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The Effects of Aging Period and Freezing Sequence on Desmin Degradation of *Longissimus lumborum, Semitendinosus,* and *Biceps femoris* Steaks

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Abstract

The objective of this study was to examine the impact of freezing and aging sequence on the degradation of desmin within three beef muscles across two aging periods. The *longissimus lumborum* (LL), *semitendinosus* (ST), and *biceps femoris* (BF) were fabricated, sliced into 1-in. steaks and assigned to one of four treatment combinations: age (21 days) then freeze, freeze then age (21 days), age (28 days) then freeze, or freeze then age (28 days). After treatment, steaks were ground and stored at -80˚F. Samples were thawed for protein extraction and desmin analysis. Sarcoplasmic proteins were removed, and then myofibrillar proteins, including desmin, were solubilized in sodium dodecyl sulfate buffer with protease inhibitors. Extracted proteins were analyzed via Western blotting. Polyacrylamide gels were prepared, and equal volumes of samples were loaded for electrophoresis. Proteins were transferred onto polyvinylidene membranes, blocked, and probed with primary and secondary antibodies. Bands were visualized using chemiluminescent detection and imaged with a FluorChem FC2 CCD camera. Band intensities were quantified to determine the percentage of intact and degraded desmin. There was no difference ($P > 0.05$) in intact desmin among the three muscles. The BF had a greater amount ($P < 0.05$) of degraded desmin. A two-way interaction ($P < 0.05$) was observed between freezing treatment and aging period when all muscles were combined. The 28-day freeze-then-age samples had a lower percentage ($P < 0.05$) of intact desmin compared to the 21-day age-then-freeze samples. This translated into the 21-day age-then freeze samples having the lowest percentage ($P < 0.05$) of degraded protein in bands 3 and 4. Overall, the freezing and aging sequence did not significantly impact degradation; however, extended aging increased degradation. In a previous study on the loins, there were no differences in instrumental tenderness. Thus, even with increased protein degradation, the change was not enough to affect tenderness.

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Introduction

Freezing is one of the most widely used practices in the industry to maintain quality and extend beef shelf life (Kim et al., 2015). Freezing is believed to improve tenderness by the well accepted mechanism of ice crystal formation and the less studied mechanism of reducing calpastatin activity. Calpastatin is the endogenous inhibitor of the prominent enzyme, μ-calpain. Therefore, calpastatin inactivation could allow for greater proteolysis during aging, potentially impacting overall palatability (Khan et al., 2016). While most of the beef industry ages meat after harvest and before freezing, reversing the order has been hypothesized to improve tenderness by enhancing proteolytic activity; however, the true impact on palatability remains unknown (Beyer et al., 2024; Setyabrata et al., 2019).

Protein degradation is critical to post-mortem tenderness improvement, heavily driven by enzymatic activity (Koohmaraie, 1996). One key structural protein, desmin, helps maintain muscle fiber integrity and is used as an indicator of tenderness during post-mortem proteolysis (Huff-Lonergan and Lonergan, 2005). During aging, desmin is degraded by proteolytic enzymes, such as μ-calpain, which weakens the cytoskeletal framework and improves tenderness (Huff-Lonergan and Lonergan, 2005). Freezing and thawing cycles can accelerate these processes by either inactivating enzymatic inhibitors or creating stress on the cytoskeleton, further promoting desmin degradation and potentially enhancing tenderness (Warner et al., 2017). In this study, desmin served as an indirect measure of μ-calpain activity to illuminate the freezing impact on calpastatin activity.

While the common aging period for beef carcasses is close to 21 days, some muscles remain tough and less palatable even at longer aging periods, leaving an opportunity to improve tenderness by reversing the freezing and aging order (Grayson et al., 2014). Therefore, the motivation for this study was to determine whether reversing the freezing and aging sequence could improve tenderness and increase proteolytic activity of particularly tougher muscles. To evaluate this mechanism, desmin was selected as a marker protein because its degradation reflects post-mortem proteolytic activity. Thus, this study aimed to assess how freezing and aging sequences influence desmin degradation in three different beef muscles.

