

A biphasic dose-response curve for o-tyrosine production in chicken meat, with a change in the slope at approximately 5 kGy, has been reported (9). The authors (9) attributed this biphasic response to a total depletion of the residual oxygen in the tissue, which occurs at 5 kGy. Under our experimental conditions, the dose response curve was linear with the dose (0-10 kGy) (3,4), suggesting either that oxygen was not limiting or that there is no oxygen effect for o-tyrosine production by irradiation.

To clarify this point, we examined the influence of different oxygen concentrations during irradiation on the levels of o-tyrosine. The results shown in Table 3 indicate that in samples irradiated under vacuum as well as under enriched oxygen atmospheres the levels of o-tyrosine are the same as those found in samples irradiated under normal atmosphere.

TABLE 3. Effect of atmosphere composition during irradiation on the radiation-induced levels of o-tyrosine in chicken breast meat irradiated (gammas) at 5 and 10 kGy.

Atmosphere Composition (%)	o-Tyrosine (ppm, dry weight)*	
	5 kGy	10 kGy
Air	1.80 ± 0.17	3.64 ± 0.20
Vacuum	1.83 ± 0.23	3.60 ± 0.33
CO ₂ :N ₂ (30:70)	1.83 ± 0.06	**
O ₂ :N ₂ :CO ₂ (45:30:25)	1.84 ± 0.10	**

* Each tabulated value represents the mean ± the standard deviation (n = 3) of o-tyrosine in parts per million, dry weight.

** Value not determined.

Postirradiation storage effect

As reported earlier (4), the levels of o-tyrosine in irradiated samples (3 kGy) stored at either 4 or 8°C until spoilage were the same as expected for fresh irradiated (0-d storage time) samples (Table 4).

Background levels

These results were published earlier (4). In summary, o-tyrosine is present in unirradiated meat at variable levels. The mean value of o-tyrosine for 18 randomly selected chickens was 0.60 ± 0.36 ppm (mean ± S.D.) dry weight. The observed 61% variability in the measured background

TABLE 4. o-Tyrosine content in gamma irradiated chicken (3 kGy) stored at 4 and 8°C.

Storage Time (d)	o-Tyrosine (ppm, dry weight)*	
	4°C	8°C
0	1.73 ± 0.20	1.93 ± 0.07
3	1.80 ± 0.10	1.80 ± 0.07
6	1.87 ± 0.20	1.70 ± 0.10
11	1.93 ± 0.20	1.73 ± 0.10
14	1.73 ± 0.14	-

* Each tabulated value represents the mean ± the standard deviation (n = 3) of o-tyrosine in parts per million, dry weight. Spoilage occurred by day 14 at 4°C and by day 11 at 8°C.

levels was greatly reduced by analyzing a pooled sample drawn from a large number of individual samples. The background levels for pooled samples were 0.33 ± 0.10 and 0.43 ± 0.05 ppm (dry weight), and they were not altered by storage at either freezing temperature or at refrigerator temperatures until spoilage occurred.

These findings are in good agreement with recently published results (13) where the background levels found in seven chickens from different suppliers fluctuated from 0.07 to 0.50 ppm. The method used in the determination of these reported background levels (13) was the GC-MS-SIM method recommended by Karam and Simic (8). However, the background levels reported by Karam and Simic (8) were <0.1 ppm per dry weight. The reason for this discrepancy is unknown but may be attributed to biological variability, such as age and feeding practices, to processing practices, or to the method used to prepare the samples prior to chromatography.

The generation of o-tyrosine by the analytical procedure is theoretically possible (6,14-16); however, in trials done with pure phenylalanine background levels of only 0.05 ppm have been found (4,12). Consequently, the discrepancies in background levels cannot be attributed to the analytical method and are most likely due to biological variability.

