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*Publication date:*  
2012

*Document version*  
Peer-review version

*Citation for published version (APA):*  
Jongberg, S., Tørngren, M. A., Nersting, L., Skibsted, L. H., & Lametsch, M. L. (2012). *Antioxidative mechanisms against protein oxidation in Bologna type sausages added Green Tea or Rosemary*. Poster session præsenteret ved IUFoST, Parana, Brasilien.

# Antioxidative mechanisms against protein oxidation in Bologna type sausages added Green Tea or Rosemary

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## Objective

To investigate the antioxidative effect on protein oxidation of the phenolic-rich plant extracts, Green Tea (GT) or Rosemary (RM), in Bologna type sausages prepared from oxidatively stressed pork.

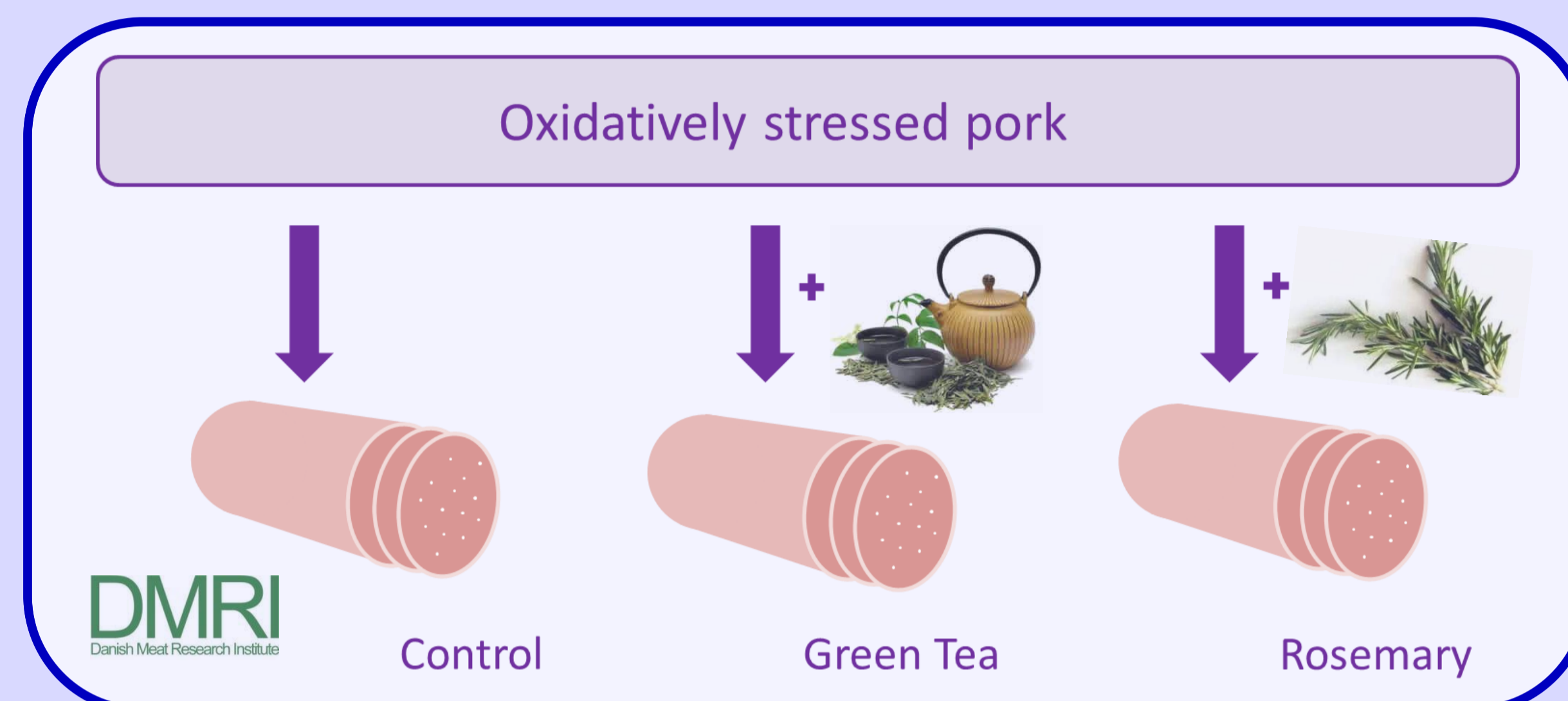
## Conclusion

- Protein carbonyl formation was inhibited by addition of the plant extracts.
- Thiol loss and protein polymerization was increased by addition of GT.
- Thiol loss and polymerization were ascribed thiol-quinone adduct formation between catechins and protein thiol groups.

## Introduction

Raw materials for meat products vary in quality and may be oxidatively stressed before processing. Addition of phenolic rich extracts to meat products protects against the formation of lipid oxidation products by scavenging radicals and chelating metals. Studies show that phenols may exert a different antioxidative activity against the formation of protein oxidation products.

The vast variety of protein oxidation mechanisms makes it difficult to predict the effect of phenolic-rich extracts against protein oxidation in meat and meat products. Previous studies have shown that some protein oxidation products may be protected by phenols while others may not.



