GREEN TEA EXTRACT ALTERS THE FUNCTIONAL PROPERTIES OF MEAT EMULSIONS BY GENERATION OF PROTEIN CROSS-LINKS

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The dose-dependent effect of green tea extract on the functional properties of meat emulsions was evaluated. Meat emulsions added 100, 500, or 1500 ppm green tea extract were prepared and stored for 1 day at 5 °C. Results showed that all concentrations of green tea inhibited lipid oxidation (TBARS) during production. However, cooking loss was enhanced and hardness was reduced in meat emulsions added 1500 ppm green tea extract. Gel electrophoresis indicated that the functional properties were altered due to increased protein cross-linking in meat emulsions with high concentration of green tea extract.

I. INTRODUCTION

The gelation properties of myofibrillar proteins are essential for the formation and stabilization of meat emulsions *(1, 2)*. It has been established that intermolecular disulfide bonds via oxidation of protein thiols play an essential role for the gel strength of heat-induced muscle protein gels *(3, 4)*. It was concluded that disulfide bridging between the surface active proteins and the proteins in the continuous gel matrix contributed to enhanced rheological and mechanical properties of the gel matrix *(5)*. Phenolic antioxidants are commonly added to meat products in order to reduce oxidation and oxidation-derived off-flavor formation during production and storage. Antioxidant protection may limit protein thiol oxidation and the resulting disulfide formation and additionally, studies have shown that phenolic compounds are able to react with thiol groups to form covalent thiol-quinone adducts (TQ-adducts) *(6)*. Quinones are generated when phenols are oxidized and has been shown to reduce total thiol concentration in beef stored under high-oxygen modified atmosphere *(7)* and in Bologna-type sausages *(8)*. It is suspected that TQ-adducts impair the gel-forming ability of the meat proteins, as it was recently found that addition of green tea extract, added as a natural antioxidant, altered the textural properties of Bologna-type sausages as detected by a sensory panel *(8)*. The objective of the present study was to determine the dose-dependent effects of green tea extract as a natural phenolic antioxidant on the oxidative stability and the texture of meat emulsions, and to elucidate the mechanisms by which the protein network is disrupted by the use of phenolic antioxidants.

II. MATERIALS AND METHODS

Meat emulsions were prepared in batches of 500 g containing 275 g lean *Semimembranosus* from pork, 12.5 g NaCl, 100 g crushed ice, 100 g canola oil, and 12.5 g water for Control meat emulsions. For meat emulsions containing green tea extract (GT20M, Dupont, Brabrand, Denmark), water was partly exchanged with 0.05, 0.25, or 0.75 g green tea extract to obtain 100, 500, or 1500 ppm green tea extract, respectively. The meat batter was prepared by mixing approximately 140 g meat, salt, 50 g crushed ice, and water or green tea extract dissolved in water for 15 sec in a food processor (Bosch CNCM20, Slovenia). Remaining ice was added, and the batter was mixed for additional 45 sec. Here after the remaining meat was added and the batter was mixed for another 30 sec. The oil was added slowly during 60 sec mixing, and the batter was finally mixed for 60 sec before it was transferred to 50 ml plastic tubes. The tubes were centrifuged at 3000 g for 10 min and heated in a water bath (80 °C) until a center temperature of 70 °C was

obtained (approximately 15 min). After heat treatment, the tubes for texture and oxidation analyses were cooled on crushed ice for approximately 30 min. Thereafter excess fluid was poured from the tubes, and they were stored in the dark at 5 °C for 1 day, where after they were vacuum packed and stored at -80 °C until analyses. Each recipe was prepared three times at different days resulting in batch A, B, and C (n=3).

Emulsion stability evaluated by cooking loss
The tubes for cooking loss determination were directly after heat treatment centrifuged at 2700 g for 3 min. The supernatant was poured into a plastic weigh boat and the weight was noted. The cooking loss was determined as (supernatant (g) / meat batter (g)) · 100 % = Cooking loss (%, w/w).

Textural properties evaluated by compression
The texture of the cold stored meat emulsions was determined using an Instron Material Testing Machine (Instron 4301, Instron, Bucks, UK). The samples were cut into 2-3 cylindrical cores and placed on a platform and each sample compressed to 30 % of its original height (strain). Maximum stress and maximum strain were recorded, and represents hardness and crumbliness, respectively.

