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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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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. The SP bound more aldehydes 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.

Keywords: Binding, bovine sarcoplasmic proteins, myofibrillar proteins, green tea, oxidation

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, Oliveira, Silva, Martins, Ventanas, & García, 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 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 were 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 et al. (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 & 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, & Were (2015) with modifications as follows. Each 0.6 mL of samples was mixed with 0.75 mL of 10g 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 532nm 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 equation 1.

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

where $[MDA]_C$ (mg/L) was the MDA concentration in buffer control sample (no protein) spiked with aldehydes, $[MDA]_{SP}$ (mg/L) was 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, Toto, & Were (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 (equation 2) after determining the internal response factor.

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

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 1ns 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; Case et al., 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., 1998). 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 30Å on iron from heme group of beef myoglobin. Specific amino acids were analyzed by centering a cubic search space with vertices of 10Å 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 further analysis of interactions. Results presented are from a snapshot of molecular dynamics that shows lowest binding energy.

2.7. Effect of green tea on tryptophan fluorescence in bovine proteins

Fluorescence spectrum of tryptophan of SP and MP samples at 300-400 nm with the excitation at 280 nm was measured on day 1, 3, 5, 7 and 9 of refrigerated storage following the method of Estévez, Kylli, Puolanne, Kivikari, & Heinonen (2008), with the following modifications: 0.1 mL of each GT-treated SP and MP was mixed with 0.9 mL of DI water prior to tryptophan determination by using a FluoroMax-4 spectrofluorometer (Horiba Scientific, Edison, NJ).

2.8. Effect of green tea on protein oxidation

Absorption spectra at 300–700 nm of bovine sarcoplasmic was determined on day 0, 1, 3, 5, 7 and 9 of refrigerated SP and the metmyoglobin and oxymyoglobin content was calculated (Tang, Faustman, & Hoagland, 2004).

Total thiol contents in beef protein homogenates were determined according to Eymard, Baron, & Jacobsen, (2009) with the following modifications: to 1 mL of each supernatant, 14.3 µL of 10 mM 5, 5′-dithio-bis (2-nitrobenzoic acid) in 0.05 mol/L sodium acetate were added and

incubated at 40 °C for 15 min. The A_{412} was used to calculate thiol content using ε of 13,600 (mol/L) $^{-1}$ cm^{-1}, and results were expressed as thiol/protein (μmol/g).

2.9. Statistical analysis

The effects of GT and bovine proteins on MDA, aldehydes and protein oxidation were analyzed using R Studio version 0.98.1074. The difference in means between treatments and days (0, 1, 3, 5, 7 and 9) was compared using ANOVA and Tukey test at significant level of 0.05.

3. Results and Discussion

3.1. Effect of green tea on malonaldehyde in bovine proteins

The bioactivity of green tea is mainly due to the phenolic compounds and the total phenolic content of GT was 134.87±1.96 mg gallic acid equivalent /g GT. The presence of bovine SP and MP resulted in decreased MDA when compared to the buffer (Table 1), indicating that MDA bound to protein homogenates. Free MDA in buffers decreased with added GT on all days of analysis ($p<0.05$) which suggests that GT also binds MDA. On day 1, MDA was bound to GT-treated SP homogenates by 0.27-0.32 mg/g SP compared to control SP binding by 0.18 mg/g SP (Fig.1A). After nine days of refrigerated storage, MDA in GT-treated SP was bound to a greater extent (0.81 mg bound MDA/g SP) than in control SP (0.33 mg bound MDA/g SP). However, no difference in MDA values was found amongst the different GT concentrations ($p<0.05$). In the presence of MP, MDA was bound to 100 mg/ml GT to a greater extent (0.35 mg/g MP) compared to control MP (0.17 mg/g MP) on day 9 (Fig. 1B). Comparing the binding capacity between the two homogenates, the MDA bound by MP was 2-fold less than what was bound by SP. The interactions of meat proteins and MDA has been demonstrated in fish and chicken (Kwon, Menzel, & Olcott, 1965). Buttkus (1967) showed that aromatic amino acids,

preferably histidine and tyrosine from fish proteins could bind MDA resulting in lower free MDA. The presence of these aromatic amino acids (tyrosine and histidine) in the SP and their absence in MP gives SP greater capacity to bind MDA than MP (Yin et al. 2011). The molecular binding simulations conducted determined potential amino acid binding sites in bovine SP myoglobin to MDA (section 3.3). The results also suggest that binding of MDA to proteins increases in the presence of GT (Table 1). The binding of GT catechins with myoglobin in addition to MDA interaction with other sarcoplasmic proteins could be the reason for lower MDA (Tang et al., 2002; Yilmaz, 2006).