## Results and Discussion

Bologna type sausages were prepared with addition of GT or RM in order to protect against protein oxidation during processing.

Protein carbonyl formation was significantly inhibited by addition of the extracts, with GT exerting the most efficient protection (Fig. 1, upper panel). On the contrary, GT increased thiol loss, indicating a prooxidative activity of the extract on thiol oxidation (Fig. 1, lower panel). Oxidation of thiols leads to protein disulfide formation, and accordingly, protein polymerization increased in the Bologna type sausages added

GT as detected by SDS-page (Fig. 2).

Determination of protein radicals showed increased radical signal intensities for Bologna type sausages added the extracts (Fig. 3). The peak to peak width ( $\Delta B_{PP}$ ) of the spectra indicated that the radicals formed in the three types of sausages were of different origin.

Previous studies have shown that oxidized phenols, the quinones, easily react to form adducts with protein thiols. Catechin, which is the dominant phenol in GT are even able to form di-, or tri-thiol adducts in effect generating protein cross-linking. Carnosic acid and carnosol, the

dominating phenols in RM contain only one possible site for thiol addition, and may therefore not contribute to protein cross-link formation.

Hence, as GT showed an antioxidative activity against protein carbonyl formation, the distinct loss of thiols observed by addition of the GT are ascribed thiol-quinone adduct (Scheme 1) formation, and not a typical prooxidative activity. Formation of di-, or tri-thiol adducts with catechins explains the phenol-mediated protein polymerization (Scheme 2), and the altered radical spectra by formation of stabilized protein-bound phenoxyl radicals (Scheme 3).

