PS2.06 Antioxidant and pro-oxidant effects of phenolic compounds on myofibrillar proteins 298.00

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Abstract— To assess the effect of added phenolic compounds (1mM), namely, gallic acid, chlorogenic acid, rutin, genistein, catechin, rutin and cyanidin-3-glucoside, and α-tocopherol on the formation of protein oxidation markers, , α-aminoadipic and γ-glutamic semialdehydes (AAS and GGS, respectively) in myofibrillar proteins (MP), was the aim of the present study. MP were extracted from porcine longissimus dorsi, suspended in 15 mM PIPES buffer and oxidized with 10 µM FeCl3 and 1 mM H2O2 while kept in a oven at 37ºC for 20 days. Both semialdehydes were analyzed by liquid chromatography-mass spectrometry (LC-MS) while the total amount of protein carbonyls was also assessed by using the dinitrophenylhydrazine (DNPH) method. As a result of the oxidation of myofibrillar proteins, the relative amounts of both semialdehydes as well as the total amount of protein carbonyls increased with time until day 15, when the highest oxidation level was found. At that point, the tocopherol and certain phenolic compounds - rutin, catechin and cyanidin-3-glucoside - inhibited the formation of protein carbonyls at different extents (11-58%). In contrast, gallic acid, chlorogenic acid and genistein promoted the formation of AAS (27-54%), GGS (19-66%) and total protein carbonyls (13-41%). The overall effect of plant phenolics on protein oxidation is influenced by numerous factors including the chemical structure of the phenolics and the result of the interactions between the myofibrillar proteins, the phenolics and other oxidant components like transition metals. Plausible mechanisms for the antioxidant and pro-oxidant effects of plant phenolics on protein oxidation are proposed in the present paper. This study highlights the complexity of redox reactions between plant phenolics and oxidizing myofibrillar proteins.

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Index Terms— antioxidant, LC-MS, plant phenolics, prooxidant, protein oxidation, semialdehydes.

I. INTRODUCTION

PROTEIN oxidation is, currently, one of the most relevant and innovative issues of study within the Food Chemistry field. However, the role of food proteins as targets for reactive-oxygen species (ROS) has been ignored for decades. Lately, numerous studies have focused on the occurrence and consequences of protein oxidation in meat and meat products [1-3]. Protein oxidation is known to affect the color, texture and nutritional value of muscle foods [1-2]. Nevertheless, further studies on this issue are required as the precise mechanisms involved in the oxidative degradation of muscle proteins and the fate of particular amino acids during handling; processing and storage of muscle foods are poorly understood. Recently, an advanced methodology for the accurate detection of specific protein oxidation products (AAS and GGS) has been developed by using LC-MS [4]. AAS is the main oxidation product from lysine while GGS derives from the oxidative degradation of arginine and proline. Both semialdehydes have been found in considerably large quantities in different meat products [3] and have been highlighted as specific protein oxidation markers [4]. Plant phenolics are bioactive compounds of increasing interest amongst food technologists and consumers. Flavonoids and phenolic acids are known to display antioxidant, anti-inflammatory and antimicrobial properties [Reviewed by Bravo, 5]. Diets enriched in flavonoids-containing foods are associated with reduced incidence of various cancer types, with the flavonoids being considered to play a relevant health-protecting role [5]. The belief that these natural substances provide an enhanced healthy value to functional food products has led to a considerably increase of health claims in foods containing phenolic-rich plant materials. However, these compounds can also display, under particular circumstances, pro-oxidant effects in food systems. As a result of that pro-oxidant action, phenolic compounds and other bioactive compounds (i.e. vitamins) have been found to exhibit harmful effects leading to an increased risk to

suffer coronary heart diseases, stroke and cancer [5]. The protective role of phenolic compounds against myofibrillar protein oxidation has been scarcely studied. In a previous paper, we reported the effect of selected phenolic compounds on protein oxidation assessed by means of fluorescence spectroscopy [6]. Whereas both antioxidant and pro-oxidant effects were reported in that paper, the specific chemical mechanisms of interaction between phenolic compounds, myofibrillar proteins and other components of the oxidative reactions (i.e. metals) remain unknown. In addition, there is no information about the effect of phenolic compounds of the formation of protein semialdehydes, AAS and GGS, from oxidizing amino acids. The aforementioned issues are addressed in the present paper.