3.2. Interaction of green tea on the interaction of bovine proteins and volatile aldehydes

The presence of SP and MP decreased the free aldehydes measured in 30 and 100 mM sodium phosphate buffers, demonstrating that the protein homogenates bound these aldehydes (Table 1). The free aldehydes were in the decreasing order: pentanal > hexanal > heptanal > octanal > nonanal, suggesting that molecules with longer carbon-chain length tended to be bound stronger to the proteins. These results are consistent with the findings by Pérez-Juan et al. (2006) who found that pork SP and MP homogenates bound to octanal and hexanal and Pérez-Juan et al. (2008) who found that pork actomyosin, a myofibrillar protein bound to octanal greater than did hexanal. The binding affinity between proteins and aldehydes thus partially depends on hydrophobic interaction (Gardner, 1979).

Bound hexanal, one of the predominant aldehydes in meat (Machiels, van Ruth, Posthumus, & Istasse, 2003; Shahidi & Pegg, 1994; Shahidi & Alexander, 1998) is shown in Fig.1C and D. There was no hexanal peak found in GT, and thus no interfering effect from GT on the results. The binding results of pentanal, heptanal, octanal, and nonanal had a similar trend as hexanal (Table 1). Significant differences were found in bound aldehydes among GT

concentration treatments in homogenates containing MP over nine days of refrigerated storage ($p<0.05$). After a week of refrigerated storage, hexanal was bound to GT-treated MP by 0.05-0.08 mg/g MP compared to 0.03 mg/g in control MP (Fig1.B). Similar to the MP-MDA binding results, GT increased the MP-volatile binding, and it was GT concentration dependent ($p<0.05$). However, no difference between concentrations was found in SP-hexanal binding (0.63-0.66 mg bound hexanal/g SP) when treated with GT after a week (Fig.1C). All protein-bound aldehydes significantly increased after seven days of refrigerated storage ($P<0.05$). Comparing the binding capacity between the two proteins, aldehydes were bound to SP (0.10-0.66 mg bound volatile/g SP) approximately 10-fold greater than those bound by MP (0.01-0.07 mg bound volatile/g MP) which was similar to results obtained by Pérez-Juan et al., (2006) who found that SP had greater ability to bind aldehydes than MP. The lowered free aldehydes measured could thus be due to the interaction with meat proteins, also shown with the molecular binding results (section 3.3). The molecular binding model was also conducted for aldehydes (section 3.3). The binding of tea phenolic compounds to meat proteins might account for enhanced antioxidant capacity towards lipids due to interactions stabilizing the protein interactions.

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

The docking calculation results for GT compounds presented in Table 2 and Fig. 5 indicate that aldehydes are able to bind to the surface of myoglobin near His, Trp and Phe residues with binding affinities in range of -1.9 to -4.1kcal/mol (Table 3). The lowest energy conformations of hexanal and nonanal docked to the surface of myoglobin near the heme group (Fig. 5). The antioxidant tea compounds bound to myoglobin with more favorable binding affinities in the range of -5.9 to -7.7 kcal/mol compared to aldehydes. All tea compounds were

bound to the surface of myoglobin near the heme pocket and His64. Interactions were facilitated by hydrophilic residues Asn66, Thr70, Lys63, Thr67 on the surface near entrance to the heme pocket, and the conformation of epicatechin gallate with lowest binding energy of -6.5 kcal/mol is presented in Fig. 5C. The most favorable conformation of theaflavin bound to the surface of myoglobin with binding energy of -7.7kcal/mol (Fig. 5D). The other tea compounds bound near His64 blocking potential aldehyde binding to that specific histidine. However, the lipid oxidation aldehydes being smaller than the tea compounds could still bind to myoglobin on the other surface histidines to form lipid oxidation-protein adducts.

