A PRELIMINARY INVESTIGATION ON THE EFFECT OF DIETARY CITRUS PULP ON PROTEIN OXIDATION IN LAMB MEAT

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Abstract – A preliminary study on the influence of dietary citrus pulp on the oxidative stability of meat proteins was done. Nine *Comisana* male lambs, aged 90 days, were fed commercial concentrate with 60% barley (Control, n=3), concentrate with 35% barley and 24% citrus pulp (24% citrus pulp, n=3) and concentrate with 23% barley and 35% citrus pulp (35% citrus pulp, n= 3) without vitamin premix. At age 158 days, the animals were slaughtered and *Longgissimus dorsi* of aged meat were packed aerobically and stored at 4°C in the dark for 0 or 6 days. Myofibrillar proteins were extracted from the meat and loss of sulfhydryl groups and cross-links with myosin heavy chains (CL-MHC) were assessed. Results showed that there was 15.9%, 6.0% and 7% thiol loss between day 0 to 6 in Control, 24% citrus pulp and 35% citrus pulp, respectively, which demonstrate antioxidative effects of citrus pulp. Neither the Control nor the 35% citrus pulp treatments showed CL-MHC indicating that protein oxidation occurred to a limited extent which could be attributed to the packaging conditions.

Key Words – Aerobic packaging, Citrus pulp, Protein oxidation

- INTRODUCTION

Delaying oxidation processes that degrade lipids and protein components of meat is important to meat industry as these affect meat flavor, texture, color, and nutritional value which in turn determine products' marketability. Specifically, muscle protein oxidation has been linked to solubility and functionality changes, as well as tenderness and juiciness deterioration [1, 2]. Oxidative changes can be delayed or inhibited by the use of food additives, however, due to their documented health risks and toxicity naturally preserved commodities are becoming more attractive to consumers.
Citrus and citrus by-products are potential sources of bioactive compounds which could be interesting to food industry due to their nutritional value and antioxidant properties. Citrus extracts protected meat lipid against oxidation [3, 4], as well as orange dietary fiber [5]. Citrus bioflavonoids possess free radical scavenging activity due to the presence of one or more hydroxyl groups in the structure [6]. Hesperidin, a citrus flavonoid, when supplemented to lamb diet, reduced the meat susceptibility to lipid oxidation for several days under refrigerated conditions [7]. It is believed that an association exists between lipid and protein oxidation reactions and that lipid derived radicals and hydroperoxides promote protein oxidation than the opposite process [1]. Therefore, if citrus derived materials were able to delay lipid oxidation in animal products it is likely that proteins also would be protected.

Citrus pulp, by-products of fruit juice industries including the peel, inside portion, seed and culled fruits [8] did not show deleterious effects to health [9, 10] and meat quality when fed to lambs [10]. According to Govaris et al. [11], bioactive compounds are preferably deposited in the tissues of animals where they are mostly needed through nutritional means rather than

when directly incorporated to muscle food. Thus, the utilization of citrus pulp, which otherwise goes to waste, would not only help reduce production cost and waste disposal, but also make use of the antioxidant components to protect lamb meat proteins against oxidative damages.

- MATERIALS AND METHODS

Animal management, diets, slaughter procedure, and muscle sampling

Nine *Comisana* male lambs, aged 90 days, with average weight of 19.76 kg (±3.84 kg) were randomly distributed into 3 groups based on dietary treatments: commercial concentrate with 60% barley (Control, n=3), concentrate with 35% barley and 24% citrus pulp (24% citrus pulp, n=3), or concentrate with 23% barley and 35% citrus pulp (35% citrus pulp, n=3), with no vitamin premix. Lambs were housed outdoors individually and during 10 days were gradually adapted to the experimental diets. Feeds were offered from 0900 h to 1900 h while water was *ad libitum.* The animals were slaughtered at age 158 days in commercial abattoir with stunning by captive bolt and exsanguinated. The carcasses were halved and after 24 h at 4°C, *longgissimus dorsi* muscle (LM) was excised from the right half, vacuum packed and aged for 4 days at 4°C. Two slices of LM from each animal were individually placed on polystyrene tray, overwrapped with oxygen permeable PVC film and stored in the dark at 4°C for 0 day (2 hours) and 6 days. After the intended storage times, meat slices were vacuum-packed and stored at -30°C prior to analysis.

Isolation of Myofibrillar Proteins

The procedure for the extraction of myofibrillar protein isolates (MPI) of meat was adopted from Jongberg et al. [19].

Loss of thiol groups

An aliquot of 10 mg lyophilized MPI was dissolved in 5% SDS (sodium dodecyl sulfate) in 0.1 M tris [tris(hydroxymethyl)-(aminomethane)] buffer at pH 8.0 in a water bath at 80°C for 1 h. The dissolved MPI was centrifuged and the supernatant was used for protein determination using the Pierce BCA Protein Kit Assay (Thermo Specific, Pierce Biotechnology Rockford, IL, USA) following the manufacturer's instructions. Measurements were done in triplicates and average protein concentration was used in the succeeding analyses. The loss of sulfhydryl groups was monitored by following the methods used by Jongberg et al. [16]. All analyses were done in triplicates and mean results were reported as nmol thiols/mg protein ± standard deviation.

