71st ICoMST
GIRONA - CATALONIA - SPAIN
3rd-8th August - 2025

UPCYCLING OF DRY-AGED BEEF CRUST INTO POTENT BIOACTIVE MATERIALS THROUGH ENZYMATIC HYDROLYSIS

Dongheon Lee, Seokjun Lee, Cheorun Jo*

Department of Agricultural Biotechnology, Center for Food and Bioconvergence, and Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul, Republic of Korea

*Corresponding author email: cheorun@snu.ac.kr

I. INTRODUCTION

As recent consumers prioritize flavorful and value-added food products, dry-aged beef has gained popularity owing to its rich flavor. During the dry aging process, moisture evaporates from the outer surface of the beef, resulting in a dark, firm, and dried crust [1]. This crust typically accounts for about 35% of dry-aged beef and is inedible, leading to economic losses for manufacturers, which is a major reason for the high price of the product [2]. To mitigate these economic losses and explore the potential of the crust as a food ingredient, previous studies have incorporated dry-aged beef crust into processed meat products, showing that it enhanced the flavor [3,4]. Nonetheless, using the crust directly in food applications faces challenges related to its texture, microbial safety, and storage. Enzymatic hydrolysis may effectively address these issues, improving the usability of the crust as a food ingredient. This process can also enhance the bioactivity of protein sources by generating various bioactive peptides. Hence, enzymatically hydrolyzing the crust could increase its potential as a bioactive material for food supplements. However, there is limited information regarding the bioactivity of hydrolysates from dry-aged beef crust. Therefore, the objective of this study was to evaluate the potential of crust as a bioactive material by assessing the bioactivity and bioactive compound content of hydrolysates from dry-aged beef crust collected at different aging periods.

II. MATERIALS AND METHODS

Beef M. *longissimus lumborum* was obtained from three 29-mon steers. The muscle from each animal was divided into five blocks (500 g each) and dry-aged for 0, 7, 14, 21, and 28 days. The aging condition was set to 4°C and 70% relative humidity. After aging, the dry-aged beef was trimmed to remove the crust which was then collected and cut into 1 cm pieces. The crust samples were lyophilized, pulverized with a mortar and pestle, and homogenized with distilled water (1:5, w/v). The resulting homogenates were neutralized and heated to 50°C. Flavourzyme® from *Aspergillus oryzae* (Novozymes, Bagsvaerd, Denmark) was added at a 2% enzyme-substrate ratio. The mixture was incubated at 50°C for 3 h, neutralized again, and then heated to 95°C for 10 min to inactivate enzyme activity. The samples were centrifuged, filtered, and lyophilized for further analysis. The degree of hydrolysis (DH) was determined using the o-phthaldialdehyde (OPA) method. The antioxidant activity of the crust hydrolysates (5 mg/mL) was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging assays. The antihypertensive activity of the sample (1 mg/mL) was measured through angiotensin-converting enzyme inhibitory (ACEI) activity. Bioactive compounds in each hydrolysate was quantitively analyzed using liquid chromatography. Statistical analysis was conducted using one-way analysis of variance.

III. RESULTS AND DISCUSSION

The DH of crust hydrolysates increased significantly as the aging period extended (Table 1). During the aging process, endogenous and microbial-origin exogenous proteases break down the myofibrils, contributing to the increased DH of crust hydrolysates. Previous literature reported that bioactive peptides are generally short, with amino acid residues less than 20, due to their bioaccessibility and ability to enter the target site [5]. Considering the high DH, dry-aged beef crust hydrolysates could serve as a valuable source of bioactive peptides. However, there were no significant differences in antioxidant and ACEI activities among the crust hydrolysates from different aging periods (Figure 1). These phenomena might be associated not only with bioactive peptides, but also with other bioactive compounds such as betaine, anserine, or carnosine. The levels of betaine and carnitine in the crust hydrolysates significantly decreased when the dry aging was extended to 14 days or more (Table 2). On the other hand, the levels of carnosine and creatine increased significantly in the crust hydrolysates from beef aged for 28 days. Betaine and carnosine are representative antioxidants found in beef, while carnitine aids in transporting long-chain fatty acids to the mitochondria, and creatine can enhance muscular performance. From the results, dry aging period influenced the contents of bioactive compounds in the crust hydrolysates, however, the overall bioactivity did not differ significantly, suggesting that the crust from various aging periods could still be effectively utilized.

Table 1 – Degree of hydrolysis (%) of Flavourzyme®-treated dry-aged beef crust hydrolysates with different aging period

Aging period (d)					P value
0	7	14	21	28	
23.61±2.68^{D}	31.49±2.94^{CD}	36.50±2.09^{BC}	45.46±6.50^{AB}	49.94±4.22^{A}	0.0002

$^{A-D}$ Different letters indicate significant differences ($p < 0.05$).

