Food Research International 89 (2016) 1038–1045

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Effect of green tea on interaction of lipid oxidation products with sarcoplasmic and myofibrillar protein homogenates extracted from bovine top round muscle

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ARTICLE INFO

Article history:
Received 31 October 2015
Received in revised form 9 January 2016
Accepted 15 January 2016
Available online 19 January 2016

Keywords:
Binding
Bovine sarcoplasmic proteins
Myofibrillar proteins
Green tea
Oxidation

ABSTRACT

The interaction between lipid oxidation products and bovine sarcoplasmic (SP) and myofibrillar protein (MP) homogenates in the presence of green tea was investigated. To monitor the effect of green tea on lipid oxidation, aldehydes were measured while effect on protein was monitored via changes in myoglobin, thiols, and tryptophan fluorescence over nine days of refrigerated storage. The presence of SP and MP decreased free aldehydes in the buffers. Sarcoplasmic homogenates bound aldehydes to a greater degree than MP. The tea compounds exhibited more favorable binding energies than aldehydes near histidine 64 close to the heme moiety of myoglobin. Addition of tea lowered tryptophan fluorescence and thiol content. The results suggested that green tea enhances the binding of bovine SP and MP to lipid oxidation products. The results also suggested that green tea can decrease rancidity by directly binding lipid oxidation products.

1. Introduction

Lipid oxidation (LOX) and protein oxidation (POX) are major causes of chemical and physical quality deterioration in meat. Lipid oxidation involves a free radical chain reaction with unsaturated fatty acids including linoleic, linolenic, arachidonic, palmitic and oleic in beef (Pavan & Duckett, 2013) to produce various alcohols, aldehydes, and ketones responsible for rancidity in beef (Saraiva et al., 2015; Shahidi & Alexander, 1998). Volatile aldehydes e.g. hexanal responsible and highly correlated with unpleasant aromas in beef, have greater binding to proteins compared to ketones which could impact perceived rancidity (Pérez-Juan, Flores, & Toldrá, 2008). Lipid oxidation compounds along with reactive oxygen species simultaneously lead to POX, altering cell structure, viscosity, emulsification, water holding capacity and texture, and lowered nutritional value (Estévez, 2011; Lund, Heinonen, Baron, & Estevez, 2011; Min & Ahn, 2005; Zhang, Xiao, & Ahn, 2013).

The interaction of lipid oxidation aldehydes with beef proteins can form protein-volatile adducts, which can negatively affect protein structure and functionality (Addis, 1986; Gardner, 1979; Gerrard & Brown, 2002; Nair, Cooper, Vietti, & Turner, 1986). The binding of aldehydes for example malonaldehyde (MDA), hexanal, and 4-hydroxynonenal to a variety of proteins has been researched (Goodridge, Beaudry, Pestka, & Smith, 2003; Lynch, Faustman, Silbart, Rood, & Furr, 2001; Pérez-Juan, Flores, & Toldrá, 2006, 2007; Pignoli, Bou, Rodriguez-Estrada, & Decker, 2009; Smith, Pestka, Gray, & Smith, 1999). These protein-volatile adducts could affect the sensory quality of foods by inhibiting perceived oxidative rancidity through conversion of lipid oxidation secondary products into non-volatile adducts (Elias, Kellerby, & Decker, 2008). In spite of the binding of beef proteins to LOX products (Pérez-Juan et al., 2006), if proteins are oxidized, they can act as lipid pro-oxidants.

Green tea (GT) has been applied to a variety of meats to minimize lipid oxidation via metal chelation and free radical scavenging by catechin and theaflavins (Tang, Kerry, Sheehan, & Buckley, 2002; Tang, Sheehan, Buckley, Morrissey, & Kerry, 2001; Yilmaz, 2006). However, the degree to which GT impacts the interaction with major beef proteins to affect LOX products has not been assessed. Sarcoplasmic and myofibrillar proteins constitute majority (>30%) of proteins in skeletal muscle, and the experimental objective was to examine the antioxidative capacity of GT when interacting with beef homogenates containing SP and MP.