3.4. Effect of green tea on myoglobin oxidation

The OxyMb decreased to a greater extent after day 3 (Fig. 2A). On the initial day of analysis, the 100 mg/mL GT-treated SP contained highest OxyMb content (63%) compared to that of the control (55%), 25 mg/mL-treated SP (60%) and 50 mg/mL-treated SP (52%). In contrast, the 100 mg/mL GT-treated SP contained lowest MetMb content (22%) compared to that of the control (28%), 25 mg/mL GT-treated SP (24%) and 50 mg/mL GT-treated SP (30%) on day 0 (Fig.2B). As expected, the MetMb increased over time as the Mb oxidized changing the Mb form of OxyMb (Fe^{2+}) to MetMb (Fe^{3+}) (Elias et al., 2008; Lynch et al., 2001). However, after day 5, GT had no effect on bovine Mb as there were similar percentages of OxyMb and MetMb in GT-treated SP and the control SP (Fig. 2).

3.5. Monitoring Effect of green tea on protein oxidation via tryptophan fluorescence

The fluorescence spectra of bovine SP and MP homogenates treated with GT over nine days of refrigerated storage is shown in Fig. 3. The GT-treated protein homogenates had lower tryptophan fluorescence intensity than the control SP and MP, and the intensity continued to decrease with higher GT concentrations. The tryptophan λ_{max} at 350 nm demonstrates that most

tryptophan residues are exposed in a hydrophilic environment (Burstein, Vedenkina, & Ivkova, 1973; Gieβauf, Steiner, & Esterbauer, 1995). The tryptophan loss which corresponded to the decrease in λ_{max} at 350 nm in the current study could be due to degradation of tryptophan residues consistent with the work of Estévez et al. (2008) and Viljanen, Kivikari, & Heinonen (2004). The tryptophan fluorescence intensity decreased over time. The data in the presence of GT could be explained by the increased LOX product binding to the SP and the MP homogenates, which while decreasing rancidity may cause damage to the proteins. The reactive oxygen species derived from LOX, such as hydroxyl radical (·OH), hydrogen peroxide (H_2O_2·) and lipid peroxyl radical (ROO·), can abstract a hydrogen atom from tryptophan and produce protein radicals (Jongberg, Torngren, Gunvig, Skibsted, & Lund, 2013). Our findings indicated that in the presence of GT, the interaction between SP and MP with LOX products may lower tryptophan fluorescence and thiol groups.

3.6. Effect of green tea on thiol oxidation in bovine proteins

After nine days, thiol content decreased 61% and 64-69% from that measured on day 0 for control SP and GT-treated SP samples, respectively (Fig. 4). This decrease may have occurred because of the oxidation of Mb in SP (Ríos-González, Román-Morales, Pietri, & López-Garriga, 2014; Romero, Ordonez, Arduini, & Cadenas, 1992) or most likely because the amino acids develop covalent bonds with green tea quinones formed by the oxidation of phenols, producing thiol-quinone adducts (Jongberg et al. 2011).

There was no significant difference in thiol content for control MP samples during the nine day storage period ($P<0.05$). Thiol loss was hastened by adding 50 and 100 mg/mL GT in MP samples, thus, no protective effect of green tea against thiol loss in the proteins was detected. Similar acceleration of thiol loss was observed when 500 ppm GT extract treated MP isolate

from Bologna type sausages was prepared from oxidatively stressed pork (Jongberg et al., 2013), and 4-methylcatechol treated raw minced beef during high-oxygen atmosphere storage (Jongberg, Lund, Waterhouse, & Skibsted, 2011).

4. Conclusions

The lipid oxidation aldehydes bind to the surface of myoglobin in many different positions, but with less favorable affinity compared to the tea compounds. The binding ability of the SP fraction was higher than that of the MP fraction for all studied lipid oxidation products. The measured lipid oxidation aldehydes in buffers were lower with addition of GT primarily through increased interaction with sarcoplasmic proteins. Our data also demonstrated the GT directly can bind these lipid oxidation products which also inhibits perceived rancidity. However the interaction of bovine proteins and GT decreased tryptophan fluorescence and thiol content, and hence, no protective effect of GT against protein even though lipid oxidation was inhibited. The dual effects of GT on lipid and protein may be concentration dependent. To ascertain if the benefits outweigh the negative effects, kinetic studies would be recommended to determine when tenderness and color are negatively affected to outweigh the benefits derived from the nutritional and sensory benefits observed with lower rancidity.

5. Acknowledgement

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Figure Captions

Fig. 1. Bound malonaldehyde in (A) sarcoplasmic /SP and (B) myofibrillar /MP and bound hexanal in (C) SP and (D) MP homogenates with added green tea infusions (GT). Uppercases represent GT treatments while lowercases represent storage time. The same uppercase letter (A-C) are not significantly different among treatments on the same day. Means with the same lowercases (a-e) are not significantly different within the same treatment with time ($p < 0.05$).

