Peptidomic analysis characterising proteolysis in thaw-aging of beef short plate

Yuri Kominami a,*, Tatsuya Hayashi b, Tetsuji Tokihiro c, Hideki Ushio a

a Department of Aquatic Bioscience, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1, Yayoi, Bunkyo-ku, Tokyo 113-865, Japan
b Division of Computer Science and Information Technology, Graduate School of Information Science and Technology, Hokkaido University, Kita 14, Nishi 9, Kitaku, Sapporo, Hokkaido 060-0814, Japan
c Department of Mathematical Sciences, Graduate School of Mathematical Sciences, The University of Tokyo, 3-8-1 Komaba, Meguro-ku, Tokyo 153-8914, Japan

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ABSTRACT

Recent studies have suggested that thaw-aging can improve sensory attributes of freeze-thawed meat. Acceleration of proteolysis is expected to promote tenderisation and improve taste; however, the details of protein degradation, including substrate proteins and cleavage sites, remain unclear. Here, we report a time course overview of the peptidome of beef short plates during thaw-aging. The accelerated degradation of key proteins for meat tenderisation, such as troponin T and desmin, was confirmed. Additionally, 11 cleavage sites in troponin T related to taste-active peptide generation were identified. Terminome analysis showed that the contribution of each protease varies depending on the substrate proteins and the thaw-aging period. Based on our results; proteases, not only calpains, but also others contributed to the degradation of myofibrillar proteins. The techniques employed indicate that meat proteolysis during thaw-aging is not constant but dynamic.

1. Introduction

Beef is one of the most important dietary protein sources worldwide. In fact, the international beef industry has grown significantly with the development of refrigeration technology. Currently, remarkable amounts of frozen beef are traded internationally as freezing can markedly extend shelf-life. However, the freeze-thaw process deteriorates the sensory quality of beef because ice crystal formation mechanically and biochemically disrupts meat tissue (Leygonie et al., 2012). Thus, quick freezing is a major strategy to prevent deterioration of sensory quality. A faster freezing rate can minimise ice crystal coarsening and effectively improve the quality of frozen meat (Leygonie et al., 2012). Many studies have revealed the relationship between freeze-storage conditions and meat quality; however, the appropriate thawing conditions have not been sufficiently investigated.

Thaw-aging is expected to improve the quality attributes of freeze-thawed meat (Balan et al., 2019; Grayson et al., 2014; Setyabrata & Kim, 2019; Shibata & Yasuhara, 1996). The magnitude of the improvements differs depending on meat species, meat cuts, aging temperature, and aging period before and after freezing; however, the acceleration of protein degradation during thaw-aging has been generally confirmed (Balan et al., 2019; Grayson et al., 2014; Setyabrata & Kim, 2019; Shibata & Yasuhara, 1996). Degradation of myofibrillar proteins, including desmin and troponin T, during thaw-aging is demonstrated by the results of western blotting (Balan et al., 2019; Grayson et al., 2014; Setyabrata & Kim, 2019); both proteins are involved in maintaining the structural integrity of myofibrils and their degradation contributes to meat tenderisation (Koohmaraei, 1996). Thaw-aging was also reported to increase free amino acids in the freeze-thawed beef (Shibata & Yasuhara, 1996). Several studies have suggested that accelerated proteolysis during thaw-aging should result in meat tenderisation and taste improvement.

