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Background Levels and Radiation Dose Yield of o-Tyrosine in Chicken Meat

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ABSTRACT

The measurement of o-tyrosine levels in poultry meat is a potential method for postirradiation dosimetry of poultry. The validity of using o-tyrosine for this purpose has not yet been established. As part of the validation process, the o-tyrosine content in unirradiated chicken meat, the radiation dose response curve, and the effects of postirradiation storage on o-tyrosine levels are examined.

In 18 individual samples, the mean background level of o-tyrosine was 0.18 ± 0.11 ppm (wet weight, 70% moisture), and the most frequent background level (60% of the cases) was between 0.05 and 0.15 ppm (wet weight, 70% moisture). In pooled samples of 10 chickens, the mean background level was 0.12 ± 0.03 ppm (wet weight, 70% moisture). The levels were not significantly affected by storage at 5°C (7 d) or by freezing the sample. The radiation dose response curve was linear within the dose range studied (0 to 10 kGy), with a slope of 0.127 ± 0.003 ppm (wet weight)/kGy. Although there was some variation in the intercept (0.132 ± 0.013), the slope was the same in all samples tested. Postirradiation storage at either 4 or 8°C until spoilage did not affect the levels of o-tyrosine. These data indicate that o-tyrosine level may be useful for determining the absorbed dose in chicken meat gamma-irradiated to doses greater than 0.6 kGy. Further validation studies are continuing.

Aromatic compounds are very reactive to hydroxyl radicals, giving rise to a mixture of isomeric hydroxylated compounds. Phenylalanine, an aromatic amino acid found in most food proteins, reacts with hydroxyl radicals generated from radiolysis of water to form three isomeric products: o-, m-, and p-tyrosine. Based on the assumption that o- and m-tyrosine are not naturally occurring amino acids, several authors (*4,6,7,11*) proposed their use as potential markers to detect irradiated food. Since o-tyrosine is easier to separate by chromatography (GC, HPLC) from p-tyrosine than is m-tyrosine (*6,7*), o-tyrosine was chosen as a potential indicator for radiation processing to have taken place.

Several studies have been conducted to assess the suitability of the proposed marker for the intended use. Different analytical methods such as GC-MS with selective ion monitoring (*6,12*), GC-MS with ion impact and selective ion monitoring (*5*) have been used, and HPLC with fluorescence detection has been particularly recommended for the determination of o-tyrosine in chicken meat (*3,9*). We found that the HPLC fluorescence method was simpler (no derivatization of o-tyrosine is needed) and faster than the GC-MS method, and did not require expensive equipment.

The background levels of o-tyrosine determined with these methods varied from <0.01 mg/kg (*8,9*) to 0.120 and 0.302 mg/kg (*3*). Even though these results are variable, they indicate that o-tyrosine is present in unirradiated chicken meat and consequently is not a "unique radiolytic product." However, if the levels of o-tyrosine in unirradiated chicken are significantly less than those produced by irradiation at commercially applicable doses, o-tyrosine would be a suitable marker (*8*).

Reported radiation yields of o-tyrosine in poultry (calculated on dry weight basis) vary from 0.05 mg/(kg.kGy) at 20°C (*9*) to 0.43 mg/(kg.kGy) at 0°C (*3*) and 1.1 mg/(kg.kGy) at 20°C (*7,8*). The discrepancies in background levels and in radiation yield may possibly be attributed to differences in the analytical methods used by each group, the conditions used during radiation processing (dose rate, temperature, availability of O_2 freshness of the product, etc.), and biological variability.

In fact, it has been reported that the amount of o-tyrosine in irradiated chicken depends not only on the dose, but also on the dose rate and the temperature of irradiation (*9,10*). This reported dose-rate dependence was observed for samples irradiated at a dose rate between 1 and 4.2 kGy/h, but the data are too sparse to permit conclusions to be drawn with confidence. Furthermore, the data are not consistent. For example, the o-tyrosine content in samples irradiated to 2.5 and 5 kGy at a dose rate of 4.2 kGy/h was 3.6 and 2.6 times higher, respectively, than the levels in the samples irradiated to the same doses with a dose rate of 1.0 kGy/h (*1*). These data imply a very strong and complex dependence of the radiation dose yield of o-tyrosine on both the dose and the dose rate, which does not seem feasible.

All these discrepancies indicate that the potential use of o-tyrosine as a marker to identify irradiated meat requires careful validation.

