



Effect of irradiation on the degradation of nucleotides in turkey meat



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ABSTRACT

The degradation of nucleotides in cured ready-to-eat (RTE) as well as uncured raw and cooked turkey meat products by irradiation were determined to evaluate the potential impact of nucleotides on the taste changes in irradiated turkey meat. Four irradiation doses (0, 1.5, 3.0 and 4.5 kGy) were applied to cured RTE and uncured turkey meat products, and the amounts of nucleotides and their degradation products were measured. Results showed that irradiation had a significant impact to the amount of nucleotides (adenosine diphosphate, adenosine monophosphate and inosine monophosphate) and the breakdown of these nucleotides (inosine and hypoxanthine) in uncured turkey meat when irradiated at < 3.0 kGy. However, significant decreases in inosine and hypoxanthine were observed when the uncured turkey meat were irradiated at > 3.0 kGy, which might attribute to uric acid and other compounds formation. The increase in K-value (the percentage of inosine and hypoxanthine over the total content of adenosine triphosphate) at lower irradiation dose in uncured cooked than raw turkey meat, which indicated that cooked meat is more susceptible to oxidation. But little effect was found on the nucleotides and nucleotides degradation products in cured RTE turkey meat products because of the antioxidant effect of sodium nitrite.

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1. Introduction

Irradiation is among the best methods to eliminate potential pathogens in meat products and prolong their shelf-life (Farkas, 2006). However, irradiation produces aqueous electron (e_{aq}^-) and hydroxyl radical ($\cdot OH$) from water molecules in meat (Thakur & Singh, 1994) and can be involved in various reactions with amino acids, protein, lipids, vitamins, nucleotides and carbohydrates to form the off-odor volatiles and off-taste compounds in meat (Simic, 1983).

The effect of irradiation on volatile production and taste changes in meat can vary depending upon fatty acid composition, protein content, amino acid composition, processing conditions used, and antioxidant content in meat. Ahn, Wolfe, Sim, and Kim (1992) reported that the phospholipid structure in muscle cell membranes are broken during cooking, and thus cooked meat are more sensitive to susceptible to oxidation-reduction environment changes than raw meat. Zhu, Lee, Mendonca, and Ahn (2004a; b); Zhu et al.

(2005) and Houser et al. (2005) found that cured ready-to-eat meat products have stronger resistances to oxidative changes than uncured meat products because nitrite serves as a strong antioxidant in cured meat products.

The degradation pathway of adenosine triphosphate (ATP) that generates intermediate compounds including adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine monophosphate (IMP), inosine (INO) and hypoxanthine (Hx) in muscle has been extensively documented (Aliani, Farmer, Kennedy, Moss, & Gordon, 2013). The role of IMP for the generation of meat odor and flavor has been demonstrated both in model system and sensory studies (Lawrie & Ledward, 2006): Sikorski and Kolakowski (2000) reported that the quality of fish can be maintained as long as IMP is not depleted. However, IMP can be degraded into inosine and hypoxanthine by enzymes (Aubourg et al., 2007; Howgate, 2006). Once inosine and hypoxanthine are formed, they can produce bitter taste, and thus they are regarded as contributors to off-flavor (Özogul, Özden, Özogul, & Erkan, 2010).

The effects of irradiation on nucleotide degradation in seafood and poultry products have been studied. However, all those studies were focused on how to minimize or eliminate the autolytic or microbial enzymes that are involved in nucleotide degradation using irradiation, and no research was done to determine nucleotides degradation or elucidate the mechanism of nucleotides

Abbreviation used: ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; IMP, inosine monophosphate; INO, inosine; Hx, hypoxanthine.

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degradation by irradiation. The objectives of this study were to 1) determine the effect of irradiation on the degradation of nucleotides in turkey meat products, and 2) illustrate the nucleotides degradation pathway under different irradiation doses.

2. Materials and methods

2.1. Sample preparation

2.1.1. Meat samples

Raw turkey breast meat, sliced RTE turkey breast rolls, sliced RTE turkey ham, and RTE turkey sausages were purchased from a local grocery store. The turkey meat products were cut to 50 g pieces and individually packaged in vacuum bags (nylon/polyethylene vacuum bags, 9.3 ml O₂/m²/24 h at 0 °C; Koch, Kansas City, MO). Cooked turkey breast meat was prepared by vacuum-packaging raw turkey breast meat (approximately 66 g each) in oxygen impermeable bags (nylon/polyethylene vacuum bags, 9.3 ml O₂/m²/24 h at 0 °C; Koch, Kansas City, MO) and heated in an 85 °C-water bath to an internal temperature of 75 °C. After draining meat juices from the bag, the cooked turkey breast meat were repackaged in vacuum bags. All meat samples were stored at 4 °C before irradiation (Min, 2006).

2.1.2. AMP and IMP model systems

Standard AMP and IMP (1% w/v) were prepared in 4-mL distilled water in 15 mL Falcon™ Conical Centrifuge Tubes, and the head-space was flushed with nitrogen gas for 5 s to minimize oxidation. AMP required a few drops of 1N hydrochloric acid to enable it to dissolve (Aliani & Farmer, 2005).

