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# Estimation of color changes in fish surface at the beginning of grilling based on the degree of protein denaturation

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ABSTRACT

Color changes at the beginning of grilling were estimated from protein denaturation. Samples of red sea bream (*Pagrus major*) were grilled under far-infrared radiation heating. The temperature and color (CIE $L^*$, $a^*$, and $b^*$ values) of the surface of the samples were monitored over time. Protein denaturation was evaluated by differential scanning calorimetry. Two peaks, which were regarded as denaturation of myosin and actin, were observed. Kinetic parameters ($E_a$ and $Z$) were calculated using the dynamic method with five heating rates (8, 10, 13, 17, and 20 °C $min^{-1}$). Measured $L^*$ value increased as protein denaturation progressed in the first 2 min of grilling. The total non-denaturation ratio using the contributions of 0.70 and 0.30 of myosin and actin, respectively is regarded as the best fit to the changes of $L^*$. Empirical equations for estimating $a^*$ and $b^*$ values were deduced based on calculated $L^*$ values and gave good approximations to the measured ones.



## 1. Introduction

Heating provides fish muscle with acceptable texture through protein denaturation. The denaturation of proteins reduces the water-holding capacity of muscle, causing shrinkage of muscle fibers and thus a harder, more compact tissue texture (Harris and Shorthose, 1988). Because excessive heating or overcooking spoils nutrients, color, and other attributes of quality, the appropriate degree of heating for the grilling process should be determined. For this purpose, information on the heat denaturation kinetics of fish muscle is important for designing the heat treatment conditions to manufacture high-quality products.

Thermal denaturation of muscle proteins such as myosin, sarcoplasmatic proteins plus collagen, and actin occurs at different temperatures (Bertram et al., 2006; Findlay et al., 1986; Gravier et al., 2006). To describe the extent of denaturation during thermal processing, the temperature dependence of the denaturation reaction rate constant must be known. Moreover, it is important to identify the factors affecting the rate constant and its temperature dependence for the design and optimization of product quality. Thermal denaturation, which involves unfolding of protein molecules, is attributed to the rupture of intermolecular hydrogen bonds and is accompanied by uptake of heat, seen as an endothermic peak in the differential scanning calorimetry (DSC) curve. The denaturation temperature of each protein and its kinetics, obtained by the DSC method, have been previously reported (Bertola et al., 1994; Findlay et al., 1989; Hastings et al., 1985; Ishiwatari et al., 2013; Kajitani et al., 2011; Thorarinsdottir et al., 2002; Wagner and Anon, 1985). In DSC measurement, the denaturation temperature is a measure of the thermal stability of the protein, and the enthalpy value, calculated from the endothermic peak, is correlated with the net content of the ordered secondary structure of the protein.

DSC has been used to study the thermal properties of fish muscle proteins and to measure the extent of denaturation under various processing conditions. Such studies have evaluated the thermal denaturation of hake myofibrillar proteins (Beas et al., 1990), the effect of processing and species variation on stability of muscle proteins (Hastings et al., 1985), kinetics of thermal denaturation of proteins in anchovy muscle (Llave, 2012), changes in protein fractions of rainbow trout gravads during production and storage (Michalczyk and Surowka, 2007), methodology of determining the ratio of myosin and actin in cuttlefish and squid (Mochizuki et al., 1995), applications of DSC to fish and fishery products (Schubring, 2009), kinetics of heat denaturation of proteins from Atlantic cod (Skipnes et al., 2008), and the effects of salt-curing, drying and rehydration on muscle proteins during processing of salted cod (Thorarinsdottir et al., 2002).

Many studies have shown that protein denaturation causes structural changes in meat and affects its physical properties such as water-holding capacity, texture, and color (Bendall and Restall, 1983; Bengtsson et al., 1976; Garcia-Segovia et al., 2007; Kong et al., 2007; Palka and Daun, 1999; Tornberg, 2005). However, in

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**Nomenclature**

| | |
|---|---|
| $a^*$ | fish surface color position on red/green axis (–) |
| $b^*$ | fish surface color position on blue/yellow axis (–) |
| $c$ | contribution of protein to color change (–) |
| $C$ | concentration of non-denatured protein (mol/g-$_{meat}$) |
| $E_a$ | activation energy (kJ mol$^{-1}$) |
| $k$ | rate constant of reaction (min$^{-1}$) |
| $L^*$ | fish surface color lightness (–) |
| $p$ | fraction of protein (–) |
| $R$ | molar gas constant (J K$^{-1}$ mol$^{-1}$) |
| $t$ | grilling time (s) |
| $T$ | surface temperature (°C) |
| $T_{max}$ | maximum peak temperature (°C) |
| $X$ | non-denaturation ratio (–) |
| $X_L$ | total non-denaturation ratio according to color changes (–) |
| $X_{tot}$ | total non-denaturation ratio according to DSC (–) |
| $Z$ | pre-exponential factor of the Arrhenius equation (min$^{-1}$) |

