Effect of Aging Time after Thawing on the Palatability of Frozen Beef

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To determine the effect of aging time after thawing on the palatability of frozen beef, several objective measurements and sensory evaluations were made on beef sirloin samples (the Longissimus thoracis muscle). The samples were aged at 0 ºC for 0, 2, 5 or 10 days after thawing. The following results were obtained:

1) Aging after thawing improved the water-holding capacity, decreased the cooking loss and increased juiciness.
2) The levels of free amino acids increased with aging.
3) The shear force and compression values decreased markedly with aging. In addition, there was a tendency for the structure of the muscle fibers to become fragile.
4) Sensory tests showed that the texture and overall palatability were improved by additional aging after thawing. However, no significant differences were found between thawed beef aged for 5 days and for 10 days.

The results suggest that before cooking, additional aging after thawing would improve beef palatability, with a minimum aging time of 5 days.

The rate of aging in the experimental samples (aged after thawing) was faster than that in the standard samples (aged at 0 ºC without freezing) as evaluated by the level of free amino acids, and the shear force and compression values.

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Keywords: frozen beef, aging time after thawing, texture, water-holding capacity, free amino acids, palatability.

INTRODUCTION

Recent liberalization in the regulations governing the importation of beef has led to a reduction in prices and contributed to an increase in consumption. Frozen beef has the advantage of being inexpensive and can be stored on a long-term basis. However, without sufficient aging, there are several problems concerning its palatability.

Beef is usually aged at a low temperature (0-5 ºC) for about 10 days, and such types of beef as Holstein have been aged for 7-10 days. However, frozen beef is not given sufficient time for aging, since it is usually frozen within 2 days after slaughter. Negishi et al. have reported that most imported frozen beef was unaged, and consumers who purchase such frozen beef usually cook it immediately after thawing.

Although aging is an important consideration in the palatability of beef, most studies on aging have used unfrozen beef. Okitani et al. have reported an improvement in the palatability of frozen beef (−20 ºC) when stored at 0 ºC after thawing, their data being based mainly on enzyme activity. Crouse et al. have demonstrated that aging beef after thawing was useful to both the packer and consumer, because it reduced shear force values. These data are informative, but not truly useful for practical purposes. From a practical point of view, it is necessary to also consider the cooking and sensory properties.

There have been few investigations on the effects of post-thaw aging on palatability. The purpose of this study is to examine the effects of additional aging time after thawing on the palatability of beef associated with its cooking properties, and the usefulness of an additional aging treatment (aging after thawing) before cooking.

MATERIALS AND METHODS

Materials

Sirloin (Longissimus thoracis muscle) samples were obtained from a Holstein steer that was 20 months of
Two days after slaughter, about 17 kg of the sirloin was cut into pieces 1.5 cm in thickness and individually vacuum-packaged. The samples were divided into two blocks, A and B. Block A was immediately frozen at -40°C and stored at the same temperature for 5 days, while block B was aged at 0°C.

Block A was thawed at 0°C for 24 h and then aged at 0°C for 0, 2, 5 or 10 days. These experimental samples were grouped according to their aging time following thawing. After aging for 5 days at 0°C, block B was then frozen to -40°C until the day before examination to provide the standard samples. The standard samples were thawed at 0°C for 24 h before being analyzed.

The experimental plan is shown in Fig. 1. This procedure was repeated three times with samples from two other steers, in which block A was aged for 0, 2, 3, 4, 5 or 10 days, and block B was aged for 5 or 10 days. This other experiment was done to obtain chemical and physical data. After aging or storing, the fat was removed from each sample and lean sirloin was analyzed.

Preparation of the cooked samples

After removing the fat, each sample was cut into pieces (5×5×1.5 cm) and grilled on a hot plate (Sanyo HPS-V95F) at 234±4°C, the sample being turned over every 30 s. The internal temperature of the sample was monitored by a copper-constantan thermocouple placed at the geometric center of the sample and was recorded with a programmable pen recorder (Rikadenki HR-2308). The temperature rose from 15 to 60°C. The raw and cooked weights were measured for each sample and used to calculate the weight loss during cooking.

Analysis of the sample

1. Drip loss

The ratio was calculated for the weight of fluid drained from a raw sample when the vacuum-package was opened to the raw sample weight before vacuum-packaging.

2. Approximate composition and pH

The raw and cooked samples were analyzed for their water, crude protein and crude fat contents by using the vacuum drying, micro-Kjeldahl and Soxhlet methods, respectively. The pH values of raw sample homogenates (about 5 g of a sample homogenized with 10 ml of distilled water at 0°C) was measured immediately after homogenization.