2. Materials and methods

2.1. Reagents

Sodium phosphate dibasic anhydrous and monobasic, HPLC water and methanol, BSA, biuret reagent and trichloroacetic acid (TCA) were purchased from Fisher Scientific (Tustin, CA, USA). Sodium azide, GC

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http://dx.doi.org/10.1016/j.foodres.2016.01.016
0963-9969/

standards, thiobarbituric acid (TBA), and 1, 1, 3, 3-tetramethoxypropane (TMP) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Preparation of green tea infusions and total phenolic content measurement

Organic, fair trade certified green tea leaves sourced from Idulgashinna estate in Sri Lanka were obtained from QTrade Teas & Herbs Company (Cerritos, CA, USA). The leaves were ground in a coffee grinder for 60 s, and passed through a 0.037 mm sieve. 6 g of GT powder was infused with 60 mL of DI water and heated at 80 °C for 30 min. Green tea infusions (GT) were then cooled to 25 °C and diluted to 100, 50 and 25 mg/mL. Total phenolic content was determined by the Folin—Ciocalteau assay according to Obuchowicz, Engelhardt, and Donnelly (2011) and expressed as mg gallic acid equivalents/g dry tea.

2.3. Preparation of sarcoplasmic and myofibrillar homogenates

Bovine top round muscle obtained from three carcasses (American Beef Packers Incorporation, Chino, CA, USA) was frozen for 48 h after slaughter. The meat was then minced through a 3 mm grinding plate attached to a Kitchen Aid food processor (St Joseph, MI, USA). The ground beef from all carcasses was then hand-mixed together for 2 min.

A 30 mM pH 7.4 sodium phosphate buffer (16 mL) containing 0.02% NaN_3 was added to 4 g of ground top round muscle to extract sarcoplasmic proteins (Pérez-Juan et al., 2006). The mixture was vortexed for 1.5 min and then centrifuged at 3000 rpm for 15 min at 4 °C. The supernatant was filtered through eight layers of cheesecloth. The precipitate was then used for MP extraction using pH 7.4 100 mM sodium phosphate buffer (28 mL) containing 0.02% NaN_3 and 0.35 mol/L KI. The mixture was again vortexed, centrifuged, and filtered using the same conditions as used for SP extraction. During the sample preparation, samples were kept on ice. The protein concentration was determined using Biuret method.

Preliminary studies showed that green tea at 0.1 g/100 g beef effectively inhibited LOX, so taking into account the percent protein concentration in beef muscle, the GT treated SP and MP samples were prepared by adding 25 μL of 25, 50 and 100 mg/mL GT into 1 mL protein samples and refrigerated prior to the various assays.

2.4. Interaction of green tea and bovine proteins on malonaldehyde

Malonaldehyde was prepared according to Kakuda, Stanley, and van de Voort (1981) with slight modifications. TMP (164 μL) was hydrolyzed in 10 g TCA/100 mL water at 70 °C for 15 min prior to diluting to a final concentration of 0.15 mM MDA. The MDA was added to samples 1:10 (MDA: sample), incubated at 4 °C, and measurements were as outlined by Lin, Toto, and Were (2015) with modifications as follows. Each 0.6 mL of samples was mixed with 0.75 mL of 10 g TCA/100 mL and centrifuged using accuSpinTM micro (Pittsburg, PA, USA) at 8000 g^{-1} for 5 min. The 0.02 mol/L TBA: supernatant (1:1) samples were incubated at 60 °C for 90 min prior to absorbance readings at 532 nm using a FLUOstar Omega Microplate Reader (BMG Labtech, Cary, NC, USA). All treatments were done in triplicate. The interaction of proteins with or without GT on MDA was expressed as bound MDA/protein (mg/g) shown in Eq. (1).

$$\text{Bound MDA/Protein} = \frac{\frac{[\text{MDA}]_C - [\text{MDA}]_{SP} + [\text{MDA}]_{EN} + [\text{MDA}]_{GT}}{[\text{MDA}]_C} \times I}{C_P} \quad (1)$$

where $[MDA]_C$ (mg/L) was the MDA concentration in buffer control sample (no protein) spiked with aldehydes, $[MDA]_{SP}$ (mg/L) was the MDA concentration in spiked protein solutions, $[MDA]_{EN}$ (mg/L) was the endogenous MDA in the protein sample (no aldehydes), $[MDA]_{GT}$ was the MDA in green tea infusion, I (mg/L) was the initial MDA concentration added to the homogenates, and C_P (mg/mL) was the protein concentration present in the protein vials.