In postmortem proteolysis, calpain-1 and -2, also known as μ- and m-calpain, respectively, are responsible for the degradation of myofibrillar proteins (Ouali et al., 2006; Pearce, Rosenvold, Andersen, & Hopkins, 2011). Additionally, cathepsins and caspases could contribute to postmortem proteolysis (Kitamura, Kudo, Chikuni, Watanabe, & Nishimura, 2010; Ouali et al., 2006; Pearce et al., 2011). Calpains are Ca2+-dependent cysteine proteases and their proteolytic activity is inhibited by calpastatin (Goll, Thompson, Li, Wei, & Cong, 2003). Freezing storage has little effect on the proteolytic activity of calpains, whereas...
freezing reduces the inhibitory activity of calpastatin to ~50% or less (Koohmariae, 1990; Whipple & Koohmariae, 1992). Accordingly, it is suggested that the degradation of myofibrillar proteins by calpains should be accelerated during thaw-aging. The higher protease activity of calpain-1 and -2 in meat after freeze-thaw than before freezing was observed (Whipple & Koohmariae, 1992). However, the contribution of cathepsins and caspasases to proteolysis during thaw-aging is unclear because the effects of freeze-thaw on proteolytic activity remains obscure (Pearce et al., 2011). Previous studies on proteolysis in meat aging have mainly consisted of two approaches: protease activity assay and quantification of remained myofibrillar proteins. These targeted approaches can only show macroscale proteolysis. To overcome limitations of the classical methodology, an omics approach has been applied to investigate postmortem evolution (Lana & Zolla, 2016; Longo, Lana, Bottero, & Zolla, 2015; Parodi, Raboni, Bendixen, de Almeida, & Mozzarelli, 2012). Proteomics and metabolomics have indicated that intracellular signal transduction leads to proteolytic processes, such as apoptosis and autophagy, which can contribute to meat tenderisation (Lana & Zolla, 2016; Longo et al., 2015; Parodi et al., 2012). Previous omics studies have mainly focused on the intracellular mechanisms related to the activation of proteases. However, the details of protein degradation, including substrate proteins and cleavage sites, have not been sufficiently investigated.

Peptidomics has been applied in food science to explore bioactive peptides, marker peptides for authentication, taste-active peptides, and allergenic peptides (Martin, Soleri, & Tagliazucchi, 2021). In contrast, we applied label-free peptidomics to characterise proteolysis during food processing (Kominami et al., 2020). Label-free peptidomics can identify and quantify free peptides generated by proteases in food. The proteolytic peptide has a characteristic sequence in the N- or C-terminus, which depends on the cleavage site specificity of the cleaved protease (Agard & Wells, 2009; Diamond, 2007). Accordingly, we developed a multiple linear regression model for analysis of the terminome (Kominami, Hayashi, Tokihiro, & Usahi, 2019); the comprehensive terminal sequence of cleaved peptides (referred to as terminome) is expressed as the linear combination of cleavage site specificity of each protease and each contributing parameter. The label-free peptidomics coupled with our mathematical model allow us to not only identify degraded proteins, but also deduce catalytic proteases that degrade them. The present study investigated proteolysis in a beef short plate during thaw-aging through the label-free peptidomics. The quantitative peptidome of the thaw-aged beef revealed degraded proteins and their cleavage sites. Additionally, the terminome analysis showed proteolytic dynamics during thaw-aging period.

2. Materials and methods

2.1. Materials

A part of frozen beef short plates imported from U.S. (gifted from Yoshinoya Holdings Co., Ltd.) was used for experiments described below. The short plate was obtained from a Holstein steer inspected by the U.S. Department of Agriculture’s inspectors. The beef satisfying a hygiene requirement was packed and frozen after a short aging period. Thereafter, it was shipped to Japan under refrigerated conditions. The imported frozen beef was inspected by the Animal Quarantine Service of the Ministry of Agriculture, Forestry and Fisheries of Japan. After the import quarantine inspection, the frozen beef was transferred to the Yoshinoya Holdings Group’s plant and stored at ~30 °C under a HACCP-based control. The frozen beef was cut into ~250 g blocks with a bandsaw and repacked under vacuum. Cutting and packing were performed in a cold room to avoid thawing of the samples. The packed beef blocks were thawed by immersing them into ice water for 1, 2, 5, 7, or 10 days. After thawing, fat layers were removed from each beef block. The specimens were quickly frozen in liquid nitrogen and stored at ~80 °C until use.