Experimental Procedures

Beef carcasses (n = 12) graded USDA Choice, A maturity were selected from a Midwestern beef plant. Trimmed strip loins (Institutional Meat Purchase Specifications [IMPS] #180) and goosenecks (IMPS #170) were collected from the right side of each carcass (NAMI, 2014). The strip loins and goosenecks were transported to North Dakota State University and fabricated the day after collection. The *semitendinosus* (ST) and *biceps femoris* (BF) were separated from the goosenecks. The strip loins, ST, and BF were denuded and sliced into 1-in. steaks. Each steak was randomly assigned a four-digit code with a tag and allocated to either age-then-freeze (AF) for 21 or 28 days or freeze-then-age (FA) for 21 or 28 days. Steaks were then randomly assigned to an assay. All steaks were aged between 34 to 40˚F in the absence of light. Steaks were blast frozen in a freezer and held at -4˚F for 91 days, then placed in a refrigerator to thaw for 24 hours prior to use (if applicable). Steaks designated for protein analysis were ground and stored in a -80˚F freezer. Samples were thawed on ice and processed for desmin extraction. Samples were washed in post-rigor extraction buffer containing protease inhibitors to remove sarcoplasmic proteins, then centrifuged. Pellets were solubilized in

phosphate buffer with 2% sodium dodecyl sulfate, homogenized, incubated 30 minutes, and clarified by centrifugation. The supernatant was then retained. Desmin degradation was assessed using myofibrillar protein extraction followed by Western blot analysis. Polyacrylamide gels (12.5% separating, 5% stacking) were prepared, and equal volumes of protein samples were loaded for electrophoresis. All gels did contain a reference sample for comparison. Proteins were transferred onto polyvinylidene membranes, blocked, and probed with primary and secondary antibodies. Bands were visualized using chemiluminescent detection and imaged with a FluorChem FC2 CCD camera under autoexposure. Band 1 was used to determine the total concentration of intact desmin, while Bands 3 and 4 were used to determine the concentration of degraded desmin. Statistical analysis was conducted using SAS with PROC GLIMMIX. The experimental unit was the individual carcass. Fixed effects included muscle, freezing treatment, and aging period. Data were analyzed as a split-split plot design, where the whole-plot factor was muscle, the subplot factor was aging period, and the sub-subplot factor was freezing treatment. An alpha level of 0.05 was set for statistical significance.

Results and Discussion

Tables 1 and 2 show the abundance of multiple molecular weights of desmin indicating different levels of degradation. Band 1 was utilized to determine the amount of intact desmin which could infer less proteolytic activity and structural degradation. Bands 3 and 4 were utilized to determine the amount of degraded desmin indicating a greater amount of proteolytic breakdown. Desmin degradation revealed an interaction between freezing treatment and aging period across all muscles combined. Specifically, the 28-day FA samples had a lower percentage ($P < 0.05$) of intact desmin compared to the 21 and 28-day AF samples, indicating more proteolytic activity occurred. Similarly, the 28-day FA steaks had a greater ($P < 0.05$) amount of degraded desmin in bands 3 and 4 compared to the 21-day AF steaks. Desmin degradation is associated with post-mortem tenderization (Huff-Lonergan and Lonergan, 2005). The greater degradation observed in the 28-day FA treatment may indicate that proteolytic systems such as μ-calpain remain active and can act more extensively when aging follows freezing, particularly over extended storage times.

There was no difference ($P > 0.05$) for intact desmin among the three different muscles. However, the BF had a greater amount ($P < 0.05$) of degraded desmin compared to the LL and ST for Bands 3 and 4. These results show a conflicting relationship of proteolytic activity; however, other studies have found similar results where the degraded desmin was more sensitive than the intact desmin (Kim et al. 2015).