SDS-PAGE of MPI

Samples from Control and 35% citrus pulp were analyzed for the presence of CL-MHC using the NuPAGE® Novex 3-8% TRIS-acetate gels according to the manufacturer's instructions and following the sample preparations published by Jongberg et al. [16].

Statistical analyses

A One-Way ANOVA (analysis of variance) was used to analyze the effects of storage time and diets on the loss of thiols. The Minitab Software version 6 was used.

- RESULTS AND DISCUSSION

Human studies on citrus flavanone glycosides, hesperidin and naringin, which were orally administered in pure form, juice, or whole grapefruit, showed evidences of absorption from the gastrointestinal tract [12]. In sheep, the hydrolysis of the glycosidic bonds of naringin, takes place prior to absorption of the aglycone naringenin from the rumen. In spite of that, the antioxidant efficacy remained to protect the plasma against lipoperoxidation [13].

Figure 1. Loss of thiol groups quantified through Ellman's reagent of MPI extracted from lamb meat fed concentrate based diet, Control, and concentrate plus either 24% citrus pulp or 35% citrus pulp stored aerobically for 0 or 6 days in the dark at 4°C.

The loss of sulfhydryl groups of cysteine amino acid side chain in proteins indicates the occurrence of oxidation, as thiol groups are susceptible to oxidation [1]. Figure 1 summarizes the results of the loss of free thiols of lamb meat samples fed three different diets. Regardless of dietary treatments, a decreasing trend in thiol concentration was observed with storage time. At day 0 the thiol content in Control and 24% citrus pulp were significantly higher than in the 35% citrus pulp groups ($p=0.002$), but 35% citrus pulp ended up with similar thiol content as Control at day 6. However, Control showed greater loss of thiols (15.9%) than 24% citrus pulp (6.0%) and 35% citrus pulp (7.0%). The reduced thiol loss in citrus pulp groups roughly demonstrates that they are less susceptible to thiol oxidation compared to the Control group. As oxidation of proteins may take place through free radical chain reaction mechanism [1] the protective effects of citrus pulp against loss of thiols may indicate the presence of bioactive components possessing radical scavenging properties, which are otherwise absent in commercial concentrate. This could also explain the results of the lipid oxidation measurements on the same meat samples which showed that meat from citrus pulp treatments were more stable than Control [14].

The cross-linking of myosin heavy chains (CL-MHC), which is one of the products of thiols oxidation [1] and have been found to increase meat toughness, can be detected through the use of gel electrophoresis [15]. Non-reduced and reduced gel electrophoreses were run in parallel to verify if cross-links were due to disulfide formation [16]. Figure 2 depicts the scanned gel images of MPI in non-reduced and reduced states comparing the effects of 35% citrus pulp at day 0 and after 6 days storage with Control. The image clearly shows the absence of any CL-MHC in non-reduced gel as well as of artifacts in reduced gel. The presence of CL-MHC has previously been observed in meat stored in high oxygen modified atmosphere package (Hi-Ox MAP) compared to non-oxygen package [15, 16, 17],

Figure 2 SDS-PAGE gels (NR=non-reduced; R=reduced) of MPI of Control (C) and 35% citrus pulp (Cp) meat samples stored for 0 and 6 days on polystyrene overwrapped with oxygen-permeable PVC film at 4°C.

suggesting that Hi-Ox MAP could induce oxidation of proteins by disulfide formation in myosin, resulting in reduced meat tenderness. The absence of CL-MHC in selected samples may suggest that meat were not highly oxidized as storage was not done in Hi-Ox MAP but rather in oxygen permeable PVC film. The low malondialdehyde (MDA) formed in the Control group after 6 days (approx. 2 mg/kg meat) also support the low oxidation levels in the stored meat [14] since slight off odors were detectable by sensory panelists at 5 mg MDA/kg lamb meat [18]. Therefore the initially low free thiols in 35% citrus pulp cannot be directly correlated to the occurrence of immediate oxidation in meat proteins since no CL-MHC were observed even until day 6, but may be linked to the reaction between thiols and the phenols contained in the citrus pulp [19].

Although protein oxidation in the meat samples took place to a limited degree, the protective effect of citrus pulp was noticeable within the storage conditions considered. The lower amount of thiols oxidized in 24% and 35% citrus pulp treatments at day 0 in comparison with Control indicates that citrus pulp bioactive components have immediate antioxidant effects to meat. The greater decrease in thiol content in Control than in citrus pulp groups at day 6 explains the extended antioxidant function of the latter at aerobic refrigerated storage.

- CONCLUSION

Dietary citrus pulp is likely to improve antioxidant capacity of lamb meat as proteins were more stable against oxidation for extended refrigerated storage held in oxygen permeable package than the concentrate fed lambs. Further analyses should be done to confirm the current findings, together with increasing the number of animals to be used in the feeding trial to make data more reliable. Nevertheless it can be concluded that citrus pulp could be used as a cheap replacement to concentrate until 35% to improve meat proteins stability.

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