Packaging Determines Color and Odor of Irradiated Ground Beef

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Summary and Implications

Irradiation of ground beef under aerobic conditions oxidized myoglobin and drastically reduced color a*-values. Under vacuum or non-oxygen conditions, however, irradiation did not influence the redness of ground beef. Also, the red color of ground beef was maintained even after the irradiated beef was exposed to aerobic conditions. Vacuum-packaged irradiated ground beef had lower met-myoglobin content and lower oxidation-reduction potential than the aerobically packaged ones. Irradiating ground beef under vacuum-packaging conditions was also advantageous in preventing lipid oxidation and aldehydes production. Vacuum-packaged irradiated beef, however, produced high levels of sulfur volatiles during irradiation and maintained their levels during storage, which resulted in the production of characteristic irradiation off-odor. Double-packaging (V3/A3: vacuum-packaging during irradiation and the first 3 days of storage and then aerobic-packaging for the remaining 3 days) was an effective alternative in maintaining original beef color (red), and minimizing lipid oxidation and irradiation off-odor. The levels of off-odor volatiles in double-packaged irradiated ground beef were comparable to that of aerobically packaged ones, and the degree of lipid oxidation and color changes were close to those of vacuum-packaged ones. Ascorbic acid at 200 ppm level was not effective in preventing color changes and lipid oxidation in irradiated ground beef under aerobic conditions, but was helpful in minimizing quality changes in double-packaged irradiated ground beef. This suggested that preventing oxygen contact from meat during irradiation and early storage period (V3/A3 double-packaging) and double-packaging+ascorbic acid combination are excellent strategies to prevent off-odor production and color changes in irradiated ground beef. Developing methods that can prevent quality changes of irradiated beef is important for the implication of irradiation, which will improve the safety of beef.

Introduction

Irradiation is among the most effective technologies in preventing pathogenic microorganisms, but negatively influences quality of ground beef. Beef color is a prime quality parameter that determines consumer acceptance. Thus, maintaining the color of irradiated beef within a normal color range is one of the most important factors if irradiation can be used as a pathogen-reduction tool in beef.

Color changes induced by irradiation are different depending on animal species, muscle type, irradiation dose, and packaging: light meat such as pork loin and poultry breast meat produce pink color while dark meat such as beef becomes brown or gray after irradiation. Studies on the mechanisms and pigments involved in the color changes of irradiated meats indicated that the pink color in irradiated light meats was mainly caused by carbon monoxide-heme pigment complexes. It is reported that the production of CO by irradiation was via the radiolytic degradation of meat components such as glycine, asparagine, glutamine glyceraldehydes, pyruvate, α-ketoglutarate and phospholipids, and the production of CO via radiolytic degradation was closely related to the structure of molecules.

Both light and dark meats produced carbon monoxide by irradiation and the amounts of carbon monoxide produced were irradiation dose-dependent. The formation of carbon monoxide-heme complex, however, could not explain the color changes in irradiated dark meat such as ground beef. While the amount of carbon monoxide produced in beef by irradiation was similar to those of light meats, the pigment content in beef is about 10 times higher than that in light meats. The amount of carbon monoxide produced from meat by 2-3 kGy of irradiation was 1-3 ppm CO/g meat, which was just enough to react with 1-2 mg Mb/g meat. Therefore, the contribution of carbon monoxide-heme pigment to the color of ground beef was much smaller than that of light meats. However, the mechanisms and causes of color changes in irradiated beef are not fully understood yet.

Ascorbic acid is a reducing agent, which inhibits myoglobin oxidation and prevents brown color development in nonirradiated beef. Packaging, which determines oxygen availability to the meat in a packaging bag, is also a critical factor that influences color of meat because the chemical conditions of heme pigments are dependent upon the partial pressure of oxygen. Therefore, use of ascorbic acid and/or modification of packaging conditions can be a good strategy to control irradiation-dependent color changes in ground beef.

Irradiation produces off-odor volatiles: the major volatile compounds responsible for off-odor in irradiated meats are sulfur compounds. Volatile sulfur compounds were produced from the radiolytic degradation of the side chains of sulfur-containing amino acids such as methionine and cysteine. The sulfur volatiles produced by irradiation had characteristic odor, and the intensity of irradiation off-

odor diminished over storage period as the sulfur volatiles disappeared during storage under aerobic conditions. Using the high volatility of sulfur compounds under aerobic conditions, an efficient packaging method that can remove irradiation off-odor in irradiated meat during storage has been devised. The method is called "double-packaging", which combines both aerobic and vacuum conditions. Double-packaging was also effective in minimizing color changes and lipid oxidation in irradiated turkey and pork.