II. MATERIALS AND METHODS

Materials All chemicals were supplied by J.T Baker (Deventer, Holland), Riedel de-häen, Sigma Aldrich (Steinheim, Germany) and Extrasynthese (Germany, France). Porcine longissimus dorsi muscle was purchased in a local supermarket in Helsinki. Methods Extraction and in vitro oxidation of muscle proteins MP were extracted from porcine longissimus dorsi muscle according to the procedure described elsewhere [6]. MP (20 mg/mL) were suspended in 15mM PIPES buffer (pH=6) containing 0.6N sodium chloride. Eight different suspensions were prepared depending on the addition of selected phenolic compounds (1mM) - gallic acid, chlorogenic acid, genistein, catechin, cyanidin-3-glucoside and rutin - including suspensions with α-tocopherol and a control group (with no phenolic compound). All protein suspensions were oxidized (10 μM FeCl3, 1 mM H2O2) while kept in a oven at 37ºC for 20 days. The chemical structures of the phenolic compounds are shown in Figure 1. Total carbonyls - DNPH method Total protein carbonyls were quantified in protein suspensions at sampling times (days 1, 4, 10, 15 and 20) according to the method described elsewhere [4]. Protein concentration was calculated from absorption at 280 nm using BSA as standard. The amount of carbonyls was expressed as nmol of carbonyl per mg of protein using an absorption coefficient of 21.0 nM-1 cm-1 at 370 nm for protein hydrazones. AAS and GGS – LC-MS analysis Samples were derivatized with p-aminobenzoic acid (ABA) and subsequently hydrolyzed with 6N HCl according to the procedure described elsewhere [4]. Hydrosylates were dried in vacuo, reconstituted with 200 L milli-Q water and filtered through PVDF syringe filter before HPLC analysis. Samples (30 L) were injected in a HPLC with a C-18 reversed phase column eluted with 2.5% acetic acid water and methanol. AAS-ABA and GGS-ABA were detected and identified by LC-ESI-MS according to their m/z values and fragmentation pattern in comparison with standard compounds [4]. The peaks corresponding to the protonated AAS-ABA and GGS-ABA were manually integrated from selected ion chromatograms and the resulting areas used as arbitrary indicators of the abundance of both semialdehydes. Results are expressed as arbitrary area units (AU). The overall effect of added phenolics on each protein oxidation measurement at day 15 was calculated as follows: [(A15 – C15)/A15] x 100, where C15 is the carbonyl content in the control sample at day 15 and A15 is the carbonyl content in the treated sample at day 15. Positive values (C15>A15) should be interpreted as percent inhibition against protein oxidation while negative values (C15<A15) denote pro-oxidant activity and should be interpreted as percent promotion in protein oxidation. Experimental design and statistical analysis All suspensions were made twice and the analysis performed in triplicate (n=6). Analysis of Variance from SPSS for Windows ver. 6.1 was used in order to study the effect of the added phenolic compounds. Pearson correlations were also calculated in order to establish relationships between parameters. Statistical significance was set at $p<0.05$.

III. RESULTS AND DISCUSSION

The MS signal for both semialdehydes as well as the total amount of protein carbonyls in protein suspensions increased throughout the oxidation assay (Figure 2). The present results confirm that lysine, proline and/or arginine from myofibrillar proteins are oxidized in the presence of Fe and H2O2 to yield GGS and AAS (Figure 3 shows lysine degradation as an example). In the present study, the reaction might be initiated by ROS derived from the Fenton reaction between Fe3+ and H2O2. The oxidative deamination from the intermediate radical molecule occurs in the presence of Fe3+ yielding the semialdehyde (Figure 3). The resulting Fe2+ could propagate the oxidative degradation to new amino acid residues through the reaction with H2O2 for the formation of additional hydroxyl radicals. The evolution of the total amount of protein carbonyls in protein suspensions is consistent with the trends observed for GGS and AAS. Both