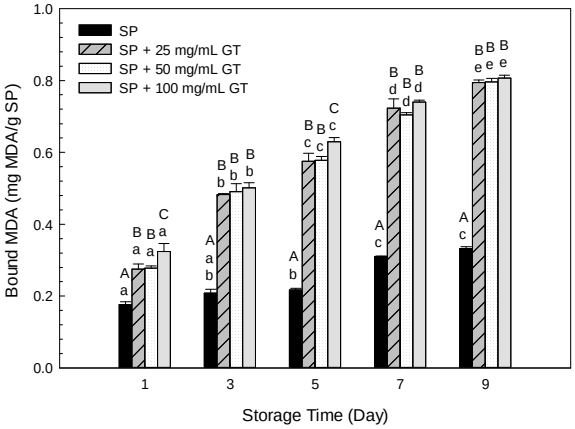
Fig. 2. Oxymyoglobin (A) and Metmyoglobin (B) of refrigerated sarcoplasmic /SP homogenates treated with green tea infusions (GT) over a nine day storage period, n=3.

Fig. 3. Emission fluorescence spectra of tryptophan of refrigerated (A) sarcoplasmic /SP and (B) myofibrillar /MP protein homogenates treated with green tea infusions (GT).

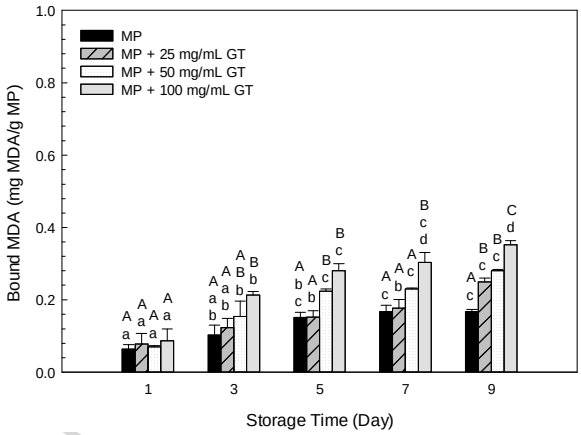
Fig. 4. Thiol Content (µmol/g protein) of refrigerated (A) sarcoplasmic /SP and (B) myofibrillar /MP protein homogenates treated with green tea infusions (GT) over a nine-day storage period. Values = means ± SD (n=3).Means with the same uppercases (A-G) are not significantly different among three treatments on the same day ($p < 0.05$). Means with the same lowercases (a-d) are not significantly different during storage time within the same treatment ($p < 0.05$).

Fig. 5. Lowest energy conformation of (A) hexanal (B) nonanal (C) epigallocatechin gallate and (D theaflavin bound to the surface of myoglobin with binding energy is -3.5, -4.1, -6.6 and 7.7kcal/mol respectively with amino acids (A)His64, Tyr67(B) His64, Tyr67(C) His64, Lys63, and Thr67 and (D)His64, Asn66, Thr70, Lys63 and Thr67.

(A)



(B)



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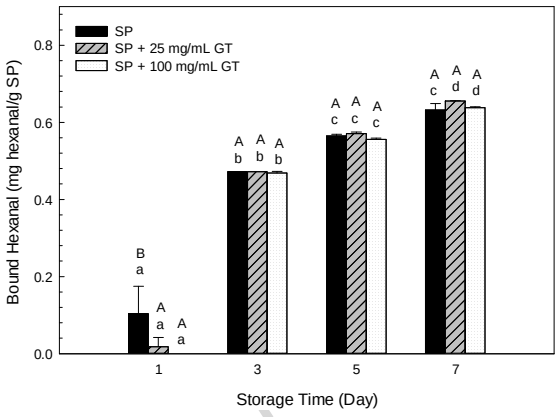