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 then either freeze dried for approximately 72 h until it reached a constant weight (samples used in the determination of background levels) or was packaged in a polyethylene pouch, (oxygen transmission rate of 8000 cm^3/m^2 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 vapor transmission rate <10 g/m^2/24 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 (<1 in.) 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 their analysis.

Irradiation

Individual or pooled samples of chicken breast in plastic pouches were placed on ice and irradiated in a ^{60}Co Gamma cell 220 (dose rate 0.157 kGy/min). Radiochromic dye dosimeters (GAF for doses <4 kGy and FWT for doses >4 kGy) were attached to each sample (*2*).

Method

o-Tyrosine was determined by HPLC/fluorescence, using an established procedure (*3*). The method involved acid hydrolysis of freeze dried samples, solid phase extraction, fractionation by HPLC, collection of a fraction, and a second chromatographic separation (HPLC) of the collected fraction. The following modifications were introduced to this method.

(i) Hydrolysis. 4.5 ml of 6 NHCl was added to 250 mg of sample in a Pierce® hydrolysis tube. The tube was cooled in liquid nitrogen, evacuated at room temperature until its contents thawed completely (~10 min), sealed, and then heated for 1 h at 150°C.

(ii) Clean-up. Hydrolyzed samples were diluted to 5 ml with distilled water and filtered through a 0.45-µm micropore filter. One ml of filtrate was loaded into a preconditioned C_{18} reverse-phase Sep-Pak^R cartridge as previously described (*3*) and eluted with 4 ml of a solution containing 1% sodium chloride, 5% acetonitrile, and 94% water. The eluted fraction was evaporated to dryness and dissolved in 500 µl of 2 N HCl.

(iii) HPLC. The HPLC columns used were the reverse-phase Selectosil^R C_{18} 5-µm analytical column, 4.6 mm i.d. x 250 mm, and the Hypersil^R C_{18} 5-µm, 4.6 mm i.d. x 250 mm (Phenomenex). Both columns performed well, although the Hypersil^R was preferred because it had a longer service life. Prior to injection, the column was equilibrated by washing it for 60 min with eluant A (1% acetonitrile, 1.5% dihydrogen phosphate, and water, pH 4) at a flow rate of 1.2 ml/min. The column was washed with eluant A for 50 min after each injection. o-Tyrosine was detected fluorimetrically (λ excitation = 275 nm, λ emission = 305 nm). The retention time was determined by using standard solutions of pure analyte and by spiking hydrolyzed samples with known amounts of o-tyrosine standard. The retention time under our conditions was ~ 9.8 min.

The quantitation was done by collecting the fraction containing the o-tyrosine, and then conducting a second chromatographic separation using as the eluant a solution containing 1% NaCl, 1% acetonitrile, and 98% water. This second chromatography yielded base-line separation of o-tyrosine. Quantitation was done by measuring the peak area; each sample was analyzed in triplicate. The column was washed at the end of each working day with solvent B (50% acetonitrile, 0.75% sodium dihydrogen phosphate, and water) for 30 min, followed by another 30 min with solvent C (80% acetonitrile and 20% water).

RESULTS AND DISCUSSION

Background levels

The background levels of o-tyrosine in unirradiated chicken breast were determined in fresh samples stored for 1, 4, and 7 d at refrigerator temperatures (~5°C), and in fresh frozen samples stored for 5 d at -30°C.

Figure 1 illustrates the o-tyrosine content in chicken breasts for a sample population of 18 individual birds. The results indicate that the background levels of o-tyrosine fluctuate between 0.06 ± 0.01 [mean ± S.D. (*3*)] and 0.42 ± 0.07 ppm (wet weight, 70% moisture). As shown in Fig. 1, 58% of the samples contained less than 0.15 ppm (wet weight, 70% moisture). The mean value of o-tyrosine for all 18 birds analyzed was 0.18 ± 0.11 ppm (wet weight, 70% moisture).

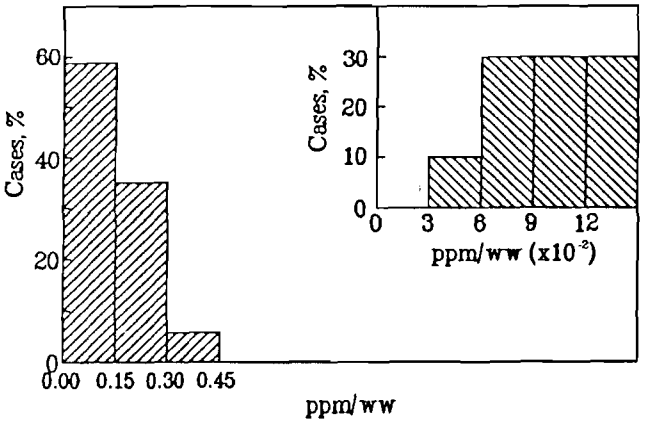

Figure 1. *Distribution of background levels of o-tyrosine in unirradiated chicken meat (n=18). Inset corresponds to distribution levels within the 0 to 0.15 ppm (wet weight) range.*