Radiation dose yield

These results were published earlier (3,4). The radiation dose response curve for o-tyrosine was determined in individual as well as pooled gamma-irradiated samples of chicken. In summary, the radiation dose response was linear within the dose range studied (0 to 10 kGy), with a slope of 0.423 ± 0.01 [mean ± S.D. (5)] ppm (dry weight)/kGy. Although there was some variation in the intercept (0.400 ± 0.03) [mean ± S.D. (5)] (dry weight), the slope was the same in all samples tested (Fig. 1).

These results fall between the already published results of 1.23 and 0.71 ppm (dry weight)/kGy for doses below and above 5.0 kGy, respectively (9) and 0.05 ppm (dry weight)/kGy (11). They are close to the reported value of 0.32 ppm/kGy (12). However, Pedersen and Fuhlendorff (12) only observed this radiation dose yield in samples irradiated with 10 MeV accelerated electrons (dose rate not reported). When samples were irradiated with gammas (dose rate not reported), they found radiation-induced o-

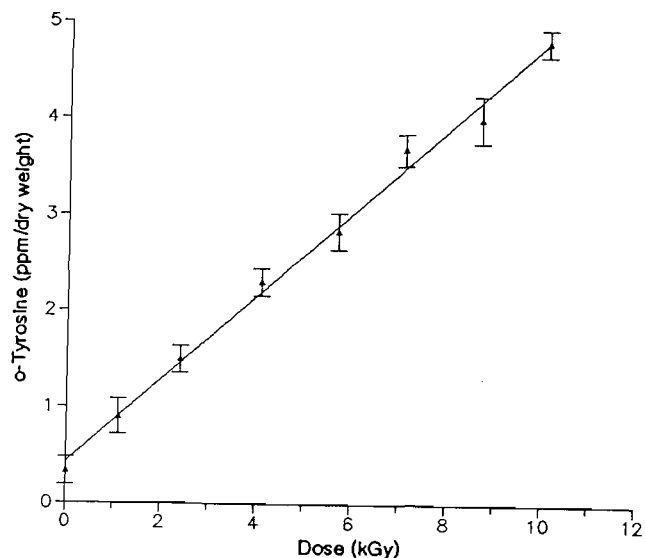


Figure 1. Radiation dose response curve of *o*-tyrosine in chicken breast meat irradiated between 0 and 10 kGy. Each plotted value represents the mean \pm the standard deviation ($n = 3$) of *o*-tyrosine in parts per million (dry weight).

tyrosine in very small amounts or not at all. These authors attributed this finding to a strong dose-rate dependency effect. However, it is not likely that a dose-rate effect can explain Pedersen and Fuhlendorff results.

In general, at high dose rates the local concentration of reactive free radicals increases ($\bullet\text{OH}$), which favors radical-radical recombination rather than radical-substrate ($\bullet\text{OH}$ -phenylalanine) reactions. If a strong dependency on dose rate existed for the radiation-induced formation of *o*-tyrosine, the radiation dose yield should be smaller at a higher dose rate. Consequently, the higher dose yield reported for electrons as compared with gammas (12) contradict this principle. Furthermore, our data indicate the absence of a dose rate effect.

CONCLUSIONS

The results reported here clearly indicate that *o*-tyrosine is present in unirradiated chicken meat, at variable levels. However, at the dose approved to treat chicken (3 kGy), the expected levels of *o*-tyrosine based on the experimentally determined radiation dose yield should be 1.8 ± 0.1 ppm (dry weight). This level is well above the reported background levels of 0.43 ± 0.08 ppm (dry weight) and supports the conclusion that this marker can be used to determine whether chicken has been irradiated or not. This marker would also meet the requirements of the Analytical Detection Methods for Irradiation of Food which has recommended as a first approach to the problem of detection of irradiated food the development of qualitative methods for monitoring trade of food and checking label declarations.

The characteristics of the radiation dose yield of *o*-tyrosine indicate that this marker could also be used to determine the absorbed dose in chicken meat irradiated either with gammas or electrons under normal or modified atmosphere. Interlaboratory testing in accordance with internationally accepted protocols is needed to fully verify the suitability of this marker for quantitative purposes.

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