Determination of the water-holding capacity (WHC)

A modification of the method developed by Penny [11] was used. About 3 g of a raw sample (1.3 cm²×1.5 cm) was precisely measured into a centrifuge tube equipped with a stainless steel basket, and centrifuged at 1,700×g and 10°C for 90 min. The ratio between the weight of the precipitate after centrifugation and the raw sample weight before centrifugation was calculated. This is considered to be the water-holding capacity (WHC). The average for 6 samples was calculated.

Determination of the cooking properties

1. Cooking loss

The ratio of the weight of the cooked sample to the raw sample weight before cooking was calculated.

2. Cooking time

This was calculated by using the following equation (Sink et al. [12]):

\[
\text{Cooking time (min/°C/100 g)} = \frac{\text{Final temperature (°C)} - \text{Initial temperature (°C)}}{\text{Uncooked weight (g)/100 (g)}}
\]

Determination of juiciness

Juiciness was determined by the press method, in which a cooked sample (1.0 cm²×1.5 cm) was precisely measured and placed between two sheets of six-ply filter paper (5.5 cm, No. 2 Toyo filter paper). The sample was then compressed to 80% of its initial height by a rheoner (Yamaden RE-3305). Each sample was placed so that the muscle fibers ran parallel to the direction of the plunger (a flat disc 3.0 cm in diameter). The ratio of the weight of the two filter papers after compression to the cooked sample weight before compression was calculated to represent the juiciness of the cooked sample. Juiciness is a measure of the volume of expressible fluids from compressed muscle, and the average for 6 samples was calculated.
Analysis of the umami taste components
1. Free amino acids (FAA)
   About 5 g of each sample was homogenized for 5 min with 10 ml of a 5% sulfosalicylic acid solution. After 1 h, the homogenate was centrifuged at 3,000 rpm for 15 min, the supernatant then being collected and the volume adjusted to 25 ml with distilled water. The resulting solution was stored at -40°C and analyzed within 2 weeks, the FAA content being determined with an amino acid analyzer (Hitachi Model 835).
2. ATP-related compounds
   Preparation of the sample solutions followed the procedure reported by Terasaki et al. Each solution being stored at -40°C and analyzed within 2 weeks. ATP-related compounds were determined by HPLC, using an Asahipak GS-320 column and a 200 nm sodium phosphate buffer (pH 3.0). The flow rate was 1 ml/min, and the ATP-related compounds were detected at 260 nm.

Analysis of physical properties
The raw and cooked samples were trimmed and then measured in the following manner.
1. Shear force (breaking strength)
   At least eight samples (1.0 cm² × 1.5 cm) of raw and cooked beef were analyzed, the shear force being determined by using a rheometer (Yamaden RE-3305). A 20-kg load sensor attached to a plunger was used to drive the blunt edge of a knife blade. Each sample was placed on the stage with the muscle fibers running perpendicular to the direction of the plunger. The plunger was depressed at a rate of 1 mm/s, and transverse sections of the raw and cooked samples were sheared.
2. Compression force (maximum strength)
   At least eight samples (1.0 cm² × 1.5 cm) of raw and cooked beef were analyzed, the compression force being determined by using essentially the same instruments under the same conditions as described for the shear force, apart from a flat round disc (3.0 cm in diameter) being used as the plunger. Each sample was placed with the muscle fibers running parallel to the direction of the plunger and compressed to 80% of the initial height.

Observation of muscle structure by optical microscopy
Small pieces (1.0 cm² × 1.5 cm) were cut from the raw and cooked samples and fixed in 10% buffered formalin (pH 7.0) for about 10 days. Each specimen was then dehydrated in 95% ethanol for 2 days and then by 90% ethanol for 1 day, before being trimmed (according to the method of Hoshino) and embedded in paraffin. Sections were cut to a 3.0-μm thickness, stained according to the Masson trichrome staining method and then observed under a microscope.

Sensory evaluation
The experimental and standard samples were cooked under the same conditions, before being evaluated on the basis of appearance, aroma, tenderness, juiciness, textural palatability, intensity of umami taste, taste palatability and overall palatability, using a seven-point scale. The sensory scores for the experimental samples were compared with those of the standard samples. A trained panel composed of 10 faculty members experienced in food preparation from Kagawa Nutrition University evaluated the samples.

All values presented are the averages from experiments repeated on samples from three different carcasses.

RESULTS AND DISCUSSION
1. Analysis of each sample
   The water, crude protein and fat contents of the samples aged for the experimental periods are shown in Table 1. There was no change in any of these parameters with aging. The pH values of the samples aged after thawing also varied little, these results agreeing with those reported by Shimada et al.

