Heat transfer analysis-based prediction of protein denaturation and umami component of meat during cooking
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ABSTRACT
The physical properties and eating quality of cooked meat are strongly affected by both the degree of protein denaturation and the amounts of Inosinic acid (IMP) which is famous as the major component of umami taste resulting from the difference temperature treatments. The objective of this work was to simulate changes in both protein denaturation and the amount of IMP by a 3D finite-element method. We targeted the vacuum-pack cooking (sous-vide) that is frequently used by professional chefs. Roast beef was cooked in the laboratory according to the sous-vide method, and we corrected the temperature history and measured the weight and size of the meat. In order to simulate changes in both protein denaturation and the amount of IMP during cooking, it is necessary to obtain the kinetics parameters. The kinetic parameters of protein denaturation were measured by DSC. IMP in the meat was quantified by HPLC. The temperature dependency of the IMP decomposition reaction was also examined in an isothermal heating experiment. DSC curve shows two endothermic peaks; myosin and actin of the fibrillar protein. The amount of remaining IMP decreased with increasing heating time at every heating temperature. However, for samples heated above 40°C, the amount of IMP was lower than that at 40°C, possibly because the activity of the enzyme that decomposed IMP had decreased. The shape model similar to the sample cooked in the laboratory was made, and the three-dimensional heat conduction analysis by the finite element method was done. Calculated temperature distribution of the meat during cooking was good agreement with that of actual cooking experiment. The protein denaturation ratio and IMP remaining ratio were able to be predicted by using the measured kinetics parameters based on the calculated temperature history.

Keywords: protein denaturation; Inosinic acid; sous-vide cooking; heat transfer analysis

INTRODUCTION
In meat cooking, various reactions may occur simultaneously with heat and mass transfer. The physical properties and eating quality of cooked meat are strongly affected by both the degree of protein denaturation and the amounts of umami components resulting from the difference temperature treatments. Inosinic acid (IMP) is famous as the major component of umami taste. It seems that the IMP content of meat is affected by the heating temperature during cooking, because IMP is decomposed by the enzyme originally present in the meat. However, there have been no reports predicting the changes in protein denaturation and umami component in accordance with heat transfer during meat cooking. The objective of this work was to simulate changes in both protein denaturation and the amount of IMP by a 3D finite-element method. We targeted the vacuum-pack cooking (sous-vide) that is frequently used by professional chefs.

MATERIALS & METHODS
Kitchen experiment: Roast beef was cooked in the laboratory according to the sous-vide method. First, the surfaces were browned in a fry pan. Next, it was stored in a refrigerator until its core temperature was 10°C or less. Next, the meat was placed in a plastic bag and vacuum-packed using a vacuum-packing machine, and it was then cooked at 80°C in a water bath. When the temperature of the core reached 58°C, it was immediately transferred to a water bath set to 2°C, and cooled until the core temperature dropped to 3°C. This stage was assumed to be the end point of the sous-vide cooking. During cooking, we corrected the temperature history for both the core and surface, and measured the weight and size of meat.

DSC measurement: Sliced round beef purchased from a supermarket was used. Meat samples of about 15mg were placed in aluminum sample pans (KIT NO, 0219–0041) and hermetically sealed. Then, the
samples were heated from 25°C to 110°C at different heating rates (β) (β = 5, 10, 15, 20°C/min) as measured by a DSC instrument (Perkin Elmer Pyris1). The peak temperatures (T_max), which were observed in the DSC curve, were determined for each run. The reference was an empty pan.

**Isothermal heating experiment for IMP analysis:** Sliced round beef was used. Meat samples were vacuum-packed, and they were heated in a water bath at a target temperature (30, 32, 35, 38, 40, 42, 45, 50, 55 and 60°C) for 15, 30, 45 and 60min. After heating, samples were quenched in iced water until they reached 20°C and then subjected to a chemical analysis.