These results indicate that there is a significant variability (61%) in the measured background levels, which may be attributed to biological variability, such as age and feeding practices, or to analytical variation (15%). The results also suggest that the most probable background level for any given sample would fall in the range from 0.05 to 0.15 ppm (wet weight, 70% moisture). In an effort to reduce the variability, the background levels were determined in pooled samples of 10 chicken breasts (two experiments). We expected that the biological variability would average out in a pooled sample, resulting in a narrower distribution of background levels. Based on the above data, we expected that the mean background level in pooled samples should be in the range from 0.05 to 0.15 ppm (wet weight). The background levels found for the pooled samples were 0.10 ± 0.03 and 0.13 ± 0.03 ppm (wet weight, 70% moisture). Thus, the pooled samples significantly reduce the variability in the background level of o-tyrosine and improved the quantitation of radiation-induced o-tyrosine.

Table 1 shows the results for o-tyrosine content in unirradiated chicken breast samples stored at refrigerator

temperature from 0 to 7 d. The data indicate that the levels of o-tyrosine are not significantly affected by storing the sample up to 7 d at refrigerator temperatures prior to analysis. The samples stored for 7 d have already reached the end of their shelf life, and so the o-tyrosine content is not affected by high levels of spoilage bacteria. The o-tyrosine levels in samples stored frozen for 5 d were the same as those found in fresh samples (data not shown).

TABLE 1. *o-Tyrosine in unirradiated chicken stored at refrigerator temperature from 0 to 7 d.*

Chicken No.	o-tyrosine (ppm, wet weight, 70% moisture)		
	0 d	4-5 d	7 d
1	0.09 ± 0.01	0.13 ± 0.04	
2	0.11 ± 0.01	0.12 ± 0.02	
3	0.06 ± 0.01	0.07 ± 0.01	
4	0.25 ± 0.02	0.25 ± 0.03	0.23 ± 0.02
5	0.23 ± 0.05		0.23 ± 0.01

Radiation Dose Yield

The radiation dose response curve of o-tyrosine was determined in individual samples (two experiments) (*3*) and in pooled samples (three experiments) of chicken breasts. Fig. 2 shows the radiation dose response curve for a pooled sample of 10 chicken breasts irradiated from 0 to 10 kGy. The data shown in Fig. 2 indicate that the radiation yield of o-tyrosine is proportional to dose. This linear dependence is described by:

$$\text{o-tyrosine (ppm)} = (0.127 \pm 0.003) \times \text{Dose (kGy)} + (0.132 \pm 0.013).$$

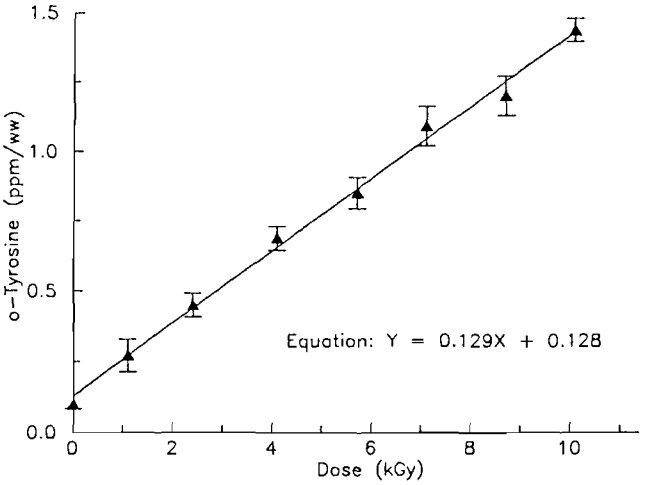

Figure 2. *Radiation dose response curve of o-tyrosine in chicken breast irradiated between 0 and 10 kGy. Each plotted value represents the mean content ± the standard deviation (n=3) of o-tyrosine in parts per million (wet weight, 70% moisture).*

The slope of the dose response curve was 0.127 ± 0.003 [mean ± S.D. (5)] ppm (wet weight)/kGy for all the samples analyzed (individual and pooled). The reproducibility of the slope for different samples indicates that the dose yield of o-tyrosine in chicken meat, gamma irradiated on ice, is independent of the origin of the sample. The intercept of 0.132 ± 0.013 [mean ± S.D. (5)] represents the content of o-tyrosine in unirradiated chicken samples obtained by extrapolating the dose responses curves. This value was compared to the background determined experimentally by analyzing the unirradiated samples used to determine the radiation dose response curves. The mean background level determined experimentally was 0.12 ± 0.03 ppm (wet weight). The results fall within the expected range for the most frequent background level of o-tyrosine in unirradiated samples, as described above.