2. Water-holding capacity (WHC) and cooking properties
   WHC of the raw samples, juiciness of the cooked samples and the cooking properties (cooking weight loss and cooking time) are shown in Table 2. WHC was improved with aging after thawing, and the juiciness of the cooked samples was also improved. This suggests that aging after thawing would increase the amount of juice expressed while chewing.
   Most of the water in a muscle cell is found in the space between the thick and thin filaments of the myofibrils. Thus, it seems likely that a change in the water content of meat reflects a change in the volume in the myofibrils. During rigor, the space between the thick and thin filaments decreases, and WHC declines. However, during postrigor, the crossbridges between the thick and thin filaments weaken and WHC improves. Therefore, it seems that WHC plays an important role in the tenderness and juiciness of meat, as well as in the weight loss during cooking.
   A significant correlation between WHC and cooking loss \( r = -0.949 \), and between WHC and juiciness.
measured by objective methods \((r = -0.955)\) was apparent. Juiciness, determined by the press method, showed a positive correlation with juiciness evaluated by the sensory test \((r = -0.976)\).

Required cooking time seemed to be less in the aged samples, our results agreeing with those of Warren et al.\(^1\)\(^8\). These results suggest that there were some changes involving proteins during aging, so we need to study this further.

### 3. Changes in FAA and ATP-related compounds in beef aged after thawing

The FAA content was increased with aging after thawing, and markedly increased in the samples aged for 5 or 10 days after thawing (Fig. 2). Asp, Cys, Met, Leu, Try and Phe were all markedly increased, in accordance with the results from Nishimura et al.\(^8\). The FAA content was similar between the raw and cooked samples. The magnitude of the increase caused by aging after thawing was not offset by a shorter cooking time, suggesting that an increase in the FAA content combined with improved juiciness and acting in synergy with ATP-related compounds (mainly 5'-IMP) may contribute to an improvement in taste palatability. The level of FAA in the samples aged for 5 days after thawing appeared to be higher than that in the standard samples. Thus, the experimental samples showed more rapid aging when compared with the standard samples. The role of peptides\(^19\) in the taste of cooked beef has recently been investigated; however, we didn't measure peptides in this present work, so this should be investigated further.

Figure 3 shows the percentage change in the level of ATP-related compounds in the aged samples compared with the day 0 values. While the IMP level was decreased with aging, about 70% of original IMP remained. Thus, the synergistic effect of Glu and IMP on taste should be considered. Similar results were

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Table 1. Analysis of samples during aging

<table>
<thead>
<tr>
<th></th>
<th>Experimental sample: aging time (days)*</th>
<th>Standard sample**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drip loss (%)</td>
<td>1.23±0.32</td>
<td>1.33±0.41</td>
</tr>
<tr>
<td>Water (%)</td>
<td>67.90±2.74</td>
<td>68.55±2.37</td>
</tr>
<tr>
<td>Crude fat (%)</td>
<td>9.25±3.89</td>
<td>9.88±3.61</td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>13.70±4.89</td>
<td>14.62±5.34</td>
</tr>
</tbody>
</table>

\*Aged at 0°C after thawing. **Aged at 0°C for 5 days.

Table 2. Effects of aging on WHC and cooking properties

<table>
<thead>
<tr>
<th></th>
<th>Experimental sample: aging time (days)*</th>
<th>Standard sample**</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHC (%)</td>
<td>67.57±2.93*</td>
<td>71.63±1.89*</td>
</tr>
<tr>
<td>Juiciness (%)</td>
<td>20.79±1.63*</td>
<td>24.85±1.68*</td>
</tr>
<tr>
<td>Cooking loss (%)</td>
<td>19.03±0.95*</td>
<td>17.90±1.36*</td>
</tr>
<tr>
<td>Cooking time (min/°C/100 g)</td>
<td>0.189±0.020*</td>
<td>0.174±0.019*</td>
</tr>
</tbody>
</table>

Different letters in the same line indicate significant differences \((p<0.05)\). *Aged at 0°C after thawing. **Aged at 0°C for 5 days.
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Fig. 2. Changes in the level of free amino acids (FAA) during aging after thawing (increase in FAA compared with the sample aged 0 days)

Experimental sample aged at 0°C after thawing for 2 days (△), 5 days (○), and 10 days (□). Standard sample aged at 0°C for 5 days (●).

seen with the cooked sample.

