APPLE PHENOLICS PROTECT AGAINST CARBONYLATION OF MYOFIBRILLAR PROTEINS

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Abstract – The effect of bioactive compounds in apple peel on the oxidation of myofibrillar proteins was investigated. Myofibrillar protein suspensions (extracted from pork loin) were prepared to which chlorogenic acid, (-)-epicatechin, phloridzin and apple peel extract were added at three concentrations (50, 100 and 200 µM). A negative control without phenolics was also included. All suspensions were oxidized during 10 days. Upon sampling time (0, 2, 5, 7 and 10 days), protein carbonylation products (α-amino adipic semialdehyde and γ-glutamic semialdehyde, AAS and GGS respectively) were determined using HPLC with fluorescence detection. During the assay, protein carbonylation products increased significantly in the control treatment, and significant effects from the phenolic treatments were found. Phloridzin had the lowest effect whereas (-)-epicatechin was the most efficient inhibitor of protein carbonylation. For pure phenolic compounds as well as the apple extract, the 200 µM concentrations had the largest antioxidant effect.

Key Words – α-Amino adipic semialdehyde, γ-Glutamic semialdehyde

I. INTRODUCTION

While protein oxidation in living tissues has been thoroughly investigated, the occurrence and impact of protein oxidation in food products has long been surpassed by the effects of lipid oxidation [1,2]. Today, oxidation of food proteins is known to influence protein functionality, nutritional value and sensory quality. In meat and meat products, the physico-chemical reactions due to protein oxidation can cause a number of changes such as decreased tenderness and juiciness, loss of essential amino acids, and altered digestibility due to decreased susceptibility for proteolytic enzymes [3]. Recent studies on the underlying chemical mechanisms and pathways of protein oxidation [2,3] are of the utmost importance to comprehend the impact on meat quality.

The formation of carbonyl compounds from amino acid side chains has been emphasized as one the most important oxidative change in proteins [3]. Under intense oxidation conditions, such as metal-catalyzed oxidation, the amino group from the amino acid side chain may be modified into carbonyl residues through deamination reactions [3,4]. α-Amino adipic semialdehyde (AAS, formed from lysine) and γ-glutamic semialdehyde (GGS, formed from arginine or proline) have been identified as the most abundant protein carbonyls in food systems [5]. The quantification of AAS and GGS as a protein oxidation indicator has been applied for several meat products such as fermented meats [6,7], raw and cooked porcine patties [5,8], and cooked ham [9].

Protein carbonylation due to oxidation can be inhibited by antioxidants. Fruits and other plant materials serve well as natural antioxidants because of their high content of phenolic compounds, and can therefore be a good substitute for conventionally synthetic antioxidants [10]. Apples are a good source of phenolic compounds, and it is known that the majority of phenolics in apples are found in the peel rather than in the flesh [11,12].

The objective of this research was to investigate the effects of apple phenolics on the oxidative stability of myofibrillar proteins in terms of carbonylation.

II. MATERIALS AND METHODS

Phenolic compounds were extracted from freeze dried apple peel using acetone:water (60:40), after

which acetone was removed using a rotary evaporator. Total phenolic content of the apple extract was measured following the Folin-Ciocalteu method [13,14], and results were expressed as millimolar gallic acid equivalents (GAE).

Myofibrillar proteins were extracted from lean pork loin with isolation buffer and NaCl [15,16]. The myofibrillar protein isolation was suspended in PIPES buffer.

Four selected phenolic compounds were added to the suspensions at three concentrations (50, 100 and 200 µM): chlorogenic acid (C50, C100 and C200), (-)-epicatechin (E50, E100 and E200), phloridzin (P50, P100 and P200) and apple extract (A50, A100 and A200). A control group without phenolic compounds was also included. All suspensions were prepared in triplicate in capped flasks and oxidized. Sampling was carried out at days 0, 2, 5, 7, and 10 for analysis.

Protein carbonylation products (AAS and GGS) were derivatized with p-amino benzoic acid (ABA) and analyzed using HPLC with fluorescence detection [5]. Results are expressed as nmol of carbonyl compound per mg of protein.