2.2. Free peptide extraction

Approximately 1 g of frozen specimen was immersed in 2 ml of ice-cold 70% EtOH (Merck Ltd., Tokyo, Japan) and quickly homogenised in a BMT-20-G tube with five glass balls (ø6 mm) using an ULTRA-TURRAX® Tube Drive (IKA® Japan K.K., Osaka, Japan) (Kominami et al., 2020). Homogenisation was performed with level 8 of 30 s and repeated three times at 1-min interval on ice. The homogenate was transferred to a new 15 ml tube on ice. The homogenising tube, including glass balls, was washed with 2 ml of ice-cold 70% EtOH, and the wash fluid was combined to the homogenate. After centrifugation at 9200 × g for 30 min at 4 °C, the supernatants were collected and passed through a 5000 MWCO filter (Vivaspin 20–5 K, Sarstedt K.K., Tokyo, Japan). The filtrates were purified using solid-phase extraction cartridges (Oasis® HLB Vac Cartridge, Nihon Waters K.K., Tokyo, Japan). Additionally, the purified extracts were desalted using GL-Tip SDB (GL Sciences, Tokyo, Japan) following the manufacturer’s instructions. The eluted peptides extracts were dried in a vacuum evaporator and dissolved in 0.1% formic acid (FA) (Thermo Fisher Scientific K.K., Tokyo, Japan). The peptide solution was clarified by centrifugation at 12000 × g for 60 min at 4 °C. The supernatant was collected and quantified by measuring the absorbance at 280 nm with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific K. K.). Each peptide sample was diluted to yield a solution with a A280 nm of 0.1.

2.3. Peptidomic analysis

LC-MS/MS analysis of free peptides was performed as previously described in Kominami et al., 2020. Briefly, 2 μl of the peptide samples were separated on an Eksigent cHiPLC® system (AB SCIEX Japan, Tokyo, Japan) equipped with a Nano cHiPLC ChromXP C18 column (75 μm × 15 cm; particle size, 3 μm; pore size, 120 Å; AB SCIEX Japan) and connected to a TripleTOF® 5600 mass spectrometer (AB SCIEX Japan). The peptide samples were analysed by information-dependent acquisition (IDA) and the sequential window acquisition of all theoretical mass spectra (SWATH) acquisition (Kominami et al., 2019).

2.4. Database search and SWATH data processing

IDA MS/MS data were subjected to database searches by ProteinPilot® software v4.5 (AB SCIEX Japan) using the Paragon algorithm. The reference proteome of bovine Bos taurus (Proteome ID: UP000009136) was downloaded from the Universal Protein Resource (https://www.uniprot.org/) (accessed May 2020). The search parameters were as follows: sample type, identification; Cys alkylation, none; digestion, none; special factors, none; ID focus, allow biological modifications.

The group files output from ProteinPilot® software were loaded to PeakView® software version 2.2.0.11391 with SWATH quantitation plug-in (AB SCIEX Japan). Specific tables of precursor masses and fragment ions were created and used to generate MS/MS peak-extracted ion chromatograms (XICs) of the fragment ions of targeted proteins. PeakView® computed a score and an FDR for each assigned peptide using retention time and spectra components; only peptides with an FDR of less than 1% and a confidence level more than 95% were used for quantitation. The peak areas for peptides were obtained by the sum of fragment ion XICs.

2.5. Terminome analysis

Sequence specificity in the peptide terminome was calculated as described previously, with a slight modification (Kominami et al., 2019). The peptides quantified in the SWATH analysis were aligned to the reference protein sequence to determine the cleavage site (P2, P1, P1’ and P2’). The peptides without P2 or P2’, whose N- or C-terminal are mapped to the next to first or last residue in the complete protein sequence, were sorted out as they are considered to be cleaved by
exopeptidases.

