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Antioxidant effect of fractions from chicken breast and beef loin homogenates in phospholipid liposome systems

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ABSTRACT

The antioxidant effects of meat fractions from chicken breast and beef loin were compared. Five meat fractions – homogenate (H), precipitate (P), supernatant (S), high-molecular-weight (HMW) and low-molecular-weight (LMW) fractions – were prepared from chicken breast or beef loin. Each of the fractions were added to a phospholipid liposome model system containing catalysts (metmyoglobin, ferrous and ferric ion) or iron chelating agents to determine the effects of each fraction on the development of lipid oxidation during incubation at 37 °C for 120 min. All fractions from chicken breast showed stronger antioxidant effects against iron-catalyzed lipid oxidation than those from beef loin. Iron chelating capacity of water-soluble LMW and water-insoluble (P) fractions from both meats were responsible for their high antioxidant capacities. High concentration of myoglobin, which served as a source of various catalysts, was partially responsible for the high susceptibility of beef loin to lipid oxidation. Storage-stable ferric ion reducing capacity (FRC) was detected in all fractions from both meats, and was a rate-limiting factor for lipid oxidation in the presence of free ionic iron. Higher antioxidant capacity and lower myoglobin content in chicken breast were primarily responsible for its higher oxidative stability than beef loin. DTPA-unchelatable compounds, such as ferrylmyoglobin and/or hematin were the major catalysts for lipid oxidation in beef loin, but free ionic iron and storage-stable FRC also played important roles during prolonged storage.

1. Introduction

Despite extensive studies for several decades, the primary catalysts for lipid oxidation in meat are still controversial. Lapidot, Granit, and Kanner (2005) suggested that metmyoglobin is a silent compound in the absence of hydrogen peroxide (H_2O_2) or lipid hydroperoxide (LOOH). However, myoglobin appeared to be the center compound in this controversy because myoglobin can be converted to ferrylmyoglobin in the presence of H_2O_2 or LOOH and serves as a major source of hematin and free ionic iron, which can initiate and propagate lipid oxidation (Min & Ahn, 2005). Ferrylmyoglobin generated by the interaction of metmyoglobin with H_2O_2 or LOOH can abstract a hydrogen atom from a bis-allylic carbon on a fatty acid chain and is a major initiator of lipid oxidation (Baron & Andersen, 2002; Baron, Skibsted, & Andersen, 1997; Hamberg, 1997). Ferrylmyoglobin can also degrade LOOH to alkoxyl or peroxyl radicals, which undergo a chain-propagation step or are decomposed to produce secondary by-products of lipid oxidation (Reeder & Wilson, 1998, 2001). Free ionic iron released from heme proteins, iron-containing proteins, or ferritin can initiate lipid oxidation in meat via the Fenton reaction in the presence of H_2O_2 or LOOH and reducing agents, such as superoxide anion ($O_2^{\cdot -}$), ascorbic acid, NAD(P)H and thiols (Ahn & Kim, 1998; Ahn, Wolfe, & Sim, 1993; Apte & Morrissey, 1987; Decker & Hultin, 1992; Kanner, Hazan, & Doll, 1988).

The activity of myoglobin as a major catalyst as well as a source of free ionic iron in the processes of lipid oxidation can be affected by the concentration of myoglobin, the presence of H_2O_2, LOOH, and reducing compounds (Baron, Skibsted, & Andersen, 2002; Gorelik & Kanner, 2001; Harel & Kanner, 1989; Lapidot et al., 2005; Rhee, Ziprin, & Ordonez, 1987). Free ionic iron can serve as a catalyst of lipid oxidation in the presence of reducing compounds or $O_2^{\cdot -}$-generating systems (Kanner, 1994; Kanner, Harel, & Hazan, 1986; Rhee, 1988; Turrens & Boveris, 1980). The status of free ionic iron is more important than the amount of ionic iron for the development of lipid oxidation (Ahn & Kim, 1998; Ahn et al., 1993). Water-soluble and water-insoluble components that influence the catalytic activities of myoglobin and free ionic iron are present in the cytosol of meat, and the balance between antioxidant and prooxidant activities of the cytosol in muscle tissues determines

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the prooxidant actions of myoglobin and free ionic iron in meat (Min & Ahn, 2009; Min, Nam, & Ahn, 2010).