The effect of apple phenolics on GGS and AAS was tested using a repeated measures linear mixed model (SAS 9.3). Days of sampling (0, 2, 5, 7 and 10) and type of treatment, as well as the interaction between the two factors were tested as fixed effects. Significant difference was considered for $P<0.05$.

III. RESULTS AND DISCUSSION

The evolution of carbonyl compounds (AAS and GGS) in the control and the 200 µM apple extract treatment are shown in Figure 1. The amount of carbonylation products in the control treatment increased significantly during the first five days of the assay, indicating that the metal-catalyzed oxidation of myofibrillar proteins occurred. After day five, the carbonyl amount did not increase or decrease significantly, which might suggest that oxidation has stopped. It is however more likely to believe that the carbonylation products are converted into secondary oxidation products under intense oxidative conditions, as has been shown by Utrera & Estévez [2].

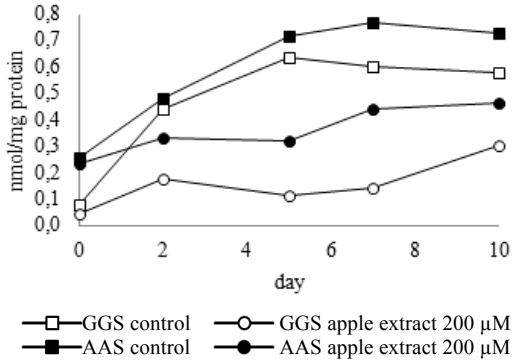

Figure 1: Formation of AAS and GGS (nmol/mg protein) during oxidation of myofibrillar proteins in the control treatment and in the presence of apple extract (200 µM)

The effects of the selected phenolic compounds and the apple extract were expressed relative to the control for both AAS (Table 1) and GGS (data not shown).

All three concentrations of chlorogenic acid as well as (-)-epicatechin significantly inhibited the formation of GGS during 10 days. Regarding the AAS formation, chlorogenic acid only had significant antioxidative effects on day 5 in all three concentrations, and on day 7 in the 50 µM concentration. All (-)-epicatechin treatments significantly inhibited AAS formation, except for the 50 µM concentration on day 2.

Table 1: Effects of apple phenolics on the formation of AAS in oxidized myofibrillar proteins, relative to control values (%). Positive values denote antioxidant activity (percent inhibition), whereas negative values denote pro-oxidant activity (percent promotion).

	day 2	day 5	day 7	day 10
C50	17.95	32.03	25.74	5.59
C100	13.21	22.05	12.62	4.24
C200	14.33	22.96	11.74	1.21
E50	27.41	42.49	51.99	36.50
E100	31.52	48.27	56.04	37.96
E200	34.65	52.87	60.99	57.17
P50	3.17	-3.09	10.27	15.83
P100	13.78	1.19	34.20	44.31
P200	15.20	25.43	47.21	40.97
A50	32.01	43.69	19.78	-18.12
A100	33.50	50.50	35.39	-25.02
A200	30.73	55.41	42.45	35.79

The phloridzin treatment only had a significant inhibition effect for GGS formation in the 200 µM concentration on day 7. The same concentration of

phloridzin was able to significantly inhibit AAS formation from day 5 to 10, whereas the AAS amount in the 100 µM phloridzin treatment was only significantly lower than in the control on day 7 and 10.

During the first five days, the three concentrations of apple extract significantly suppressed formation of both AAS and GGS, closely following the trend of (-)-epicatechin. From then on, the carbonyl content in the 50 and 100 µM apple extract treatments became significantly higher than in the (-)-epicatechin treatments and even the control (AAS in 100 µM apple extract on day 10), suggesting that the phenolics in the apple extract were no longer sufficiently stable and active.

IV. CONCLUSION

V.

Apple phenolics have the potential to protect myofibrillar proteins against the formation of carbonyl compounds. Formation of GGS was significantly inhibited by 100 and 200 µM apple extract, whereas only the 200 µM apple extract was able to significantly inhibit AAS formation during 10 days.

Further research will include testing other concentrations and extraction methods of apple, in order to find the optimal conditions to use phenol-rich apple as a natural antioxidant in meat products. Furthermore, the formation of secondary oxidation products will be investigated in order to understand the effect of apple phenolics on the carbonylation pathway more profoundly.

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