The peak area of peptides is summed according to the amino acid in P2, P1, P1', and P2'. Each sum of area $\mathcal{S}_{aa}$ ($aa \in \{P2, P1, P1', P2\}$; $\mathcal{S}_{aa}$ represents amino acid) coordinates a matrix $20 \times 4$. The $\mathcal{S}_{aa}$ is then divided by the frequency of each amino acid in the entire sequence of substrate proteins $X_{aa}$.

$$Y = \begin{pmatrix} 1/\mathcal{S}_{Gly} & 0 & 0 & 0 \\ 0 & 1/\mathcal{S}_{Pro} & 0 & 0 \\ \vdots & \vdots & \vdots & \vdots \\ 0 & 0 & 1/\mathcal{S}_{His} & 0 \end{pmatrix} \begin{pmatrix} P^2_{Gly} & P^1_{Gly} & P^1_{Pro} & P^2_{Gly} \\ P^2_{Pro} & P^1_{Pro} & P_1 & P^2_{Pro} \\ \vdots & \vdots & \vdots & \vdots \\ P^2_{His} & P^1_{His} & P^1_{Gly} & P^2_{His} \end{pmatrix}$$

Let $y_{an}$ be an element in the matrix $Y$.

$$Y = \begin{pmatrix} y^2_{Gly} & y^1_{Gly} & y^1_{Pro} & y^2_{Gly} \\ y^2_{Pro} & y^1_{Pro} & y_1 & y^2_{Pro} \\ \vdots & \vdots & \vdots & \vdots \\ y^2_{His} & y^1_{His} & y^1_{Gly} & y^2_{His} \end{pmatrix}$$

The sum of elements in matrix $Y$ is scaled to 1 as follow.

$$Y = \frac{1}{\sum_{n \in \mathcal{E}} Y_n} Y$$

Specificity matrices representing the cleavage pattern of proteases can be obtained from the MEROPS database (http://merops.sanger.ac.uk/index.shtml). The protease specificity matrix can be obtained from the MEROPS database (http://merops.sanger.ac.uk/index.shtml). The protease specificity matrix from SWATH data, and data visualisation were performed on the proteomic analysis combined with 2D-electrophoresis (Laville et al., 2009). Additionally, proteomic changes combined with meat shear force indicated accelerated degradation of glycolytic enzymes during beef tenderisation (Laville et al., 2009). An increase in the fragment peptides of glycolytic enzymes was also observed in thaw-aged beef (Fig. 1a). Therefore, the glycolytic enzymes, including GAPDH, CK, and β-enolase, were also degraded in the beef during thaw-aging; however, the degradation can proceed in a different manner from post-mortem aging. Ice crystal formation causes intra- and extracellular structural changes, translocation of proteases, and denaturation of proteins in freeze-thawed meat. This should result in different dynamics of protein degradation from those of postmortem aging.

Troponin T and desmin, the key proteins for meat tenderness, were also degraded in the thaw-aged beef (Fig. 1b). The degradation of nebulin, titin, vimentin, and actinin during thaw-aging was also confirmed. As these proteins play a crucial role in maintaining myofibril integrity, their degradation contributes to meat tenderisation (Frontera & Ochala, 2015). Based on our results, extending thaw-aging can promote myofibrillar degradation and lead tenderness of the freeze-thawed meat. Pittner et al. demonstrated a chronological degradation of titin, nebulin, desmin, cardiac troponin T, and SERCA1 in pig limb for up to 10 days postmortem (Pittner et al., 2016). In the present study, most of proteolytic fragments from both myofibrillar and sarcoplasmic proteins simultaneously increased for up to 7 days of thaw-aging. The quantity of most peptides peaked on day 7 of thaw-aging, regardless of protein species. This discrepancy can be attributed to the freezing process. As previously discussed, ice crystal formation during storage via freezing affects the protease activity in meat. The freeze-storing process could perturb the chronological protein degradation observed in post-mortem and accelerate deregulated protein degradation during thaw-aging.