**Quantification of IMP:** The raw and heated samples were prepared by following method. The sample (2g, after heating weight) was homogenized with 5ml of a 10% perchloric acid (PCA). The homogenate was centrifuged (14,000×g at 4°C) for 10min, and the supernatant was collected. After removing the proteins from this supernatant by adding 5ml of a 10% PCA and centrifuging at the same condition, the supernatant was collected again. The obtained supernatants were combined, and the volume was adjusted to 25ml with 10% PCA. Then, after adjusting to pH7.0 with 10M-KOH and 1M-KOH, it was centrifuged (16,000×g at 5°C) for 2min and the supernatant was diluted 10-fold with distilled water. This sample solution was used for determining Inosinic acid (IMP). IMP in the meat was quantified by HPLC, using an Asahipak GS-320HQ column and a 0.2M sodium dihydrogen phosphate buffer (pH2.9). The flow rate was 0.6ml/min, and IMP was detected at 260 nm.

**Three-dimensional heat transfer analysis:** An unsteady-state 3D heat transfer analysis was conducted. The boundary condition was assumed that the sample surface immediately reaches an ambient temperature, because it was regarded that the thermal resistance of packing film was extremely small and heat transfer coefficient was large enough. Moreover, in order to calculate it, the Galerkin finite element method was used. A computational domain was created with each element using a FEMAP. In order to render a faithful simulation, the length and the volume after model for simulation was adjusted to the experimental one.

**RESULTS & DISCUSSION**

**Sous-vide cooking:** The core of the meat took 41 minutes to reach the target temperature of 58°C, although the temperature of the surface rose to the ambient temperature immediately. When the meat was transferred to a water bath for cooling, the core temperature did not immediately fall, but instead rose to 62°C. After this rise, the temperature decreased slowly. The changes in the size and weight of the meat before and after cooking are shown in Table 1. The change in both width and height was smaller than in length. That is, shrinkage of the meat was confirmed only in the direction of length. Moreover, the weight-loss was only 15%. This value is smaller than the general cooking method. It is estimated to be 20% or higher.

<table>
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<th>Table 1. Size and weight of meat sample before and after cooking</th>
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<td>Before cooking</td>
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<td>Change of ratio (%)</td>
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**DSC measurement and kinetics of thermal denaturation of meat protein:** The sample thermogram shows two endothermic peaks. From published literature, the first peak corresponds to myosin and the second one to the actin of the fibrillar protein. When the heating rate was 10°C·min⁻¹, the maximum peak temperature (T_max) of each endothermic peak was 56.3°Cand 73.2°C. As the heating rate of the scans increased, each T_max shifted to a higher temperature. When the thermal denaturation rate of meat protein is assumed to be proportional to the concentration of non-denatured protein, (that is, a first-order reaction), the reaction kinetic equation is given by Eq. (1), where k is the rate constant of reaction. Integration of the differential equation Eq. (1) gives Eq. (2), where C₀ is the concentration of non-denatured protein at initial and Cᵣ is that at time t, and X is the non-denaturation ratio, which is a dimensionless parameter. Temperature dependency of the reaction rate constant k is represented by the following Arrhenius equation, which is shown by Eq. (3). Where E_a is the activation energy, R is the gas constant, and T is the temperature.

\[
\frac{dC}{dt} = -kC
\]  (1)
\[ \ln \left( \frac{C}{C_0} \right) = \ln X = -kt \quad (2) \]
\[ k = Z\text{e}^{\frac{E_a}{RT}} \quad (3) \]

These Arrhenius parameters were estimated using the Ozawa method, which is conducted by DSC in nonisothermal conditions. The relation between the heating rates \( \beta \) and \( T_{\text{max}} \) was observed from the DSC measurement, and is shown by Eq. (4).

\[ \ln \left( \frac{\beta}{T_{\text{max}}} \right) = \ln \left( \frac{ZR}{E_a} \right) - \left( \frac{E_a}{RT_{\text{max}}} \right) \quad (4) \]

The plots according to Eq. (4) give a straight line whose slope is used to determine the activation energy, and intercept cloud is used to determine the pre-exponential factor. The calculated changes of non-denaturation ratio of each protein during heating from 25°C to 85°C at a rate of 10°C \cdot \text{min}^{-1}, it turned out that actin doesn’t begin to denature, even when the temperature rises to around 65°C, while myosin began to denature at around 35°C. Moreover, the denaturation of myosin has already finished at around 65°C. In other words, the denaturation temperature was different depending on the kind of the protein.