TBARS analysis
The TBARS values were determined by TBARS analysis according to Vyncke *(9)* and Sørensen & Jørgensen *(10)*.

Protein cross-linking
The myofibrillar proteins from the meat emulsions were separated by SDS-PAGE as described by Nieto, et al. *(11)*.

Statistical data analysis
Statistical analysis were performed using R© version 2.12.1, The R Foundation for Statistical Computing (ISBN 3-900051-07-0). Data were analyzed by analysis of variance (ANOVA) using a linear model with mixed effects, with Replicates (A, B, C) as random effects, and with Recipe (Control, 100 ppm GT, 500 ppm GT, 1500 ppm GT) as systematic effect. Letters (a-d) denotes significant difference ($P < 0.05$) between treatments.

III. RESULTS AND DISCUSSION

The water holding capacity of meat emulsions added green tea extract was evaluated by cooking loss immediately after production (Figure 1, left panel). Cooking loss was found to be significantly higher in meat emulsions with added 1500 ppm green tea extract, while neither addition of 100 nor 500 ppm green tea extract altered the water holding capacity as compared to the Control meat emulsion.

The oxidative stability of the lipid fraction of the meat emulsions was evaluated by the formation of secondary lipid oxidations products as determined by thiobarbituric acid reactive substances (TBARS) (Figure 1, right panel). Addition of 100, 500, and 1500 ppm green tea extract were able to significantly inhibit the formation of TBARS during production, as compared to the Control meat emulsion.

The textural properties of the meat emulsions were evaluated by two parameters: Stress (N) and Strain (%) (Figure 2). Stress represents the force needed to break the meat emulsion, and is a measure of firmness/hardness. Strain represents the distance in percentage the probe needs to travel though the meat emulsion before it breaks, and is a measure of crispiness/crumbliness.

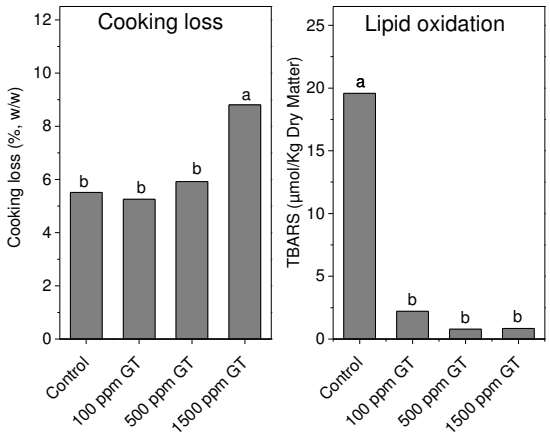

Figure 1. **Left panel:** Cooking loss (%, w/w) in meat emulsions added 0 (Control), 100, 500, or 1500 ppm green tea extract (GT) at day 0 (n=3). **Right panel:** Secondary lipid oxidation products as determined by TBARS (umol/kg DM) in the same meat emulsions stored at 5 °C for 1 day (n=3).

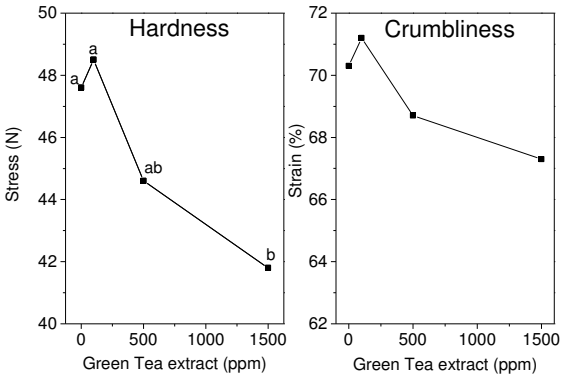

Figure 2. Hardness (Stress (N)) and Crumbliness (Strain (%)) of meat emulsions added 0 (Control), 100, 500, or 1500 ppm green tea extract (GT) stored at 5 °C for 1 day (n=3).