semialdehydes are known to be the main protein carbonyls in biological samples and comprise around 90% of total carbonyl compounds in BSA subjected to metal catalyzed in vitro oxidation [7]. The results obtained in this study, including the significant correlations found between DNPH measurements and GGS (0.79; $p<0.01$) and AAS (0.73; $p<0.01$), contribute to support that both semialdehydes could be highly representative of the total amount of carbonyl compounds formed during metal catalyzed oxidation of myofibrillar proteins. The overall effects of added phenolic compounds and α-tocopherol on myofibrillar proteins oxidation at day 15 are shown in Figure 4. At that point, α-tocopherol and certain phenolic compounds - rutin, catechin and cyanidin-3-glucoside - inhibited the formation of AAS, GGS and the total protein carbonyls at different extents (11-58%). The antioxidant activity of plant phenolics is mainly derived from their ability to act as radical scavengers and metal chelators [5]. It is generally accepted that the effectiveness of plant phenolics as antioxidants is related to the stabilization of the phenoxyl radical by delocalization of an electron and the absence of suitable sites for O2 attack, and it is enhanced by the presence of hydroxyl groups [5]. In the present study the aforementioned phenolics could have inhibited the oxidative degradation of myofibrillar proteins by i) partially inhibiting the Fenton reaction and the subsequent formation of ROS through the chelation of iron and ii) by scavenging the ROS eventually formed from Fe3+ and hydrogen peroxide. In agreement with a previous study, the α-tocopherol displayed the more intense protective action against protein oxidation. In contrast, gallic acid, chlorogenic acid and genistein promoted the formation of AAS (27-54%), GGS (19-66%) and total protein carbonyls (13-41%). These results are in agreement with previous studies carried out by Akagawa & Suyama [8] and ourselves [6] who reported pro-oxidant actions for some of the aforementioned compounds. Whereas the pro-oxidant effect of phenolics has been reported, the exact mechanisms are still to be fully clarified. Recently, Akagawa et al. [9] reported that phenolics undergo an auto-oxidation process in the presence of transition metals leading to the formation of the corresponding quinones which are able to promote lipid and protein oxidation. Furthermore, the same authors highlighted that those quinone structures are co-factors in several lysyl-oxidase enzymes and themselves might be able to display amine-oxidase activities. The conversion of chlorogenic and gallic acid and genistein into their corresponding quinone forms would trigger their ability to promote the oxidative degradation of the lysine, proline and arginine side-chains and hence, the formation of AAS and GGS. The influence of transition metals on plant phenolics' pro-oxidant effect, described as metal-initiated pro-oxidant activity, depend upon a number of factors including the chemical structure of the plant phenolic, their dose, and the presence of other oxidation promoters. In this sense certain phenolics might be particularly prone to be oxidized and act as effective promoters of the oxidative degradation of proteins. Consistently with the present results, Rietjens et al. [10] reported that phenolics with a phenol-type structure in the B-ring (i.e. genistein) are more prone to undergo auto-oxidation than 3'-4' catechol-type structures (found in rutin, catechin and cyanidin-3-glucoside) and the resulting quinone structures show particularly intense pro-oxidant potential. In summary, plant phenolics can display, as redox-active compounds, both antioxidant and pro-oxidant actions in systems containing myofibrillar proteins. The overall effect of plant phenolics on protein oxidation is the result of their ability for radical scavenging and their pro-oxidant activity which depends on multiple and complex factors.

IV. CONCLUSION

The present results highlight the complexity of the mechanisms involved in the interactions between myofibrillar proteins and plant phenolics. The overall effects of plant phenolics include beneficial results derived from antioxidant actions and potential harmful effects towards meat quality and consumer's health derived from their pro-oxidant activities. Taking into account that the overall effect of a single phenolic compound or a mixture of those in a complex system such as a meat product is mostly unpredictable, solid scientific support should be required before any health claim is attached to a functional muscle food.

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Figure 1. Chemical structure of phenolics compounds tested in the present paper.

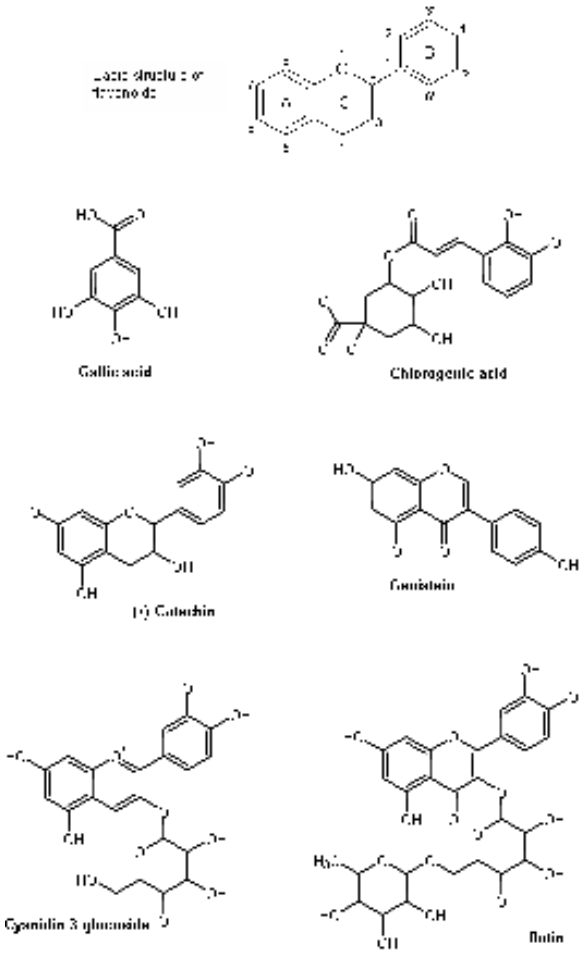


Figure 2. Chemical structure of phenolics compounds tested in the present paper.