*Greek symbol*

| | |
|---|---|
| $\beta$ | heating rate (°C min$^{-1}$) |

*Subscripts*

| | |
|---|---|
| 0 | initial |
| 1 | myosin |
| 2 | actin |
| $b$ | during browning color formation |
| $c$ | calculated |
| $m$ | measured |
| $M$ | maximum |
| $up$ | step characterized by an increase in $L^*$ value |
| $tot$ | total |
| $down$ | step characterized by a decrease in $L^*$ value |

spite of some recent works that reported on the color changes of fish during the grilling process (Llave et al., in press; Matsuda et al., 2013; Nakamura et al., 2011), the relationship between the progressive color changes during grilling and the progression of protein denaturation of the muscle, affected by the heat applied, at the beginning of the grilling process remains unclear.

Analysis of the color changes of fish during the grilling process is necessary to determine the degree of cooking required for effective cooking, and thus to obtain grilled fish with attractive colors. Since color changes during grilling involve four steps: (1) protein denaturation, (2) water evaporation, (3) a browning reaction, and (4) a carbonization reaction (Nakamura et al., 2011), this study aims to estimate the surface color changes of fish during the beginning of grilling from the degree of protein denaturation. DSC measurements were performed to obtain information on the thermal protein denaturation behavior of fish muscle, and the denaturation degree was compared and correlated with color changes during the beginning of the grilling process for accurate color prediction.

## 2. Materials and methods

### 2.1. Raw material

Red sea bream (*Pagrus major*) was used for the samples in this study because it is a fish species commonly used for grilling. It was purchased from a fish market on the day of the experiment. The skin and bones were removed, and the ordinary muscle of the fillets was cut into 4 × 5 × 2-cm pieces for all experiments. The samples were wrapped in wrapping film and refrigerated at 5 °C for no more than 30 min before use.

### 2.2. Moisture and crude fat contents

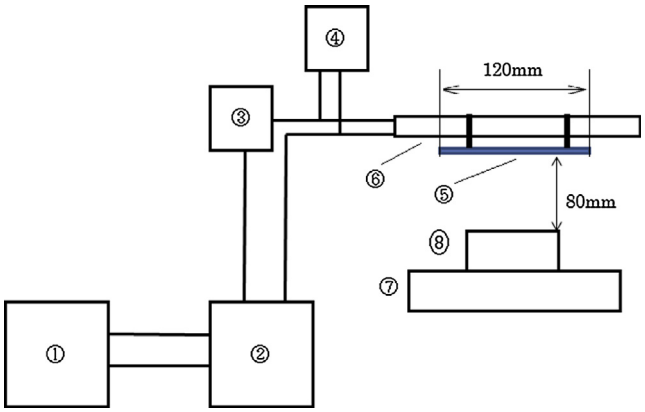
The moisture and crude fat contents of fish samples were quantified. Sample pieces were ground with a mortar and pestle. Crude fat content was determined by the Bligh & Dyer method using a 10-g portion of the ground sample. Moisture content was determined on a wet basis by drying a 5-g piece of the ground sample at 40 °C to a constant weight by the gravimetric reduced pressure method at 0.09 MPa. The result was expressed as the mean value of at least five samples, excluding the maximum and minimum values.

### 2.3. Experimental conditions

A schematic diagram of the far-infrared (FIR) experimental apparatus used in this study, which was a manually assembled laboratory-scale oven, is shown in Fig. 1. The FIR heater (100 V/750 W; electric ceramic plate heater PLC-328, Noritake Co., Aichi, Japan) was 12 × 12 cm. The infrared energy radiated downward from the heater. The samples were positioned approximately 8 cm below the heat source on an electronic balance. The radiation energy, measured by a radiation sensor (RF30 Captec, Villeneuve d'Ascq, France) at the same sample position, was 2.4 × 10$^4$ W m$^{-2}$. Sampling of the grilled fish was conducted at prescribed times of 0, 20, 30, 40, 60, 80, 100, 120, 240, 360, 480 and 600 s.

### 2.4. Temperature measurements

The surface and center temperatures of the samples were measured using a K-type thermocouple ($\phi$ = 0.5 mm). The heater temperature was also measured using a K-type thermocouple ($\phi$ = 2.0 mm). A personal computer, a datalogger (Thermodac 5001A, Eto Denki Co., Tokyo, Japan), and software (Thermodac-E/Ef 2.6, Eto Denki Co.) were used to collect the temperature data.




1. Power supply-unit (100V). 2. Power controller. 3. Ammeter. 4. Voltmeter. 5. Infrared heater. 6. Heater holder. 7. Electronic balance. 8. Sample

**Fig. 1.** Experimental grilling apparatus of the FIR heater.