4. Changes in physical properties of beef aged after thawing

Table 3 shows changes in the shear force and compression values caused by aging after thawing for both the raw and cooked samples. The shear force value, which is the force required to break the muscle fibers when the plunger sheared the sample perpendicularly to the muscle fibers, indicates the ease with which the muscle fibers could be broken. The compression value, the force required to compress the muscle fibers to 80% of their initial length, indicates the degree of cohesiveness in the muscle structure. Both these values were significantly reduced with aging after thawing, most markedly in the samples aged for 5 or 10 days after thawing. This suggests that tenderness would be improved with aging after thawing. Shear force had a significant correlation with the "ease for biting off with the first

Table 3. Effect of aging on textural properties

<table>
<thead>
<tr>
<th>Shear force value (kg)</th>
<th>0</th>
<th>2</th>
<th>5</th>
<th>10</th>
<th>Standard sample**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw</td>
<td>2.43±0.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.01±0.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.63±0.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.56±0.23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.59±0.36&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cooked</td>
<td>3.28±0.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.06±0.68&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.40±0.56&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.22±0.66&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.60±0.52&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Compression value (kg)</td>
<td>9.13±1.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.96±0.91&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.12±0.81&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.55±0.75&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.32±0.79&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Raw</td>
<td>12.72±1.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.33±1.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.21±1.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.83±1.39&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.89±1.41&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cooked</td>
<td>13.28±1.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.96±1.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.31±1.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.93±1.36&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.12±1.42&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Different letters in the same line indicate significant differences (p<0.05). *Aged at 0°C after thawing. **Aged at 0°C for 5 days.

Fig. 3. Changes in the level of ATP-related compounds during aging after thawing

The increase is shown in comparison with sample aged for 0 days. Aging times (days) after thawing for the experimental samples are indicated in parentheses. std: standard sample aged at 0°C for 5 days. HPLC conditions: Asahipak GC-320 column, 200 mm sodium phosphate buffer (pH 3.0), 1 ml/min flow rate, ATP-related compounds detected at 260 nm.
Fig. 4. Structural changes during aging after thawing

Longitudinal sections of muscle fibers were viewed by optical microscopy (×400) after Masson trichrome staining. Raw sample: ①, experimental sample aged for 0 days after thawing; ②, experimental sample aged for 10 days after thawing; ③, standard sample aged at 0°C for 5 days. Cooked sample: ④, experimental sample aged for 0 days after thawing; ⑤, experimental sample aged for 10 days after thawing; ⑥, standard sample aged at 0°C for 5 days.
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Compression was strongly correlated with tenderness \( (r = -0.980) \), this result agreeing with Shimada et al. Meat generally becomes tough with heating due to the coagulation of proteins. Steak is an example requiring a short-time cooking procedure that does not allow the meat to be tenderized due to the conversion of collagen to gelatin. Therefore, the length of aging of beef after thawing can greatly influence its tenderness.

5. Muscle structure

Structural changes in the samples aged after thawing are shown in Fig. 4. In the longitudinal sections of the muscle fibers, cracks perpendicular to the muscle fibers were increased with aging. In the transverse sections, the area of the muscle fiber was increased with aging, and gaps between the fibers were increased (gaps began to appear 2 or 3 days after thawing). These structural changes indicate that aging after thawing promoted fragmentation and loosening of the muscle fibers. An improvement in the sensory-evaluated meat tenderness was accompanied with these structural changes, as well as a decrease in the shear force and compression values.

6. Changes in the sensory properties of cooked samples of aged beef

Figure 5 shows changes in the sensory properties of the cooked samples. Beef palatability (tenderness, juiciness, texture, taste palatability, and overall palatability) were improved with the progress of aging time after thawing.

Textural parameters such as tenderness and the “ease of biting off with the first contact by the teeth” both showed a strong correlation with the overall palatability \( (r = 0.996 \text{ and } r = 0.982, \text{ respectively}) \). With Holstein steers, tenderness has been demonstrated to influence the overall palatability score. This accords with our findings. Juiciness had a high correlation with textural palatability \( (r = 0.994) \), which is considered to be related to structural changes such as myofibrillar fragmentation and improved retention of meat juices. Juiciness was also correlated strongly with the intensity of umami taste and taste palatability, as well as with overall palatability. Thus, juiciness appears to be related to the retention of umami taste, leading to an improvement in taste palatability.

There was no significant difference in overall palatability between the 5- and 10-day-aged samples. This suggests that a minimum aging time of 5 days after thawing is required to improve frozen beef palatability.

7. Comparison between the rate of aging in the experimental samples and standard samples

The rate of aging in the experimental samples (aged for the same period of 5 days as with the standard samples) seemed to be higher than in the standard samples, so we compared the two conditions.
The results show that the rate of aging in the experimental samples was higher than that in the standard samples on the basis of free amino acids levels, and shear force and compression values.

CONCLUSION

This study examined the effects of aging time after thawing on the palatability of frozen beef.

The results indicate that aging after thawing improved beef palatability when aged for a minimum of 5 days. The same concept may also be applicable to other types of cooked meat.

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REFERENCES