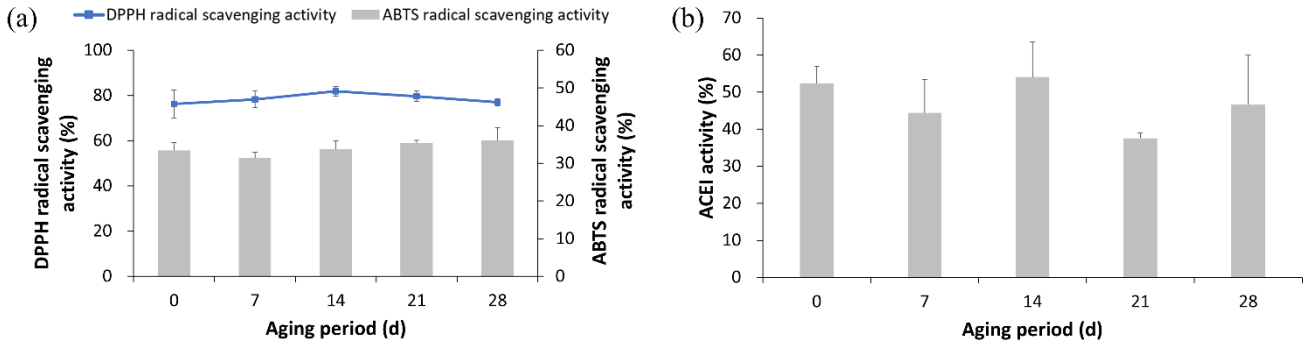


Figure 1. Antioxidant (a) and antihypertensive activities (b) of Flavourzyme®-treated dry-aged beef crust hydrolysates with different aging period. The sample concentration was 5 and 1 mg/mL for antioxidant and antihypertensive assays, respectively. DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid; ACEI activity, angiotensin converting enzyme inhibitory activity.

Table 2 – Bioactive compound content (mg/g) of Flavourzyme®-treated dry-aged beef crust hydrolysates with different aging period

Compound	Aging period (d)					P value
	0	7	14	21	28	
Betaine	2.63±0.23^{A}	2.20±0.14^{AB}	1.74±0.05^{C}	1.89±0.03^{BC}	2.07±0.23^{BC}	0.0002
Carnitine	18.14±0.83^{AB}	18.36±0.95^{A}	15.61±0.65^{C}	16.17±0.75^{BC}	14.68±0.86^{C}	0.0087
Carnosine	19.49±1.14^{B}	19.69±0.49^{AB}	19.21±1.00^{B}	19.86±0.38^{B}	21.90±0.88^{A}	0.0005
Creatine	20.54±1.09^{B}	20.04±0.23^{B}	18.52±0.39^{B}	19.72±0.90^{B}	23.63±1.05^{A}	0.0009

$^{A-C}$ Different letters within the same row indicate significant differences ($p < 0.05$).

IV. CONCLUSION

Dry-aged beef crust hydrolysates exhibited strong *in vitro* antioxidant and ACEI activities, comparable to those found in fresh beef hydrolysates (day 0). Additionally, the levels of carnosine and creatine in the crust hydrolysates significantly increased at the late phase of dry aging. Therefore, the crust could be upcycled into potent bioactive materials for food supplements through enzymatic hydrolysis.

ACKNOWLEDGEMENTS

This work was supported by Ascending SNU Future Leader Fellowship through Seoul National University.

REFERENCES

1. Xue, S.; Setyabrata, D.; Bonham, C. C.; Kim, Y. H. B. (2021) Evaluation of functional and chemical properties of crust from dry-aged beef loins as a novel food ingredient. Meat Science, 173: 108403.
2. Lee, J. A.; Kim, H. Y.; Seoul, K. H. (2022) Quality properties of dry-aged beef (Hanwoo cattle) crust on pork patties. Foods 11(15): 2191.
3. Park, B.; Yong, H. I.; Choe, J.; Jo, C. (2018) Utilization of the crust from dry-aged beef to enhance flavor of beef patties. Food Science of Animal Resources, 38(5): 1019-1028.
4. Lee, J.; Kim, H. Y. (2021) Effect of *Hanwoo* crust on the physicochemical properties of emulsion-type sausages. Food Science of Animal Resources, 41(3): 440-451.
5. Toldrá, F.; Reig, M.; Gallego, M.; Mora, L. (2023) Bioactive peptides in meat and meat products. Meat and Muscle Biology, 7(3): 16243.