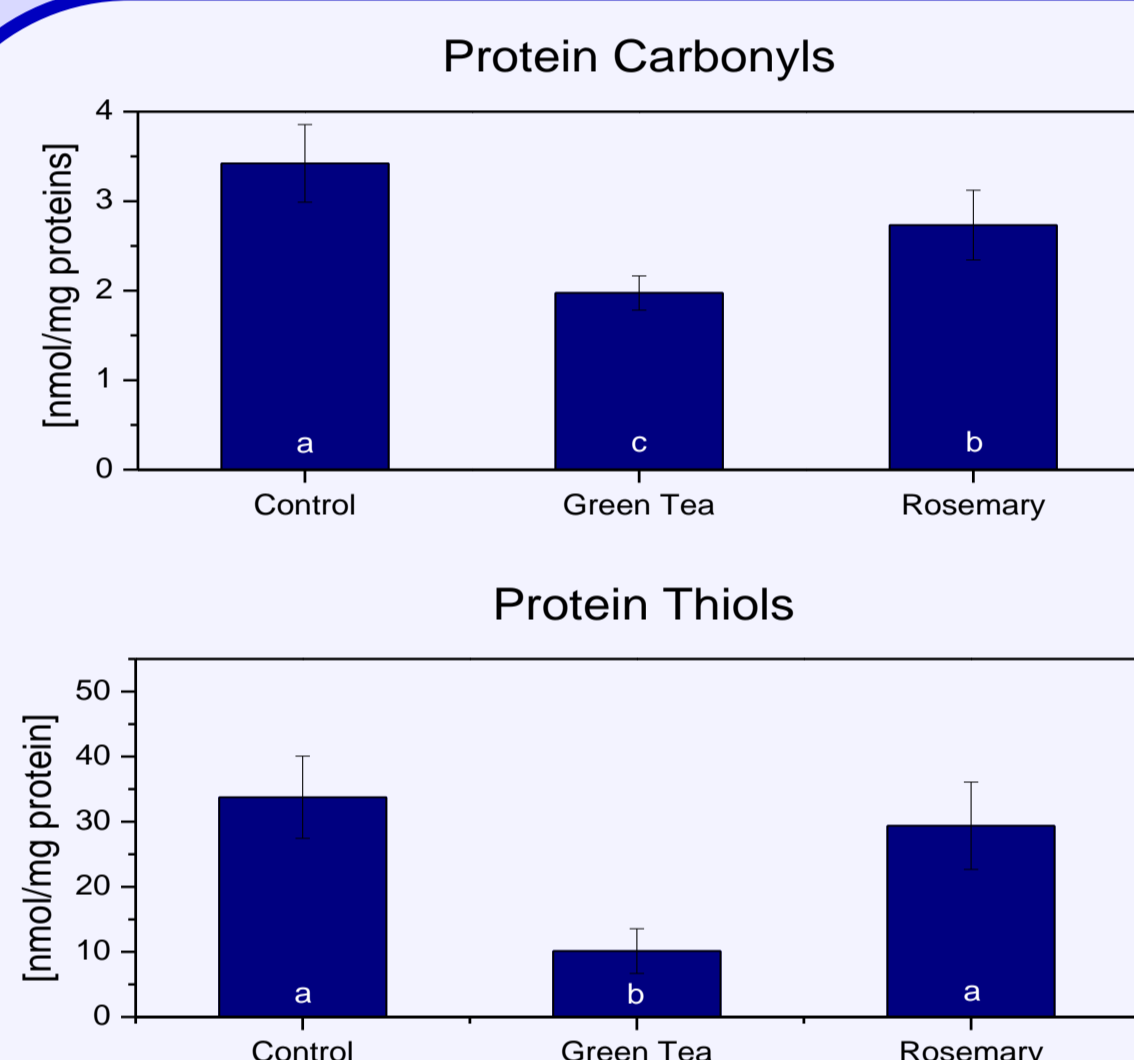


Figure 1. Protein carbonyls (upper panel) and protein thiols (lower panel) in Bologna type sausages added Green Tea or Rosemary extract.