2.5. Gas chromatography (GC) measurement of interaction of green tea, aldehydes and bovine proteins

Each 9 mL of GT (25 and 100 mg/mL) treated SP and control samples were incubated with 10 mg/L pentanal and hexanal and 12.5 mg/L heptanal, octanal and nonanal final concentration while GT treated MP samples were incubated with 2 mg/L of all aldehydes at final concentration. All aldehydes were below detection limit (all were bound by proteins) after a week when lower concentrations were used. Based on preliminary studies, the concentration of spiked aldehydes was thus increased from 1 to 10 mg/L for C_5 and C_6 and 12.5 mg/L for C_7–C_9 aldehydes in SP samples and to 2 mg/L of all aldehydes in MP samples to monitor changes over expected refrigerated storage time of meat. After 24 h at 4 °C, 4.75 mL sample was mixed with 0.25 mL of 2 mg/L 4-heptanone as the internal standard (IS). The GC analysis was conducted as outlined by Lin et al. (2015), using an 8:1 splitless inlet. The peak area was integrated using ChemStation software (Agilent Technologies, Inc. Santa Clara, CA, USA). The aldehydes bound by proteins and/or GT were expressed as bound mg aldehydes/g protein (Eq. (2)) after determining the internal response factor.

$$\text{Bound aldehyde}\left(\frac{\text{mg}}{\text{g}}\right)\text{Protein} = \frac{\frac{[A]_B - [A]_P + [A]_{UN} + [A]_{CF}}{[A]_B} \times I}{\text{protein concentration}\left(\frac{\text{g}}{\text{mL}}\right) * 1000(\text{mL})} \quad (2)$$

where $[A]_B$ refers to aldehyde concentration in buffer control sample (no protein) spiked with aldehydes, $[A]_P$ refers to aldehyde concentration in spiked protein sample, $[A]_{UN}$ refers to aldehyde concentration in unspiked protein sample (no aldehydes), $[A]_{CF}$ refers to aldehyde concentration in tea and I refers to initial concentration of aldehydes added (mg/L).

2.6. Binding of aldehydes and tea compounds on bovine myoglobin by molecular modeling studies

Homology modeling structure of the apoprotein of beef myoglobin was attained (http://www.ebi.ac.uk/pdbsum/1Z2H). Coordinates of the heme group were taken from a protein data bank of beef hemoglobin (1G09.pdb) Heme group was placed into the heme pocket of apoprotein in the position similar to the X-ray structure of myoglobin in 1MBN.pdb. Modified protein structure was solvated in a box of about 9000 TIP3P water molecules. We performed 5000 steps of steepest decent energy minimization followed by 1 ns equilibration molecular dynamics. Next 10 ns production molecular dynamics runs were executed. The MD simulations were performed with an Amber 10 program and Amber99SB force field (Case et al., 2004, 2008). Periodic boundary conditions with constant pressure constraints, Langevin thermostat at 300KParticle Mesh Ewald (PME) approach was used to model electrostatic interactions. Ten snapshots from last 1 ns of molecular dynamics trajectory were saved and used for subsequent docking using Autodock Vina (Trott & Olson, 2010). Ligand and receptor files were prepared based as described in the Autodock Tools (ADT) documentation program (Morris et al., 2009). The ADT assigned polar hydrogens, united atoms Kollman charges, and solvation parameters.

The entirety of the molecular surface was considered by centering a search space of 30 Å on iron from heme group of beef myoglobin. Specific amino acids were analyzed by centering a cubic search space with vertices of 10 Å on the target amino acid residues. An exhaustiveness of 100 was used for the entirety of the molecular surface and an exhaustiveness of 20 was employed for the individual amino acids. The pose with lowest binding free energy was aligned with receptor for