Several taste-active peptides were already found in beef products. For example, the delicious peptide “KGDEESLA” has been isolated from papain treated beef meat (Yamasaki & Maekawa, 1978). In addition, the peptide “APPPPAEVHEVHEVH”, a degraded fragment of troponin T, has been identified as a sourness-suppressing peptide (Nakai, Nishimura, Shimizu, & Arai, 1995). Nakai et al. (1995) found that the peptide “APPPPAEVHEVHEVH” increases in beef longissimus during chilled storage with and without freeze-thaw process. In our results, the delicious peptide “KGDEESLA” was not found; however, the sourness-suppressing peptide “APPPPAEVHEVHEVH” was identified in a thaw-aged beef short plate (Fig. 1b). In accordance with the results of Nakai et al. (1995), the peptide increased as the thawing period increased. In addition, an increase in other peptides containing the sequence “APPPPAEVHEVH” during thaw-aging was found. Okumura, Yamada, and Nishimura (2004) indicated that “APPPPAEVHEVH” is the key sequence for sourness-suppression. The sour-suppressing effects are suggested to be caused by competitive interaction of the peptide with sour taste receptors or sour taste substances (Okumura et al., 2004). The increase in the peptides containing the sequence “APPPPAEVHEVH” during thaw-aging can improve the sensory attributes of freeze-thawed beef. Kitamura et al. (2005) determined the N- and C-terminal sequences of troponin T fragments >25 kDa in aged and calpain-hydrolysed pork and found four cleaved sites: Glu21-Ala22, His37-Glu38, Glu43-Glu44, and Thr51-Ala52. In the present study, 11 cleavage sites in troponin T, including His38-Glu39, were determined de novo (Fig. S1). Kitamura et al. (2010) indicated that cathepsin B and L partially contribute to the degradation of troponin T during postmortem aging of pork. Herein, the peptides derived from troponin T with different sequence patterns in the N- and C- terminals also increased in addition to a significant increase in peptides are summarised in Fig. 1. A total of 114 free peptides were quantified and identified as digests of 28 proteins. Many sarcoplasmic proteins, such as glyceroldehyde-3-phosphate dehydrogenase (GAPDH) and creatine kinase (CK), were identified as degraded proteins in the thaw-aged beef. The degradation of GAPDH, CK, β-enolase, and heat shock protein β-1 was also identified in beef during postmortem aging through the proteomic analysis combined with 2D-electrophoresis (Laville et al., 2009). Additionally, proteomic changes combined with meat shear force indicated accelerated degradation of glycolytic enzymes during beef tenderisation (Laville et al., 2009). An increase in the fragment peptides of glycolytic enzymes was also observed in thaw-aged beef (Fig. 1a). Therefore, the glycolytic enzymes, including GAPDH, CK, and β-enolase, were also degraded in the beef during thaw-aging; however, the degradation can proceed in a different manner from post-mortem aging. Ice crystal formation causes intra- and extracellular structural changes, translocation of proteases, and denaturation of proteins in freeze-thawed meat. This should result in different dynamics of protein degradation from those of postmortem aging.

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the peptide “APPPADEVHEVHEEVH” during thaw-aging. Such finding indicates that troponin T is degraded by various proteases with different active sites. Thus, several protease species would contribute to the degradation of troponin T, and their proteolytic action changes during thaw-aging. Such time-dependent changes in protease contribution should occur not only in the degradation of troponin T, but also in the degradation of other sarcoplasmic or myofibrillar proteins and cause dynamic changes in the meat peptidome. The peptidomic changes could affect sensory attribute of the thaw-aged meat because the meat peptidome may include uncharacterised taste-active peptides. Recent peptidomic analyses of fish meat or scraps identified novel umami peptides (Liu, Zhu, Wang, Zhou, Chen, & Liu, 2020; Shiyan, Liping, Xiaodong, Jinlun, & Yongliang, 2021). Novel taste-active peptides obtained in this study from the beef peptidome should be explored in future work.