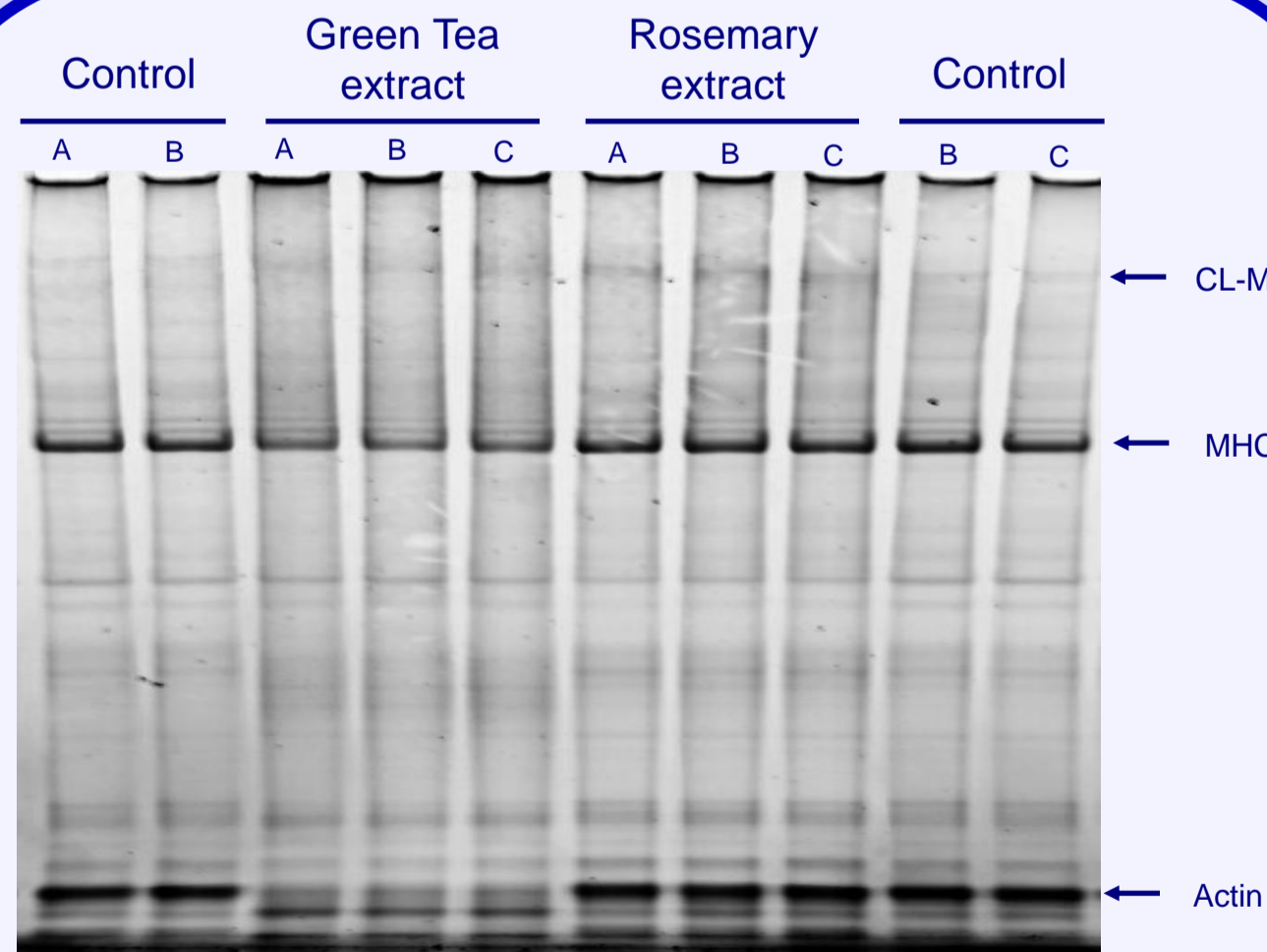


Figure 2. Representative SDS-page gel of myofibrillar proteins extracted from Bologna type sausages added Green Tea or Rosemary extract. MHC: myosin heavy chain; CL-MHC: cross-linked MHC.