Addition of 100 ppm green tea extract seemed to increase stress and strain in the meat emulsion, while higher concentrations tended to reduce the two textural parameters. Addition of 1500 ppm green tea extract significantly reduced stress as compared to the Control meat emulsion indicating that the emulsions hardness was altered.

The myofibrillar proteins from the meat emulsions were separated by gel electrophoresis in both their non-reduced and reduced state for evaluation of protein disulfide cross-linking (Figure 3). The pixel intensity was quantified to estimate the concentration of monomer MHC and the degree of reducible MHC cross-links.

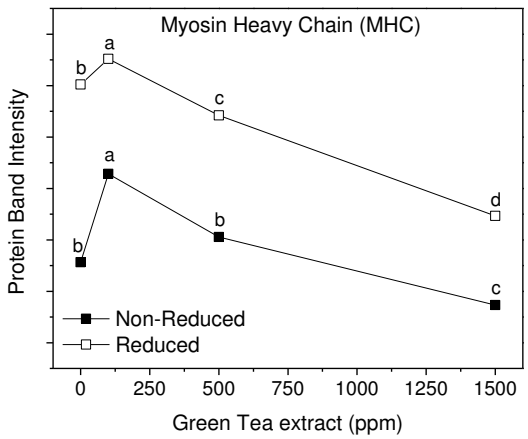

Figure 3. Protein band intensity of myosin heavy chain (MHC) in non-reduced or samples reduced by dithiotreitol (DTT) from meat emulsions added 0 (Control), 100, 500, or 1500 ppm green tea extract (GT) stored at 5 °C for 1 day (n=3).

Gel electrophoresis showed that the intensity of monomer MHC was more intense in samples after treatment with DTT as compared to the non-reduced samples. This indicates that protein disulfides were reduced to yield a higher concentration of monomer MHC, and indirectly that protein disulfide cross-links were generated during the production of the meat emulsions.

The concentration of monomer MHC was evaluated by comparing the intensities of the non-reduced samples. A high MHC band intensity indicates a low degree of cross-linking. The results showed significantly higher MHC intensity in the meat emulsions added 100 ppm green tea extract and significantly lower MHC intensity in meat emulsions added 1500 ppm green tea extract as compared to the Control meat emulsion (Figure 3). This indicates that meat emulsions added 100 ppm green tea extract was less subjected to MHC cross-linking as compared to the Control, and that 1500 ppm green tea extract resulted in increased MHC cross-linking.

The degree of reducible MHC cross-links was evaluated by the pixel intensity of MHC after treatment by DTT (Figure 3). The MHC levels were close to similar (P = 0.0458) for the Control meat emulsion and the meat emulsion added 100 ppm green tea extract. In contrast, addition of 500 or 1500 ppm green tea extract showed significantly lower MHC levels after reduction as compared to the Control (P = 0.022 and 0.0000 for 500 ppm and 1500 ppm, respectively). This indicate that especially for the high concentrations of green tea extract, part of the MHC in the meat emulsions was lost due to non-reducible protein polymerization.

In summary, the results indicated that while lipid oxidation were equally affected by the concentrations of green tea extract used in the present study, the formation of MHC cross-linking were affected in a dose-dependent manner by the green tea extract.

With respect to the functional properties, 1500 ppm green tea extract was found to elevate the cooking loss and lower the hardness of the meat emulsions, while 100 ppm green tea extract seemed to slightly increase hardness and crumbliness. Accordingly, 100 ppm green tea

extract was found to protect against protein cross-linking, while the higher concentrations seemed to promote both reducible and non-reducible protein cross-linking. Increased cross-linking of proteins has previously been found to reduce the gelling properties and water holding capacity of meat emulsions *(1)*. Meanwhile, phenolic compounds have recently been found to form protein-phenol adducts in meat systems leading to increased protein cross-linking *(8, 12)*. Thus, hazardous effects may be generated on the functional properties of meat products if the concentration of such phenolic compounds is not controlled.

IV. CONCLUSION

Green tea extract added to meat emulsions protected against lipid oxidation, but when added in higher concentrations the cooking loss and textural properties were altered due to the increased generation of protein cross-links. However, addition of low concentration Green Tea extract may increase the functional properties, while still protecting against lipid oxidation.

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