THE EFFECTS OF STORAGE CONDITIONS ON PROTEIN OXIDATION OF RENDERED BY-PRODUCTS

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I. INTRODUCTION

The use of rendered by-products in animal feed, especially in the growing pet food market, is widespread and provides a cost-effective source of protein. These sources are exposed to extreme temperatures to ensure microbial stability before being incorporated into the pet food chain. While it is known that the high heat of rendering can increase protein oxidation, the effects of storage of rendered products at elevated temperature has not been carefully examined. Over the years, interest in the mechanisms and health effects of protein oxidation has increased but currently, there is very little information known on the amount of protein oxidation present in rendered products after the rendering process. Furthermore, little is known about how storage conditions or further processing of rendered products will change the degree of protein oxidation. By understanding the extent of oxidation, processors can better utilize antioxidants or other processing techniques to mitigate protein oxidation. This in turn could add additional value compared to products containing higher levels of protein oxidation. In other studies, heat has been used to induce protein oxidation [1,2,3]. We hypothesized that as rendered by-products were exposed to elevated temperatures for extended periods of time, protein oxidation would increase. Therefore, the objective of this study was to characterize how time and temperature can impact the amount of protein oxidation in rendered by-products.

II. MATERIALS AND METHODS

The protein source evaluated was dried plasma from a commercial renderer. Dried plasma was subjected to a 3x4+2 factorial design with temperature (45°C, 65°C, or 100°C) over time (24, 48, 72, or 96 hours) in addition to two control samples with two temperatures (-20°C and -80°C). Each treatment was replicated five times. After subjected to heating, samples were vacuum packaged and stored at -80°C until analyzed. When proteins are oxidized, the most common product formed during the process is carbonyls [4]. Furthermore, these products of oxidation are irreversible in comparison to other products of oxidation such as disulfides. Therefore, in our study, oxidation was assessed through a carbonyl assay, using the DNPH method [5]. In this method, samples were dissolved into solution and homogenized at a concentration of 15 mg protein/ ml of water. 1 ml of solution was transferred to 6 individual tubes. 4 ml of HCL was added to 3 tubes and 4ml 10mmol DNPH HCL was added to the remaining 3. Following a 1 hour incubation period, 5 ml of 20%w/v TCA was added to stop the reaction, and placed in a water bath for 10 min. Samples were then centrifuged at 3100xg for 10 min at 4°C. Once supernatant was discarded, the pellet was dissolved in 10%w/v TCA. It was then centrifuged at 3100xg for 10 min at 4°C. Once supernatant was discarded, the pellet was dissolved in 1:1 Ethanol: Ethyl Acetate. It was then centrifuged at 3100xg for 10 min at 4°C. This step was repeated. Supernatant was discarded again and the pellet was dissolved in 6M Guanidine HCL. Samples were placed in a hot water bath at 37°C and vortexed every 5 minutes. Samples were centrifuged at 3700xg for 10 min at 20°C. Three control tubes that did not contain DNPH were used to determine protein content with the Biuret protein assay. The 3 tubes that did contain DNPH were read for absorbance at 365nm. Final carbonyl content was corrected for protein concentration. Data was analyzed using SAS GLM procedure (v 9.4, SAS Institute, Cary, NC, USA) with time, temperature, and time x temperature as fixed effects.

III. RESULTS AND DISCUSSION

After rendering, dried plasma contained 2.70 and 3.02 nmol carbonyl/mg of protein when samples were maintained at -20°C or -80°C, respectively. As temperature increased for extended periods of time, a significant increase in carbonyls/mg protein was observed. Samples exposed to 45°C, increased from 3.19

nmol/mg of protein at 24 hours to 5.94 nmol/mg of protein at 96 hours (p<.0001). At 65°C, carbonyl levels increased from 3.60 nmol/mg of protein at 24 hours to 5.87 nmol/mg of protein at 96 hours (p<.0001). When plasma was held at 100°C, carbonyls increased from 7.65 nmol/mg of protein at 24 hours to 15.23 nmol/mg of protein at 96 hours (p<.0001). Results are summarized in figure 1. These results are consistent with other studies where heat increased carbonyl content in cooked meat and plant proteins [1,2,3]. It is important to consider that carbonyl levels will vary based on the protein source. Therefore, it is more important to consider the change in carbonyls rather than the concentration itself.

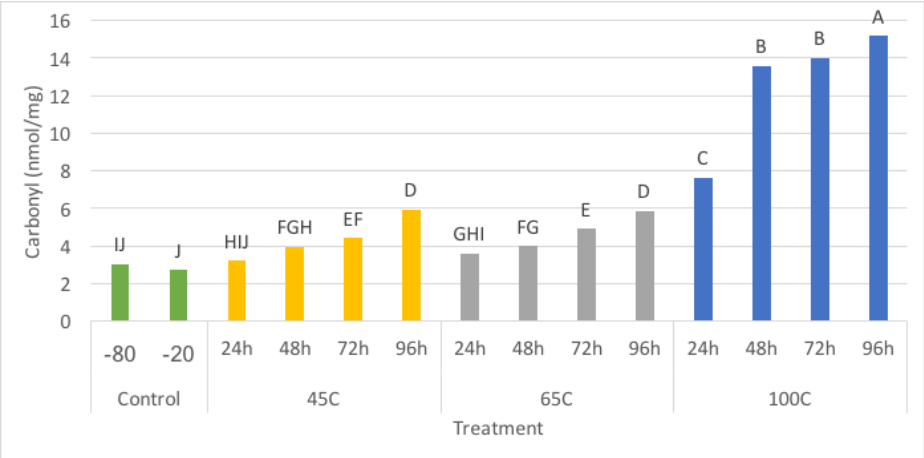

Figure 1. Summary of carbonyl levels in plasma for various time and temperatures. [A-J] Significant difference between means ($p < 0.05$).

IV. CONCLUSION

Heat damage during extreme storage conditions of rendered proteins can further increase carbonyl levels, indicating an increase in protein oxidation. Therefore, care should be taken in storing and processing of dried plasma to limit exposure to heat for extended periods of time. Future investigation should also examine the level of protein oxidation in other rendered by-products. Furthermore, it is known that absorption of oxidized proteins can cause damage in the small intestine and other organs [6] and result in oxidative stress. Therefore, effects of oxidized protein from rendered by-products on overall animal health should be analyzed. Understanding how oxidized proteins in animal feed will affect their health and performance will allow processors that reduce oxidation to add value to their products.

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