The objective of this study was to determine the effect of ascorbic acid and double-packaging on color, oxidation-reduction potential, lipid oxidation, met-myoglobin formation, and volatiles production in irradiated ground beef during storage. Although maintaining attractive color in irradiated ground beef is very important, little work has been done to improve the color of irradiated beef. This study can be of help in understanding the causes and mechanisms of color changes and developing an efficient method to control quality changes in irradiated ground beef.

Materials and Methods

Sample preparation

Beef loins from 4 different carcasses were obtained from Meat Laboratory at Iowa State University. Muscles taken from each carcass were treated as a replication. Each replication was ground separately through a 3-mm plate. Seven different treatments were prepared: 1) aerobically packaged, non-irradiated, 2) aerobically packaged, irradiated, 3) double-packaged I (A3/V3: aerobically packaged during irradiation and the first 3 days of storage and then vacuum-packaging for the next 3 days), irradiated, 4) vacuum-packaged, irradiated, 5) double-packaged II (V3/A3: vacuum-packaging during the irradiation and the first 3 days of storage and then aerobic packaging for the next 3 days), irradiated, 6) ascorbic acid-added, aerobically packaged, and irradiated, and 7) ascorbic acid-added, double-packaged II (V3/A3), and irradiated.

For ascorbic acid-added treatments, ascorbic acid was dissolved in minimal amount of distilled water and added at 200 ppm to the ground beef (final concentration) and mixed for 1 min in a bowl mixer. The mixed meat samples were ground again through a 3-mm plate to ensure even distribution of ascorbic acid. For rest of the treatments, water with no ascorbate was added, mixed, and ground at the same conditions as in ascorbic acid-added treatments.

Ground beef (approximately 75 g) was individually packaged in an oxygen-permeable bag (polyethylene, 4 x 6, 2 MIL), an oxygen-impermeable bag (nylon/ polyethylene, 9.3 mL O_2/m^2/24 h at 0°C), or double-packaged. For double-packaging, ground beef was individually packaged in an oxygen permeable bag and then vacuum-packaged in a larger oxygen-impermeable bag.

The packaged ground beef were irradiated at 4.5 kGy using a Linear Accelerator Facility with 10 MeV energy and 10.2 kW power level. The average dose rate was at 83.5 kGy/min. Alanine dosimeters were placed on the top and bottom surfaces of a sample and were read using a 104 Electron Paramagnetic Resonance Instrument to check the absorbed dose. The dose range absorbed at meat samples was 4.449 to 4.734 kGy (max/min ratio was 1.06). Aerobically packaged non-irradiated ground beef was used as a control. The samples were stored at 4 °C for 6 days. Color, met-myoglobin, oxidation-reduction potential, lipid oxidation, and volatiles of the samples were determined during the storage.

Color measurement

CIE color values were measured on the surface of meat samples using a LabScan colorimeter with a 1.225-cm aperture. The colorimeter was calibrated against a black and a white reference tiles covered with the same packaging bags used for samples. The color L* (lightness)-, a* (redness)-, and b* (yellowness)-values were obtained. An average value from 2 random locations on each sample surface was used for statistical analysis.

Met-myoglobin contents

Meat sample (1g) was homogenized with 9 mL of 0.04 M phosphate buffer (pH 6.8, 4 °C) using a Brinkman Polytron (Type PT 10/35) for 10 s at high speed. Meat homogenate (1 mL) was centrifuged at 8,000 × *g* for 1 min and the absorbances of the supernatant were immediately measured at 525, 572, and 700 nm using a spectrophotometer.

Oxidation-reduction potential (ORP)

To reduce the deviation of ORP values depending on the location of a meat patty, meat homogenate was used to determine ORP. Meat sample (5 g), 15 mL deionized distilled water, and 50 μL butylated hydroxytoluene (7.2% in ethanol) were placed in a 50-mL test tube and homogenized using a Brinkman Polytron for 15 s at high speed. The ORP values of homogenates were determined using a pH/ion meter equipped with a platinum electrode filled with an electrolyte solution (4 M KCl saturated with AgCl).

2-Thiobarbituric acid-reactive substances (TBARS)

Lipid oxidation was determined using a TBARS method. Minced sample (5 g) was placed in a 50-mL test tube and homogenized with 15 mL of deionized distilled water (DDW) using a Brinkman Polytron (Type PT 10/35) for 15 s at high speed. The meat homogenate (1 mL) was transferred to a disposable test tube (13 x 100 mm), and butylated hydroxytoluene (7.2%, 50 μL) and thiobarbituric acid/trichloroacetic acid [20 mM TBA and 15% (w/v) TCA] solution (2 mL) was added. The sample was mixed using a vortex mixer, and then incubated in a 90 °C water bath for 15 min to develop color. After cooling for 10 min in cold water, the samples were vortex mixed and centrifuged at 3,000 x *g* for 15 min at 5 °C. The absorbance of the resulting upper layer was read at 531 nm against a blank

prepared with 1 mL DDW and 2 mL TBA/TCA solution. The amounts of TBARS were expressed as mg of malonedialdehyde per kg of meat.