3.2. Terminome analysis

Figs. 2 and 3 show the sequence specificity in the terminome of non-myofibrillar and myofibrillar peptides, respectively. The sequence specificity of the overall terminome is shown in Fig. S2. The total cleavage pattern in beef proteins became multifarious as the thaw-aging period increased (Fig. S2). The sequence specificity in the terminome of non-myofibrillar proteins (Fig. 2) also became multifarious and slightly differed from the whole terminome (Fig. S2). There was a general tendency to cleave non-myofibrillar proteins with hydrophilic amino acids at the P2 or P1 position and hydrophobic amino acids at the P1’ or P2’ position (Fig. 2). However, there was a more specific tendency to cleave myofibrillar proteins (Fig. 3). Many proteases that cleave myofibrillar proteins bind to His at the P1 position, Cys at the P1’ position, and/or Pro at the P2’ position. Based on a comparison of cleavage patterns in non-myofibrillar and myofibrillar proteins, proteases, whose P1 active site can bind to His in the S1 subsite and/or its P1’ active site can bind to Cys in the S1’ subsite, can cleave both proteins in the thaw-aging of beef short plate.

A linear combination of $X_{ec}$ expression, the characteristic matrix of the beef terminome on the nth day of thaw-aging, is demonstrated intuitively in Fig. S3. The matrix $Y_n$ calculated from whole, non-myofibrillar, or myofibrillar peptidome on the nth day is shown in the xlsx file (Supplementary Material). In addition, the protease specificity matrix $X_{ec}$ is also attached as the xlsx file (Supplementary Material). Tables S1, 1, and 2 show the estimated proteolytic contribution to the degradation of whole proteins, non-myofibrillar and myofibrillar proteins in beef short plate during thaw-aging. The numbers in Tables S1, 1, and 2 are not indicative of protease activity; instead, they are weights of proteases for expressing the peptide terminome by a linear combination. Based on Table S1, it is suggested that not only calpains, but also other proteases participate in meat proteolysis. Table 1 and 2 indicate the contribution of each protease to the proteolysis changes during thaw-aging and a clear difference is observed between the degradation of non-myofibrillar and myofibrillar proteins. The changes in the contribution of calpains are notable because calpains have been discussed as

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**Fig. 1.** Quantification of the proteolytic peptide generation from non-myofibrillar (a) and myofibrillar (b) proteins in beef short plate during thaw-aging. The data bar indicates the peak area of each peptide. *1 [1Ac] indicates N-terminal acetylation. *2 BLAST search identified titin.
Fig. 2. Sequence specificity in the terminome of peptides derived from non-myofibrillar proteins in beef short plate thaw-aged for 1 (a), 3 (b), 5 (c), 7 (d), and 10 (e) days.

Fig. 3. Sequence specificity in the terminome of peptides derived from myofibrillar proteins in beef short plate thaw-aged for 1 (a), 3 (b), 5 (c), 7 (d), and 10 (e) days.
Table 1
The calculated contribution of each protease to the degradation of non-myofibrillar proteins in beef short plate during thaw-aging.

| Thaw-aging period (day) | 1 | 2 | 5 | 7 | 10 |
|-------------------------|---|---|---|---|----|
| f$_{4.21.1}$             | 0.909094 | 0.126061 | 0.138668 | 0.145303 | 0.127706 |
| f$_{4.21.4}$             | 0.244172 | 0.255843 | 0.226056 | 0.215239 | 0.175831 |
| f$_{4.22.1}$             | 0.180748 | 0.182343 | 0.165635 | 0.174406 | 0.155095 |
| f$_{4.22.15}$            | 5.43E–06 | 3.48E–05 | 0.033828 | 0.017353 | 0.085028 |
| f$_{4.22.16}$            | 0.213317 | 0.184644 | 0.17826 | 0.162481 | 0.262784 |
| f$_{4.22.36}$            | 0.042981 | 0.040404 | 0.040502 | 0.02636 | 0.049416 |
| f$_{4.22.5A53}$          | 0.226878 | 0.178241 | 0.184448 | 0.2034 | 0.146802 |
| f$_{4.22.5}$             | 4.4E–06 | 0.03063 | 0.028079 | 0.037737 | 0.001838 |