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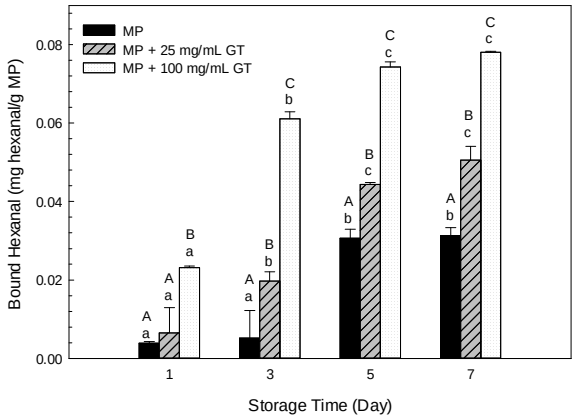
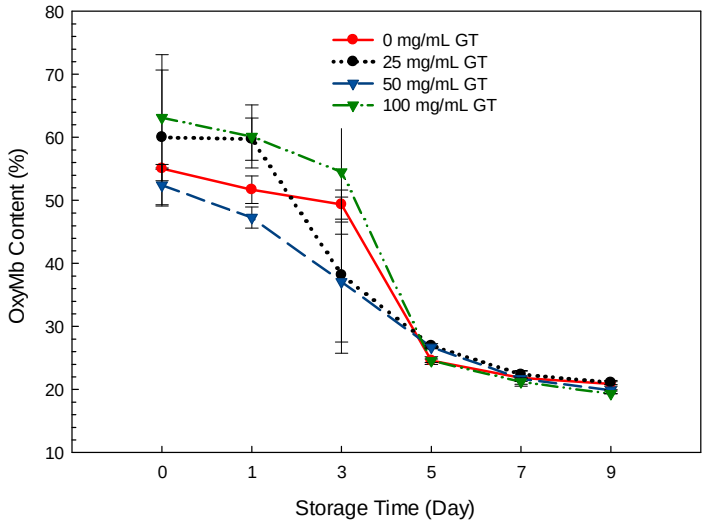


Fig. 1

A) Oxymyoglobin in sarcoplasmic homogenates



B) Metmyoglobin in sarcoplasmic homogenates

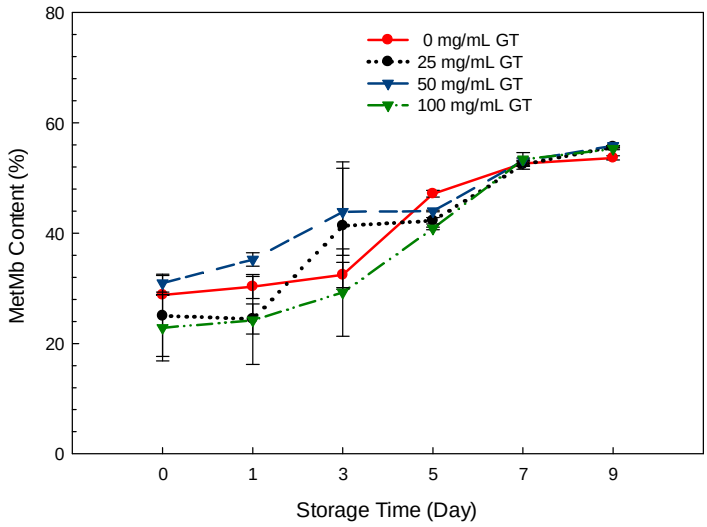
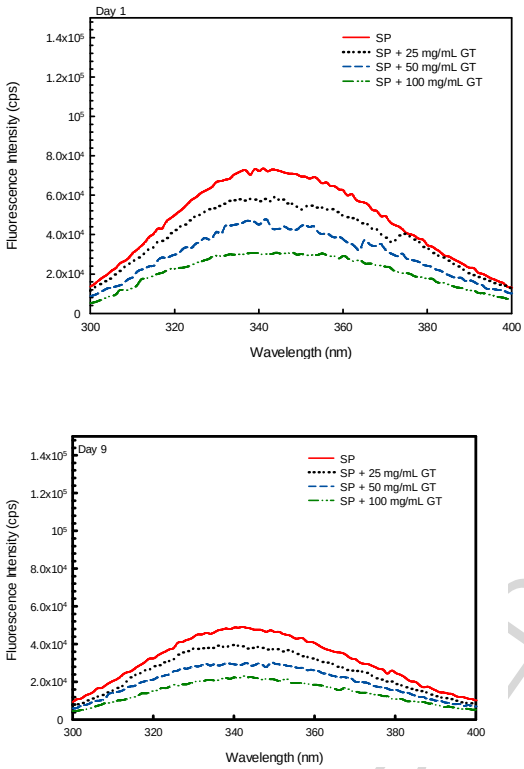


Fig. 2.