Volatile compounds

Volatiles of samples were analyzed using a Solatek 72 Multimatrix Vial Autosampler/Sample Concentrator 3100 connected to a GC/MS. Sample (3 g) was placed in a 40-mL sample vial, flushed with helium gas (40 psi) for 3 s, and then capped airtight with a Teflon*fluorocarbon resin/silicone septum. The maximum waiting time for a sample in a loading tray (4 °C) was less than 2 h to minimize oxidative changes before analysis. The meat sample was purged with He (40 mL/min) for 14 min at 40 °C. Volatiles were trapped using a Tenax/charcoal/silica column and desorbed for 2 min at 225 °C, focused in a cryofocusing module (-80 °C), and then thermally desorbed into a column for 60 s at 225 °C. An HP-624 column (7.5 m, 0.25 mm i.d., 1.4 μm nominal), an HP-1 column (52.5 m, 0.25 mm i.d., 0.25μm nominal), and an HP-Wax column (7.5 m, 0.250 mm i.d., 0.25 μm nominal) were connected using zero dead-volume column connectors. Ramped oven temperature was used to improve volatile separation. The initial oven temperature of 0 °C was held for 1.5 min. After that, the oven temperature was increased to 15 °C at 2.5 °C per min, increased to 45 °C at 5 °C per min, increased to 110 °C at 20 °C per min, and then increased to 210 °C at 10 °C per min and held for 2.25 min at that temperature. Constant column pressure at 22.5 psi was maintained. The ionization potential of MS was 70 eV, and the scan range was 19.1 to 350 m/z. The identification of volatiles was achieved by the Wiley library. The area of each peak was integrated using ChemStation™ software and the total peak area was reported as an indicator of volatiles generated from the samples.

Statistical analysis

A completely randomized design with 7 treatments and 4 replications was used. Data were analyzed using the generalized linear model procedure of SAS software. Student-Newman-Keul's multiple range test was used to determine significant differences between the mean values of treatments. Mean values and standard error of the means (SEM) were reported. Significance was defined at $P< 0.05$.

Results and Discussion

Color values of irradiated ground beef

The color of aerobically packaged ground beef was significantly influenced by irradiation. Electron beam-irradiation at 4.5 kGy reduced the CIE L*-, a*-, and b*-values of ground beef at Days 0 and 1, but the extent of decrease in a*- and b*-values were greater than that of L*-value. L-values in all irradiated meat gradually increased with storage except for A3/V3 treatment. The decrease of a*-values in ascorbic acid-added, aerobically packaged irradiated ground beef was the greatest at Day 0. After 1 day of storage, however, the a*-values in irradiated ground beef under aerobic and A3/V3 conditions were the lowest. After 6 days of storage, color a*-value of irradiated meat with V3/A3 double-packaging+ascorbate treatment was the highest, and irradiated meat with vacuum-packaging, V3/A3 double-packaging, or ascorbic acid treatment showed higher a*-values than nonirradiated control. The decrease of b*-values in irradiated ground beef was greater under vacuum than aerobic conditions.

The visual color of aerobically packaged ground beef changed from a bright red to a greenish brown immediately after irradiation, which should be unattractive to consumers. The color of ground beef was more stable when irradiated and stored under vacuum conditions. One day after irradiation, the color defects of irradiated ground beef was getting worse under aerobic conditions and the a*-values of aerobically packaged irradiated ground beef decreased by approximately 3 units from Day 0. However, the a*-values of irradiated ground beef under vacuum conditions did not change much and were significantly higher than those under aerobic conditions at Day 1. At Day 6, aerobically packaged non-irradiated control discolored to dark brown due to pigment oxidation. The degree of discoloration at Day 6 was the most severe in aerobically packaged or A3/V3 double-packaged irradiated ground beef. When irradiated ground beef was vacuum-packaged, V3/A3 double packaged, or ascorbic acid-added, the a*-value of irradiated ground beef were greater than that of non-irradiated control at Day 6. Addition of ascorbic acid to ground beef at 200 ppm (w/w) was also effective in reducing color changes by irradiation in aerobically conditions. This suggested that elimination of ascorbic acid as well as oxygen during irradiation is very important to minimize color changes in irradiated ground beef during storage.