**Changes in the amount of IMP in the meat:** The amount of remaining IMP decreased with heating time during the isothermal experiments. However, as for the sample heated above 40°C, the amount of remaining was lower than that at 40°C, possibly because the activity of enzyme that decomposed IMP had decreased. From published literature, when the meat of pH 6.0 is heated at 50-54°C, the activity of enzyme becomes 50% or less. In other words, in order to calculate the amount of remaining of IMP, it is necessary to consider the enzymatic activity in IMP decomposition reaction.

**Kinetics of the IMP decomposition reaction with the enzyme activity:** When the IMP decomposition reaction rate including the enzyme activity is assumed to be proportional to the concentration of remaining IMP of meat, (that is, a first-order reaction), the reaction kinetic equation is given by Eq. (5), where \( k \) is the rate constant of reaction and \( A_E \) is a dimensionless parameter for the enzyme activity. \((0 \leq A_E \leq 1.0)\) When the heat treatment temperature is 40°C or less, it can be assumed that the enzyme activity \( A_E \) is constant. That is Eq. (5) gives Eq. (6), moreover, integration of the differential equation Eq. (6) gives Eq. (7), where \( C_0 \) is the initial amount of IMP in row meat and \( C_t \) is that at time \( t \), and \( X_t \) is the remaining IMP ratio, which is a dimensionless parameter. The plots experimental value according to Eq. (7) give a straight line whose slope is used to determine the rate constant of reaction at heating temperature. Then, the activation energy and the pre-exponential factor of the IMP decomposition reaction were determined by using the Arrhenius plot.

\[ \frac{dC_\perp}{dt} = -kC_\perp A_E \quad (5) \]
\[ \frac{dC_i}{dt} = -kC_i \quad (6) \]
\[ \ln \left( \frac{C_t}{C_0} \right) = \ln X_t = -kt \quad (7) \]

However, it is necessary to consider the decreasing of enzyme activity during heating temperature above 40°C. When the enzyme activity decrease reaction rate is assumed to be proportional to the remaining enzyme activity at time \( t \), (that is, a first-order reaction), the reaction kinetic equation is given by Eq. (8), where \( k_0 \) is the rate constant of reaction. The rate constant when the difference between the calculated changes of remaining IMP ratio that assumed that the enzyme activity doesn't decrease and experiment value is the smallest was estimated by the golden section method. Then, the activation energy and the pre-exponential factor of the enzyme activity decrease reaction were determined by using the Arrhenius plot.
Three-dimensional heat transfer analysis and prediction of protein denaturation and remaining IMP of meat during cooking: Figure 1 shows the comparison between measured value and the calculated temperature history of meat at the core. The ambient temperature was used 80°C for the heating period and 2°C for the cooling period. The change in the temperature was good agreement between the measured and calculated. Next, the changed of protein denaturation and the remaining IMP were simulated based on the calculated temperature history.

![Figure 1. Comparison between measured and calculated temperature history of meat at the core during sous-vide cooking.](image)

【Protein denaturation distribution in meat】 As for the myosin, the thermal denaturation progress at 60°C or less, and the non-denaturation ratio have already reached 0 at the end of the heating period during cooking. In other words, denaturation of myosin was completed throughout the sample. However, it is understood that actin hardly denatures on the inside, although denaturation was completed only at the surface region.

【IMP distribution in meat】 The remaining IMP ratio at the surface region was almost the same as the initial at the end point of the sous-vide cooking, because the sample surface immediately reaches an ambient temperature which is deactivation temperature for the IMP decomposition enzyme. However, it turned out that the remaining IMP ratio on the inside was about a half on the surface, because the enzyme activity decreases little by little.

CONCLUSION

The distribution of protein denaturation and IMP in meat during sous-vide cooking were analyzed. It was clearly shown that a feature of sous-vide cooking was that the internal actin hardly denatures, although whole proteins complete denaturation on the surface region. Moreover, in the central of meat IMP decreased to half of its initial value, although that remained height at the surface region.

REFERENCES