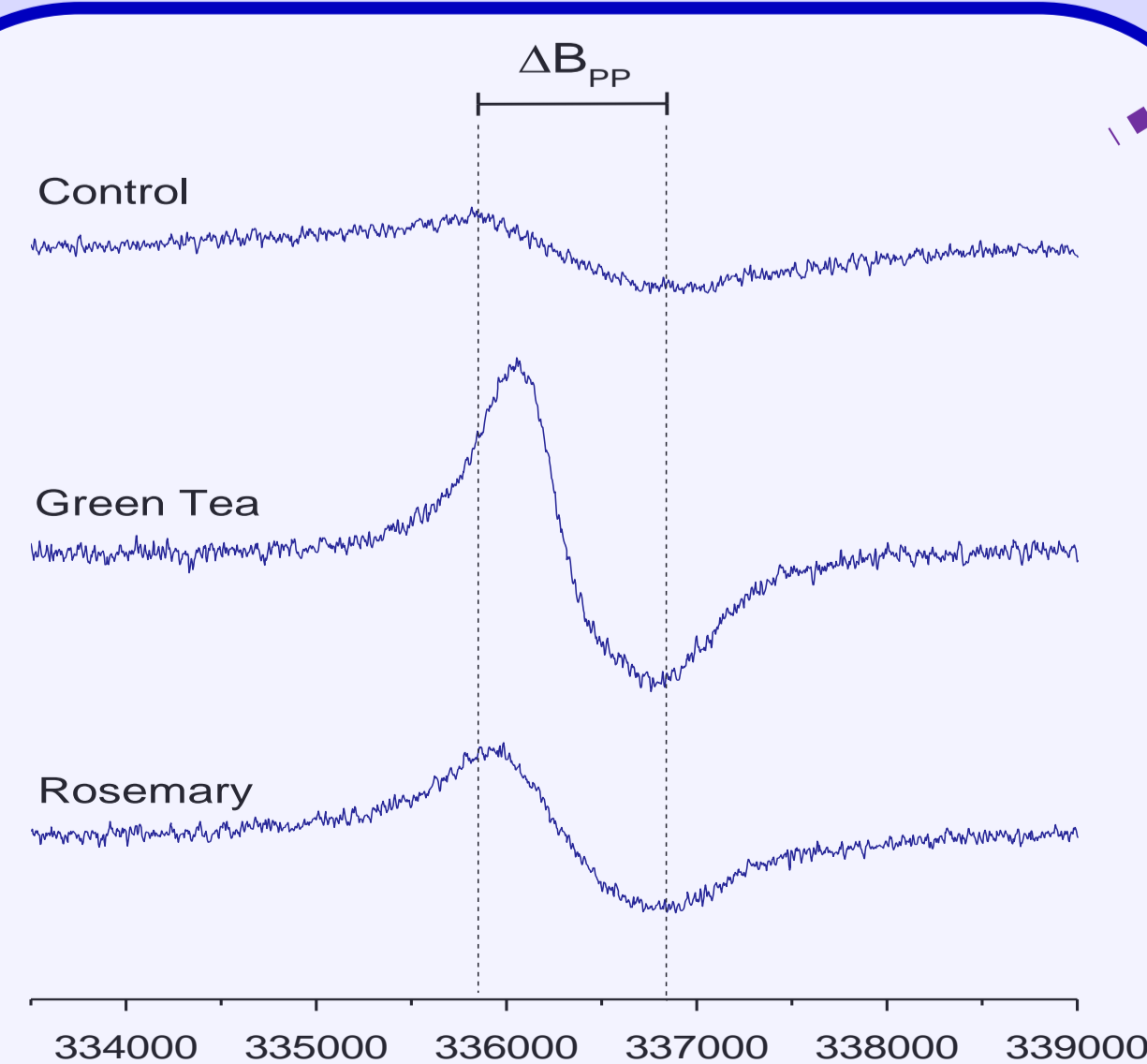
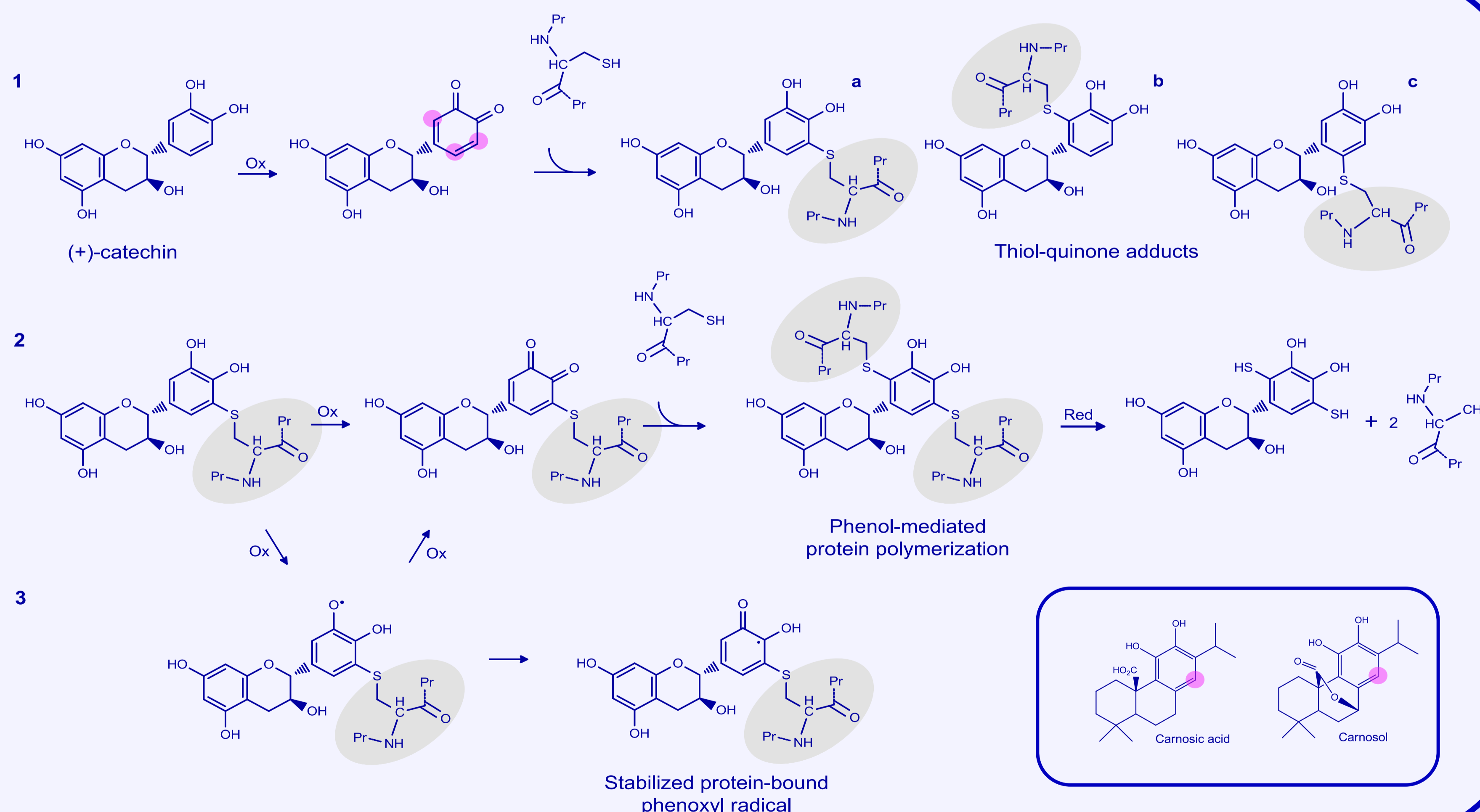


Figure 3. ESR spectra of myofibrillar protein extracted from Bologna type sausages added Green Tea or Rosemary extract.

## Experimental

Bologna type sausages were prepared from oxidatively stressed pork (UV, 5 °C, 48 h). One batch were prepared by traditional recipe, a second batch was added Green Tea extract (500 ppm total phenolics), and a third batch was added Rosemary extract (400 ppm total phenolics). The sausages were cooked until a center temperature of 75 °C, and rapidly chilled.

The myofibrillar proteins were isolated (MPI) by excessive wash, freeze-dried, and kept frozen until analyses.

**Protein carbonyls** were quantified spectrophotometrically after derivatisation with dinitrophenylhydrazine (DNPH).

**Protein thiols** were quantified spectrophotometrically after derivatisation with 5',5'-dithiobis(2-nitrobenzoic acid) (DTNB).

**Protein cross-linking** was examined by SDS-page of reduced (data not shown) or non-reduced samples.

**Protein radical** formation was investigated by mounting the MPI in the cavity of an electron spin resonance (ESR) spectrometer.

## Acknowledgements

The authors thank DuPont Nutrition and Biosciences ApS for providing the plant extracts, and the Danish Agency for Science, Technology and Innovation for granting the project, ref. 11-117033.