DTPA is an excellent chelating agent for both ferrous and ferric ion. DFO chelates only ferric ion and inhibits its catalyzing activities (Kanner & Harel, 1987; Rahhal & Richter, 1989). However, DFO serves as an electron donor, suppresses catalytic activity of ferrylmyoglobin, and interrupts free radical chain reaction of lipid oxidation (Kanner & Harel, 1987; Rice-Evans, Okunade, & Khan, 1989). Thus, DFO can be more efficient inhibitor of lipid oxidation than DTPA (Gutteridge, Richmond, & Halliwell, 1979).

The susceptibility of meat from different animal species to lipid oxidation is different, and chicken breast is much less susceptible to lipid oxidation than beef loin (Min & Ahn, 2009; Min, Nam, Cordray, & Ahn, 2008). High total antioxidant capacity, high myoglobin reducing capacity, low myoglobin concentration and its lipoxygenase-like activity, and low free ionic iron concentration were responsible for the high oxidative stability of chicken breast (Min & Ahn, 2009; Min, Cordray, & Ahn, 2010; Min et al., 2008). The objective of this study was to evaluate the antioxidant and prooxidant effects of meat fractions from chicken breast and beef loin in a phospholipid liposome model system in the presence of catalysts (metmyoglobin, ferrous, and ferric ions) or chelating agents (DFO and DTPA).

2. Materials and methods

2.1. Chemicals and reagents

Metmyoglobin (from equine skeletal muscle), ferrous ammonium sulfate, ferric chloride, diethylenetriamine pentaacetic acid (DTPA), desferrioxamine (DFO), linoleic acid, 2-thiobarbituric acid (TBA), ferric chloride, Chelex-100 resin (50–100 dry mesh, sodium form), and butylated hydroxytoluene (BHT) were purchased from Sigma (St. Louis, MO). All other chemicals were of reagent grade. Deionized distilled water (DDW) by Nanopure Infinity™ ultrapure water system with ultraviolet (UV) light (Barnstead, Dubuque, IA) was used for the preparation of all reagents and buffers. All DDW and buffers were treated with Chelex-100 resin to remove any free metal ions before use.

2.2. Preparation of fractions from meat homogenates

Eight beef loins were obtained from a local packing plant 6d post-slaughter. Two loins were pooled and treated as a replication. Each loin was trimmed off any visible fat and connective tissues, and each replication was ground separately through a 3-mm plate twice. Twelve 8-week-old broiler chickens raised on a corn-soybean meal diets were slaughtered according to the USDA guidelines, and breast meats were separated from the carcasses 24 h after slaughter. The breast meats from 3 birds were pooled and used as a replication. Muscles for each replication were ground separately through a 3-mm plate twice.

The ground meat was homogenized with three volumes of 50 mM acetate buffer (pH 5.6) using a high speed homogenizer (Brinkman Polytron, Model PT 10/35, Westbury, NY) for 15 s at speed setting 7. A portion of the homogenate (H) was centrifuged at 15,000 × *g* for 30 min at 4 °C. After centrifugation, the supernatant was filtered through a Whatman No. 1 filter paper twice and used as a supernatant (S) fraction. A portion of S fraction was ultrafiltered by centrifugation through a Centricon Plus-20 centrifugal filter (MW cut-off of 10,000; Millipore, Billerica, MA). The filtrate was collected as a low-molecular-weight (LMW) fraction. The retentate was recovered, made to the initial volume with acetate buffer, ultrafiltered two more times through a Centricon Plus-20 centrifugal filter to remove any remaining low molecular weight substances, and then used as a high molecular weight (HMW) fraction. The precipitant was re-suspended in three volumes of 50 mM acetate buffer (pH 5.6) and centrifuged to remove remaining water-solubles. After washing two more times with acetate buffer, the precipitant was suspended in three volumes of 50 mM acetate buffer (pH 5.6) and used as a precipitant (P) fraction (Fig. 1). All fractions were stored at 4 °C until analyzed and all analyses were finished within 3 days after preparations.