It has been reported that the radiation-induced formation of o-tyrosine in chicken meat depends on the availability of oxygen (*8*). These authors observed a change in the slope of the dose response curve of o-tyrosine production in chicken meat. They attributed this biphasic response of yields to a total depletion of the residual oxygen in the tissue, which occurred at 5 kGy. The dose response curve was linear under our conditions, with no evidence of a change of slope (Fig. 2). This indicates either that oxygen was not limiting under the conditions of our experiment, or that there is no oxygen effect for o-tyrosine production by irradiation. Since the latter possibility would be contrary to the claim reported in (*8*), further work is required to determine the correct interpretation.

Our work does not confirm claims that o-tyrosine is formed chemically at several points during the sample preparation procedure and, therefore, may not be useful as a marker for poultry irradiated at the approved levels (*1*). The results reported here for the decrease in the variability of the background levels of o-tyrosine in pooled samples indicate that the amount produced during the extraction procedure, if any, is of a minor significance.

Postirradiation storage effect

Pooled samples of fresh chicken breast were irradiated to a nominal dose of 3 kGy and stored at either 4°C for 0, 3, 6, 10 and 14 d, or at 8°C for 0, 3, 6 and 11 d, before determination of o-tyrosine. The samples were prepared in a way which precluded resampling. Consequently, the small differences in the irradiation dose delivered to the replicate samples did not allow a direct comparison of the o-tyrosine levels in samples stored for different time periods. To circumvent this problem, the levels found in stored samples were compared with those calculated for 0-d of post-treatment storage using the dose response equation described earlier. The levels of o-tyrosine in samples stored at 4 and 8°C were found to be stable for the periods studied. As shown in Table 2, the o-tyrosine content in samples stored at 8°C for up to 11 d was the same as expected for fresh irradiated (0-d storage time) samples.

High levels of spoilage bacteria (>10^8) clearly do not alter the o-tyrosine levels in irradiated chicken meat since the samples stored at 8°C for 11 d were spoiled.

CONCLUSIONS

The results indicate that o-tyrosine is present in unirradiated chicken meat at variable levels. This variability, possibly due to biological factors, can be greatly reduced by analyzing a pooled sample drawn from a large

TABLE 2. o-tyrosine content in irradiated chicken stored at 8°C.

Storage time (d)	Dose (kGy)	o-tyrosine[1] calculated (ppm)*	o-tyrosine observed (ppm)*
0	3.15	0.53 ± 0.02	0.58 ± 0.02
3	3.35	0.56 ± 0.02	0.54 ± 0.02
6	2.85	0.49 ± 0.02	0.50 ± 0.03
11	3.05	0.52 ± 0.02	0.52 ± 0.03

* ppm, wet weight 70% moisture.

[1] Value calculated from radiation dose response equation determined for irradiated unstored samples.

number of individual samples. The results reported here also demonstrate that these background levels are not altered by storage at either freezing temperatures or until spoilage occurs at refrigerator temperatures.

The radiation dose response curve for the formation of o-tyrosine is linear (within the dose range studied from 0 to 10 kGy), with a slope of 0.127 ± 0.003 ppm (wet weight, 70% moisture)/kGy and an intercept of 0.130 ± 0.013 ppm (wet weight, 70% moisture). Postirradiation storage at either 4 or 8°C until spoilage does not affect the levels of o-tyrosine. These data indicate that the o-tyrosine method, at its present state of development, may be useful to determine the absorbed dose in chicken meat gamma irradiated on ice at doses no lower than 0.6 kGy. This lower dose limit is based on the assumption that the limit for confident detection of a real increase (due to irradiation) in o-tyrosine is 50% above the average background level found in pooled samples.

The evidence presented here is promising but is still not sufficient to fully establish the validity of using o-tyrosine as a marker to detect and quantify radiation treatment of meats. Further work is required to examine the effects of radiation processing conditions (such as dose rate, radiation temperature, and oxygen availability during irradiation) on the levels of o-tyrosine formation.

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