These results demonstrate that increasing the aging period in combination with a freeze-then-age sequence promotes greater overall degradation of the desmin protein. This outcome suggests that underlying biochemical changes are occurring at the structural protein level.

Implications

The results indicate reversing the typical aging and freezing order increases desmin degradation only when combined with additional aging time.

Acknowledgments

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References

Beyer, E.S., L.K. Decker, E.G. Kidwell, A.L. McGinn, M.D. Chao, M.D. Zumbaugh, J.L. Vipham, and T.G. O'Quinn. 2024. Evaluation of fresh and frozen beef strip loins of equal aging periods for palatability traits. Meat and Muscle Biology 8(1):16903, 1-13. doi: *https://doi.org/10.22175/mmb.16903*

Grayson A.L., D.A. King, S.D. Shackelford, M. Koohmaraie, and T.L. Wheeler. Freezing and thawing or freezing, thawing, and aging effects on beef tenderness. 2014. Journal of Animal Science. 92(6):2735-2740. *doi: 10.2527/jas.2014-7613*

Huff-Lonergan, E., and S.M. Lonergan. 2005. Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. Meat Science 71(1):194–204. *doi:10.1016/j.meatsci.2005.04.022*

Khan M.I., S. Jung, K.C. Nam, and C. Jo. 2016. Postmortem aging of beef with a special reference to the dry aging. Korean Journal for Food Science of Animal Resources 36(2):159-69. *doi: 10.5851/kosfa.2016.36.2.159*

Kim, Y.H., C. Liesse, R. Kemp, and P. Balan. 2015. Evaluation of combined effects of ageing period and freezing rate on quality attributes of beef loins. Meat Science 110:40–45. *https://doi.org/10.1016/j.meatsci.2015.06.015*

Koohmaraie, M. 1996. Biochemical factors regulating the toughening and tenderization processes of meat. Meat Science 43(Supplement1):193-201. *doi:10.1016/0309-1740(96)00065-4*

Setyabrata, D., and Y. H. Brad Kim. 2019. Impacts of aging/freezing sequence on microstructure, protein degradation and physico-chemical properties of beef muscles. Meat Science 151:64–74. *https://doi.org/10.1016/j.meatsci.2019.01.007*

Warner, R.D., P.L. Greenwood, D.W. Pethick, and D.M. Ferguson. 2010. Genetic and environmental effects on meat quality. Meat Science 86:171-183. *doi: 10.1016/j.meatsci.2010.04.042*

North American Meat Institute. 2014. The meat buyer's guide. 8th ed. North American Meat Institute, Washington D.C.

Table 1. Least square means of relative band concentrations of desmin within steaks with two freezing treatments, two aging periods, and three muscles

Freezing treatment	Aging period	Band 1[1]	Band 3[2]	Band 4[2]
Age-then-freeze samples	21	1.01[a]	0.12[b]	0.24[b]
	28	0.92[ab]	0.14[ab]	0.34[ab]
Freeze-then-age samples	21	0.78[bc]	0.14[ab]	0.32[ab]
	28	0.59[c]	0.17[a]	0.44[a]
SEM[3]		0.07	0.01	0.04
P-value		<0.05	<0.05	<0.05

[abc] Means within the same column without a common superscript differ ($P < 0.05$).

[1] Band 1: Intact desmin.

[2] Bands 3 and 4: Degraded desmin.

[3] Standard error (largest) of the least squares means.

Table 2. Least square means of relative band concentrations of desmin within steaks within three muscles

Muscle	Band 1[1]	Band 3[2]	Band 4[2]
Biceps femoris	0.77	0.27[a]	0.74[a]
Longissimus lumborum	0.90	0.10[b]	0.16[b]
Semitendinosus	0.80	0.06[c]	0.10[b]
SEM[3]	0.06	0.01	0.04
P-value	0.32	<0.05	<0.05

[abc] Means within the same column without a common superscript differ ($P < 0.05$).
[1] Band 1: Intact desmin.
[2] Bands 3 and 4: Degraded desmin.
[3] SE (largest) of the least squares means.