(A)



(B)

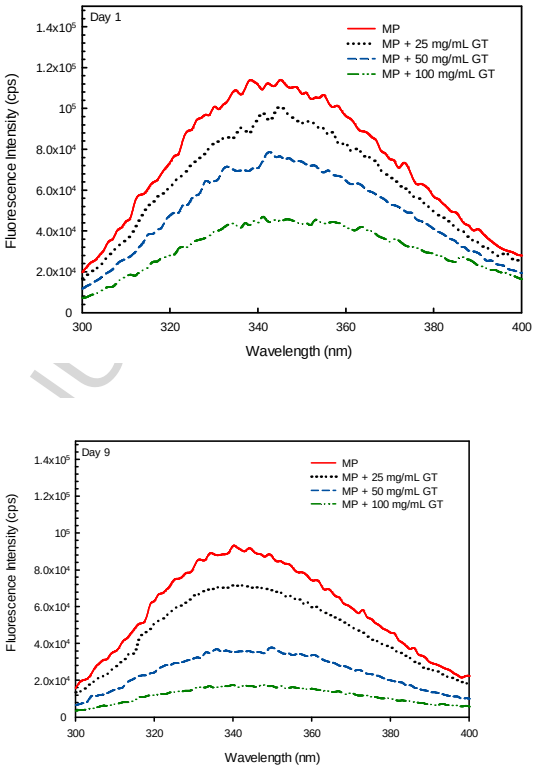
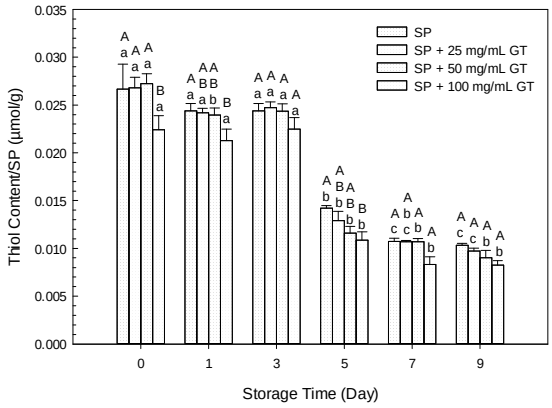


Fig. 3.

(A)



(B)

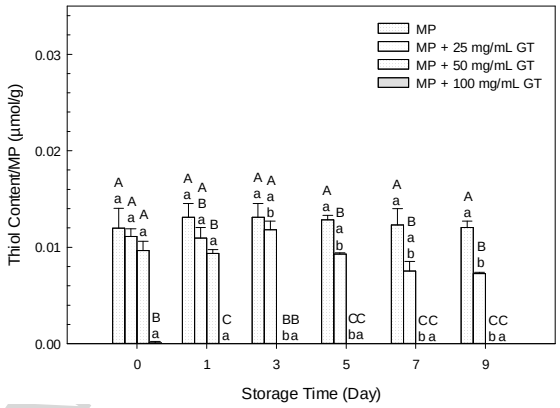
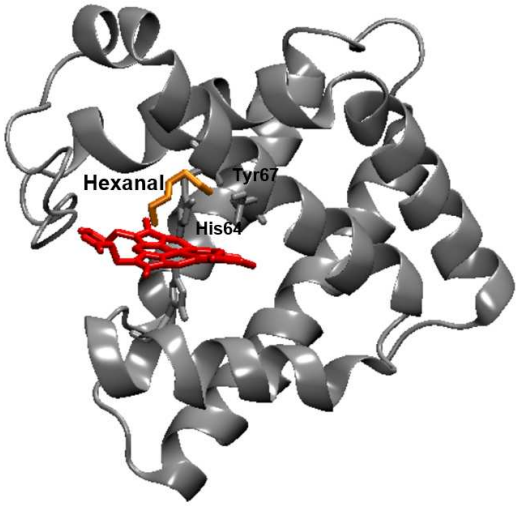
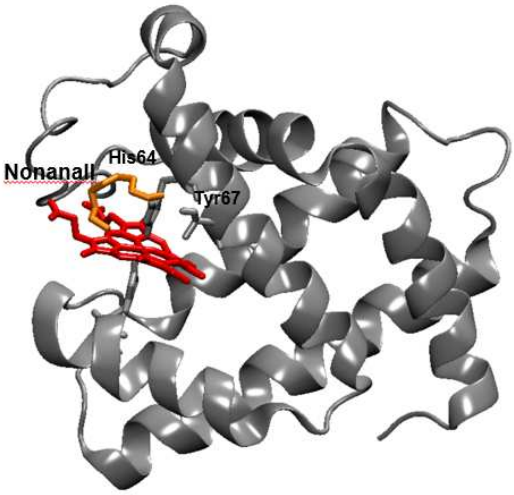