2.2. Irradiation

The packaged meat and solutions were irradiated at four target dose levels (0, 1.5, 3.0 and 4.5 kGy) using an electron beam accelerator (Titan Corp., San Diego, CA) with 10 MeV energy and 5.6 kW power level. Alanine dosimeters were placed on the top and bottom surfaces of a package and read using a 104 Electron Paramagnetic Resonance Instrument (Bruker Instruments Inc., Billerica, MA) to check the absorbed dose. Following irradiation, packaged meat samples and solutions were immediately placed in coolers with crushed ice and transported to our lab and stored in a refrigerator at 4 °C. Nucleotides and nucleotides degradation products were determined on the day of irradiation.

2.3. Analysis of nucleotides, inosine and hypoxanthine

Nucleotides and nucleotides degradation products were measured using the HPLC method of Aliani and Farmer (2005) with some modifications. Three grams of minced meat were weighed into a 50 ml test tube and homogenized with 0.5 mL of an internal standard (10 mM xanthine) and 6 mL of 0.6 M perchloric acid using a Polytron homogenizer (Type PT 10/35, Brinkman Instruments Inc., Westbury, NY, USA) for 15 s at high speed. The precipitated proteins were removed by centrifugation at 3900 × g for 5 min. The supernatant was filtration through a Whatman No. 54 filter paper and the pH adjusted to pH 5.5 by dropwise addition of 6 M potassium hydroxide to precipitate potassium perchlorate, which was removed by centrifugation (11 min, 3900 × g). The supernatant was filtered through a Whatman No. 54 filter paper, held at 4 °C and analyzed on an HPLC system equipped with a diode array detector (Agilent 1100 Series HPLC system, Agilent Technologies, Wilmington, DE, USA). An aliquot of extract (1 µL) was injected using an auto-sampler and the nucleotides were separated on a Synergi Fusion-RP HPLC column (4 µm particle size, 80 Å pore size,

150 mm × 4.6 mm i.d., Phenomenex, Manchester, UK). A two-solvent mobile phase was used for elution: solvent A was a methanol/water mixture (60:40) and solvent B was aqueous KH₂PO₄ (0.02 M, adjusted to pH 5.5 with 1 M potassium hydroxide). All solvents were filtered through a 45 µm membrane filter (Millipore) and degassed using helium for 30 min before use. The binary gradient consisted of 3–20% A (97–80% B) for 16 min, 20% A (80% B) for 5 min. The column was regenerated at the end of each run by reversing the solvent gradient from 20 to 3% A (80–97% B) in 5 min. Detection was done at 254 nm (Aliani et al., 2013).

K-value, I-value and H-value were calculated as description of Özogul et al. (2010) and Saito, Arai, and Matsuyoshi (1959): it is expressed as a percentage of the content of the last two final compounds of the ATP catabolic pathway (Inosine, Hypoxanthine) over the total content of ATP and its degradation products: ATP, ADP, AMP, IMP, Inosine and Hx. The formulas are as follows:

$$\text{K-value (\%)} = \frac{[(\text{Inosine} + \text{Hypoxanthine}) / (\text{ADP} + \text{AMP} + \text{IMP} + \text{Inosine} + \text{Hypoxanthine})] \times 100}$$

$$\text{I-value (\%)} = \frac{[\text{Inosine} / (\text{ADP} + \text{AMP} + \text{IMP} + \text{Inosine} + \text{Hypoxanthine})] \times 100}$$

$$\text{H-value (\%)} = \frac{[\text{Hypoxanthine} / (\text{ADP} + \text{AMP} + \text{IMP} + \text{Inosine} + \text{Hypoxanthine})] \times 100}$$

2.4. Statistical analysis

Six packages of samples (replications) were used for each treatment. Data were analyzed by the GLM procedure of SAS (SAS 9.1 version) for different treatments. The differences in the mean values were compared by Tukey's multiple comparison method, and mean values and standard deviation of the means (SD) were reported ($P < 0.05$).

3. Results and discussion

3.1. Effect of irradiation on the degradation of nucleotides in raw and cooked turkey breast meat

Irradiation significantly impacted the nucleotides degradation of raw turkey meat: 27% decrease in ADP under irradiation doses from 0 to 1.5 kGy, 7% decrease in IMP from 1.5 to 3.0 kGy irradiation, and 1.4-/1.1-fold increase of inosine and hypoxanthine from 0 to 3.0 kGy irradiation, respectively. As the irradiation dose increased further, the concentration of inosine and hypoxanthine decreased more ($P < 0.05$). The removal of a phosphate group from ADP by irradiation increased the amount of AMP, inosine and hypoxanthine in 1.5 kGy-irradiated cooked turkey meat. The degradation of ADP and AMP did not occur rapidly at irradiation dose between 1.5 and 4.5 kGy. However, a significant decrease in IMP (from 19.96 to 17.34 µmol/g) was observed at irradiation dose between 3.0 and 4.5 kGy, and significant decreases in inosine and hypoxanthine were also observed at irradiation dose from 1.5 to 4.5 kGy ($P < 0.05$) (Fig. 1).

ADP and AMP were reported to be present only in trace amounts in irradiated (3 kGy) and non-irradiated sea bass muscle (Reale et al., 2008). Similar results were found in this study: ADP and AMP remained at low concentrations (<2.7 µmol/g) in irradiated raw and cooked turkey meat samples, but cooked turkey meats had higher levels of ADP and AMP because of dehydration and condensation effects during cooking (Lawrie & Ledward, 2006). Cooked meat is more susceptible to oxidation than raw meat

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