2.3. Lipid oxidation potential (LOP)

Lipid oxidation potential (LOP) of catalysts (metmyoglobin, Fe(II), and Fe(III)), chelating agents (DFO and DTPA), fractions from chicken breast and beef, and the mixtures of the catalysts or chelating agents with the fractions were determined in the phospholipid liposome model system. Metmyoglobin, ferrous ammonium sulfate, ferric chloride, DTPA, and DFO solution dissolved in 50 mM acetate buffer (pH 5.6) were mixed with each fraction at 1:1 (v/v) ratio just before analyses to make their final concentrations at 1.0 mg/ml, 5 μg/ml, 5 μg/ml, 2 mM, and 2 mM, respectively. The phospholipids from egg yolk was used to prepare the liposome model system following the method described previously (Min & Ahn, 2009). The fatty acid composition of the phospholipids used in this study is shown in Table 1. Briefly, an aliquot of phospholipids dissolved in chloroform were transferred to a volumetric flask and evaporated under nitrogen gas to make a thin film on the flask wall. Each fraction was added to the phospholipid-coated flask and then the flask was shaken vigorously for 2 min to make fraction-liposome solution with final concentration of 3 mg phospholipids per milliliter fraction.

The liposome solutions containing the meat fraction were transferred to scintillation vials and incubated at 37 °C for 120 min to accelerate lipid oxidation. Lipid oxidation in the liposome solution was determined at 0, 15, 30, 60, 90, and 120 min after incubation. After adding 10 μl of 6% BHT in ethanol to stop lipid oxidation, an aliquot (0.5 ml) of sample was mixed with 1 ml of TBA/TCA solution (15 mM TBA/15% trichloroacetic acid (TCA; w/v)) and incubated in a boiling water bath for 15 min. After cooling, the mixture was centrifuged at 15,000 × *g* for 10 min. The absorbance of the supernatant was determined at 531 nm against a reagent

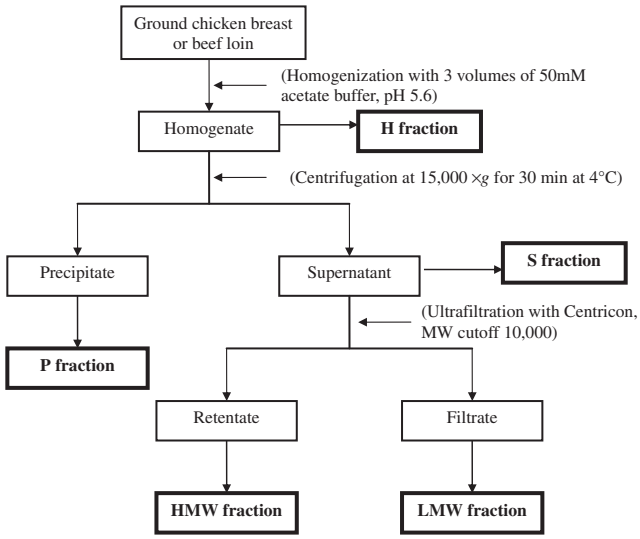

Fig. 1. Flow diagram of fraction preparation from raw chicken breast and beef loin. Abbreviations: H, homogenate fraction; P, precipitate fraction; S, supernatant fraction; HMW, high molecular weight fraction from supernatant fraction; LMW, low molecular weight fraction from supernatant fraction; MW, molecular weight.