Table 2
The calculated contribution of each protease to the degradation of myofibrillar proteins in beef short plate during thaw-aging.

| Thaw-aging period (day) | 1 | 2 | 5 | 7 | 10 |
|-------------------------|---|---|---|---|----|
| f$_{4.21.1}$             | 0.025826 | 0.072913 | 0.088541 | 0.040411 | 0.072724 |
| f$_{4.21.4}$             | 1.49E–05 | 0.038934 | 0.022113 | 6.71E–07 | 1.49E–06 |
| f$_{4.22.1}$             | 9.19E–06 | 3.42E–06 | 1.48E–06 | 3.81E–07 | 9.48E–07 |
| f$_{4.22.15}$            | 0.126891 | 0.212004 | 0.262245 | 0.32018 | 0.271037 |
| f$_{4.22.16}$            | 0.628087 | 0.56852 | 0.544464 | 0.539224 | 0.573889 |
| f$_{4.22.36}$            | 0.057187 | 0.068122 | 0.086262 | 0.071558 | 0.082343 |
| f$_{4.22.5A53}$          | 0.162529 | 0.039502 | 2.79E–05 | 1.66E–06 | 4.99E–06 |
| f$_{4.22.5}$             | 8.0E–07 | 1.35E–06 | 4.97E–07 | 0.025023 | 5.18E–07 |

the leading character in meat proteolysis. The contribution of calpains was found to decrease rather than increase (Table S1). Furthermore, the contribution of calpains to the degradation of non-myofibrillar proteins was constant (Table 1), but it was decreased in the degradation of myofibrillar proteins (Table 2). Such findings do not necessarily mean that calpains are not important in myofibrillar degradation after freeze-thaw. Instead, this finding suggests that proteases, besides calpains, such as cathepsin and caspase, should also strongly contribute to the degradation of myofibrillar proteins after freeze-thaw. Instead, this finding suggests that proteases, besides calpains, such as cathepsin and caspase, should also strongly contribute to the degradation of myofibrillar proteins. These results indicate that non-myofibrillar and myofibrillar proteins clearly differ in their susceptibility to trypsin-like or chymotrypsin-like proteases.

4. Conclusion
Herein, peptidomic analysis of thaw-aged beef short plates was performed using nanoLC-MSMS to assess meat proteolysis during thaw-aging. Many peptides derived from myofibrillar proteins were identified. In particular, several cleavage sites in troponin T were identified, and a deeper insight into the generation process of the taste-active peptide “APPAAPEVEVHEEHV” was obtained. Additionally, terminome analysis revealed the inconstant proteolysis in beef short plate during thaw-aging. Future exploration of the taste-active peptides in beef peptidome or the effects of freezing and/or thaw-aging condition on proteolytic dynamics will further our understanding of meat proteolysis during thaw-aging. Future exploration of the taste-active peptides in beef peptidome or the effects of freezing and/or thaw-aging condition on proteolytic dynamics will further our understanding of meat proteolysis during thaw-aging and allow for the improvement of freeze-thawed meat quality.

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CRediT authorship contribution statement
Yuri Kominami: Conceptualization, Funding acquisition, Project administration, Resources, Investigation, Data curation, Writing – original draft. Tatsuya Hayashi: Data curation. Tetsuji Tokihiro: Data curation. Hideki Ushio: Supervision, Funding acquisition.

Declaration of Competing Interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.jfochms.2021.100051.

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