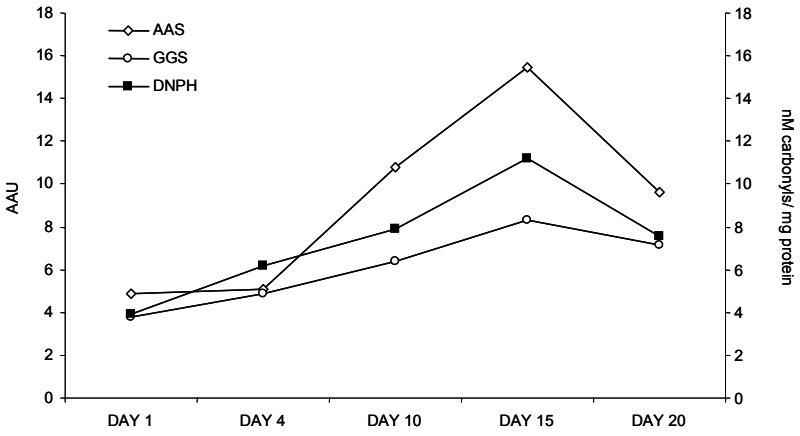


Figure 3. Formation of α-amino adipic semialdehyde (AAS) from lysine.

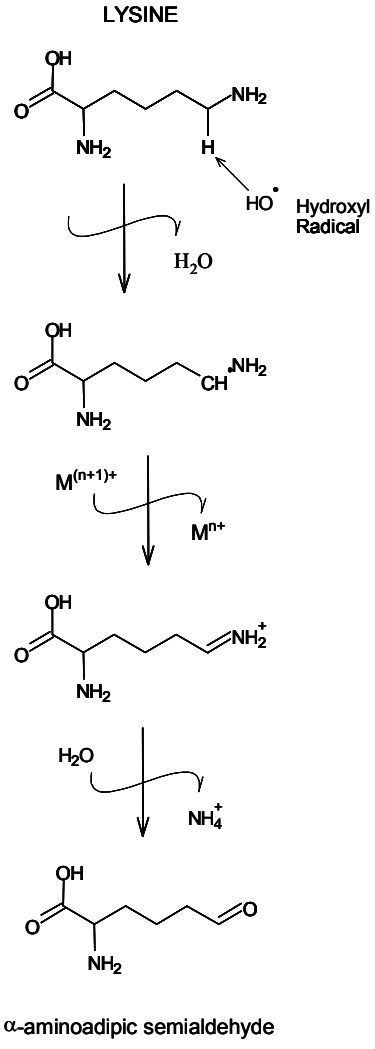
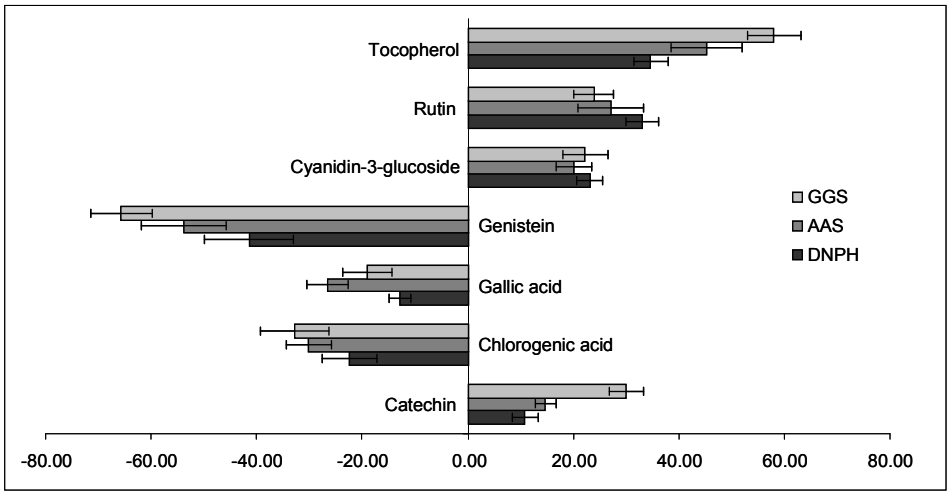


Figure 4. Overall effects[1] of added phenolics compounds and α-tocopherol on protein oxidation at day 15.



[1]Positive values denote antioxidant activity (percent inhibition against protein carbonyl formation) while negative values denote pro-oxidant activity (percent promotion of protein carbonyl formation).