When the irradiated ground beef was added with ascorbic acid and V3/A3 double-packaged, the color of ground beef was the best among the treatments after 6 days of storage. The a*-value of ground beef with ascorbic acid + V3/A3 double-packaging treatment was greater than that of the non-irradiated aerobically packaged beef due to the synergistic effect of ascorbic acid and vacuum conditions during the first 3 days of storage period, which helped maintaining heme pigments in reduced state.

Forms of heme pigments in irradiated ground beef

Ground beef irradiated under aerobic conditions produced higher amounts of met-myoglobin than those irradiated under vacuum conditions at Day 1, and the proportion of met-myoglobin in meat and color a*-value was negatively correlated. Aerobic conditions during storage further increased the percentage of met-myoglobin, but addition of ascorbic acid helped slowing down met-myoglobin formation. Vacuum conditions during storage reduced the level of met-myoglobin in irradiated ground beef. This suggested that the brown discoloration of ground beef upon irradiation under aerobic conditions was caused

by the met-myoglobin formation, and the color was totally different from that of irradiated pork or poultry breast, which was pink or red The formation of CO-heme complex, however, could not explain the color changes in beef after irradiation. The production of carbon monoxide in beef by irradiation was similar to that of light meats, but the pigment content in beef is much higher (> 10-fold) than that in light meats. Therefore, the contribution of CO-heme pigment to the color of ground beef was much smaller than that of the light meats.

Because the color intensity of ferrous heme pigment is stronger than that of ferric form, oxidation-reduction potential (ORP) plays a very important role on the color changes of meat. Both vacuum conditions and ascorbic acid were effective in maintaining low ORP values in irradiated ground beef. It is speculated that free binding sites of myoglobin can react with free radicals such as hydroxyl or sulfuryl radicals produced by irradiation and forms met-myoglobin and sulf-myoglobin responsible for brown and green color, respectively. However, the lowered ORP values by vacuum conditions and/or ascorbic acid maintained heme pigments in ferrous state and stabilized the color of irradiated ground beef. At Day 6, vacuum-packaged irradiated ground beef had lower met-myoglobin content and ORP values than the V3/A3 double-packaged ones, indicating that the color of vacuum-packaged irradiated ground beef could be bloomed when it is exposed to aerobic conditions even after 6 days of storage. Although the chemical forms of heme pigments in irradiated beef have not been clearly identified, the color changes in irradiated ground beef could be reverted to the desirable red color if the reducing power of meat is maintained by vacuum conditions and/or ascorbic acid.

Lipid oxidation of irradiated ground beef

The TBARS values of ground beef irradiated and stored under aerobic conditions were not different from that of control at Day 1, but the rate of lipid oxidation was faster in irradiated than non-irradiated control as the storage time increased. Vacuum-packaged irradiated beef was resistant to lipid oxidation during the 6 days of storage. Double-packaged irradiated ground beef showed significant differences in TBARS values depending on whether they were aerobic/vacuum or vacuum/aerobic packaged. V3/A3 double-packaged irradiated ground beef had lower TBARS values than the A3/V3, indicating that hydroxyl radicals produced by irradiation can initiate lipid oxidation but cannot propagate without the presence of oxygen. Addition of ascorbic acid at 200 ppm was not effective in preventing lipid oxidation when the irradiated beef was aerobically packaged.

When irradiated ground beef was stored for 6 days under aerobic conditions, significant amounts of volatile aldehydes (propanal, pentanal, and hexanal) were produced in irradiated ground beef. Hexanal, a major indicator of lipid oxidation, was the most prominent aldehyde, and large amounts of hexanal were detected in aerobically packaged ground beef at Day 6. Addition of ascorbic acid was not effective in inhibiting production of volatile aldehydes in irradiated beef at Day 6 as shown in TBARS values. However, vacuum packaging or double-packaging+ascorbic acid combination was effective in minimizing the production of volatile aldehydes in irradiated ground beef. Therefore, lipid oxidation of irradiated ground beef was highly dependent upon the availability of oxygen to meat during storage. Addition of 200 ppm ascorbate to double-packaged ground beef was helpful in slowing down the development of lipid oxidation in irradiated ground beef.

Sulfur volatiles of irradiated ground beef

Irradiated ground beef produced significant amounts of sulfur-methyl ester ethanethioic acid and dimethyl disulfide. The amounts of sulfur volatiles in aerobically packaged irradiated ground beef, however, were much smaller than those in vacuum-packaged ones at Day 1. Aerobically packaged and double-packaged irradiated ground beef did not have sulfur-methyl ester ethanethioic acid and only a small amount of dimethyl disulfide was detected at Day 6. Most of the sulfur volatiles in irradiated ground beef disappeared after 6 days of storage under aerobic or double-packaged conditions because they were highly volatile.