Fig. 4

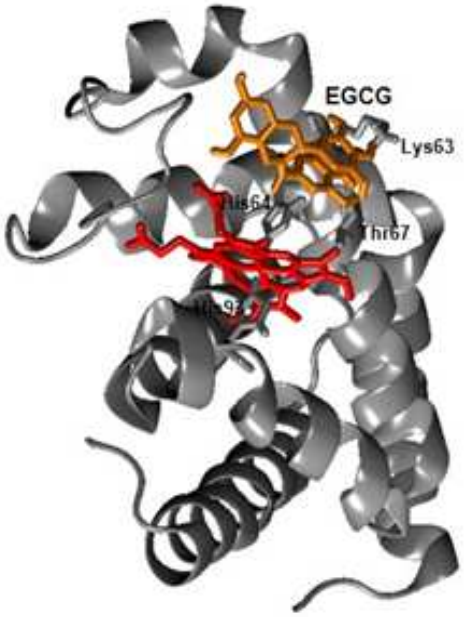
A) Hexanal | $C_6H_{12}O$ | *CID* 6184
Binding energy: -3.5 kcal/mole



B) Nonanal| $C_9H_{18}O$ | *CID* 31289
Binding energy: -4.1 kcal/mol



C) Epigallocatechin gallate | $C_{22}H_{18}O_{11}$ | *CID* 65064, Binding energy: --6.5 kcal/mol



D) Theaflavin| $C_{29}H_{24}O_{12}$ | *CID* 114777, Binding energy:-7.7kcal/mol

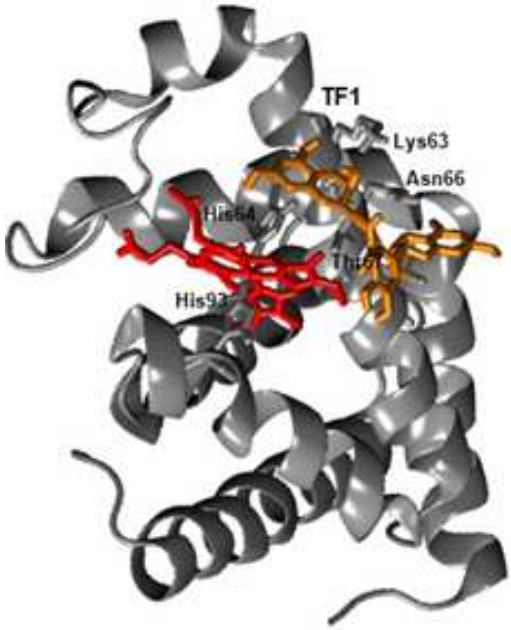


Fig. 5

Tables

Table 1 Free aldehydes (%) in refrigerated 30 mmol/L sodium phosphate buffer extracted sarcoplasmic proteins homogenates and 100 mmol/L sodium phosphate buffer extracted myofibrillar proteins homogenates treated with green tea infusions (GT) incubated with aldehydes on day 7.

MDA and Volatile Compounds	Presence of Proteins	Free Volatile Concentration in 30 mmol/L Sodium Phosphate Buffer (%)			Free Volatile Concentration in 100 mmol/L Sodium Phosphate Buffer (%)		
		Control (No GT)	25 mg GT /mL	100 mg GT /mL	Control (No GT)	25 mg GT /mL	100 mg GT /mL
Malonaldehyde	No Protein	100 ± 0.00	54.15 ± 0.76	30.46 ± 0.76	100 ± 0.00	76.08 ± 0.98	58.68 ± 1.14
	+Protein	73.98 ± 0.37	41.07 ± 0.55	41.31 ± 0.57	69.33 ± 1.10	57.47 ± 1.88	43.68 ± 1.88
Pentanal	No Protein	100.00 ± 0.00	98.71 ± 1.82	74.55 ± 0.07	100.00 ± 0.00	100.00 ± 0.00	71.70 ± 0.85
	+ Protein	35.15 ± 2.51	32.57 ± 0.04	34.51 ± 1.37	79.11 ± 1.34	69.07 ± 4.20	39.95 ± 0.19
Hexanal	No Protein	100.00 ± 0.00	92.18 ± 1.61	65.17 ± 0.35	100.00 ± 0.00	94.14 ± 0.46	62.52 ± 0.74
	+ Protein	29.34 ± 3.07	24.95 ± 0.06	27.75 ± 0.58	66.20 ± 2.20	45.43 ± 3.81	15.70 ± 0.21
Heptanal	No Protein	100.00 ± 0.00	84.02 ± 0.37	57.35 ± 0.34	100.00 ± 0.00	86.39 ± 0.07	52.55 ± 1.43
	+ Protein	17.35 ± 2.96	14.97 ± 0.03	16.70 ± 0.65	64.36 ± 3.21	32.54 ± 3.71	7.75 ± 0.02
Octanal	No Protein	100.00 ± 0.00	66.50 ± 0.01	43.47 ± 0.30	100.00 ± 0.00	64.93 ± 2.18	33.29 ± 1.66
	+ Protein	10.35 ± 2.16	7.98 ± 0.06	12.26 ± 0.33	55.76 ± 3.43	20.92 ± 2.74	4.46 ± 0.51
Nonanal	No Protein	100.00 ± 0.00	42.50 ± 0.52	26.97 ± 0.57	100.00 ± 0.00	32.41 ± 7.19	13.93 ± 1.01
	+ Protein	6.83 ± 1.59	4.03 ± 0.01	5.60 ± 0.37	55.57 ± 4.14	18.93 ± 1.70	4.80 ± 0.37

Values= means ± SD. Malonaldehyde n=3, and volatile aldehydes n=2.

Table 2. Docking results of aldehyde and tea compounds binding to myoglobin

Tea and aldehyde compounds	Binding energies (kcal/mol)
Key tea bioactive compounds	
Theaflavin| $C_{29}H_{24}O_{12}$ | *CID* 114777	-7.7
Theaflavin-3-gallate | $C_{36}H_{28}O_{16}$ | *CID* 22833650	-7.2
Theaflavin-3′-gallate	-7.8
Theaflavin-3,3′-digallate | $C_{43}H_{32}O_{20}$ | *CID* 21146795	-7.7
(-)-Epicatechin | $C_{15}H_{14}O_6$ | *CID* 72276	-5.9
Epigallocatechin | $C_{15}H_{14}O_7$ | CID *72277*	-5.9
Epicatechin gallate | $C_{22}H_{18}O_{10}$ | CID *107905*	-6.7
Epigallocatechin gallate | $C_{22}H_{18}O_{11}$ | CID *65064*	-6.6
Key aldehyde compounds from beef binding to Histidine 64	
Malondialdehyde | $C_3H_4O_2$ | CID *10964*	-3.1
Pentanal | $C_5H_{10}O$ | CID *8063*	-3.2
Hexanal | $C_6H_{12}O$ | CID *6184*	-3.6
Heptanal | $C_7H_{14}O$ | CID *8130*	-3.7
Octanal | $C_8H_{16}O$ | CID *454*	-4.1
Nonanal | $C_9H_{18}O$ | *CID* 31289	-4.0

Table 3 Binding energy (kcal/mol) of lipid oxidation aldehydes on amino acid residues of beef myoglobin

Binding site	Malondialdehyde | *C_3H_4O2* | CID *10964*	Hexanal | *$C_6H_{12}O$* | CID *6184*	Nonanal | $C_9H_{18}O$ | *CID* 31289
Histidine			
His 24	-2.6	-2.5	-2.4
His 36	-2.8	-3.2	-3.5
His 64	-3.1	-3.6	-4.0
His 81	-2.0	-2.5	-2.6
His 88	-3.0	-3.5	-4.1
His 93	-3.3	-3.0	-2.9
His 97	-2.1	-2.4	-2.6
His 119	-2.4	-2.1	-2.4
His 152	-2.5	-3.3	-3.3
Aromatic amino acids			
Trp 7	-2.2	-2.6	-2.6
Trp 14	-1.9	-2.3	-2.0
Tyr 103	-2.8	-3.7	-3.6
Phe 43	-2.6	-3.5	-3.2
Phe 46	-2.6	-2.9	-2.6
Phe 106	-2.7	-3.3	-3.7
Phe 123	-2.6	-2.8	-1.9
Phe 138	-2.4	-1.3	0.6
Phe 151	-2.6	-3.1	-3.3

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HIGHLIGHTS

- Aldehydes with longer carbon-chain length were bound stronger to the proteins.
- Binding aldehydes to sarcoplasmic proteins was stronger than with myofibrillar.
- Aldehydes bind to myoglobin surface with less favorable affinity than tea phenols