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Determination of the Maximum Internal Temperature of Previously Heated Saury (*Cololabis saira*) Meat

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Attempts have been made to assess the maximum internal temperature (MTT) of heated saury ground meat. 80 g of meat was heated at selected temperatures with different holding times. Proteins were extracted with 0.9% NaCl, then prepared a visually clear filtrate. It was possible to detect the MIT of previously heated saury meat up to 65°C by coagulation method and 70°C by SDS-PAGE and DSC analysis. No coagulum was observed in filtrates prepared from 70°C heated meat and this might be due to the insufficient concentration of soluble proteins. Raw meat showed a wide variety of protein bands in SDS-PAGE with molecular weight between 19 and 53 kDa. However, only a single protein band with molecular weight of 33 kDa was detected at 65°C which could be responsible for producing coagulum at 65°C. All protein bands disappeared at 70°C. Saury raw meat had four endothermic DSC peaks. As the temperature of heat treatment increased, peaks gradually disappeared and thermogram of meat previously heated at 70°C was featureless. Results of SDS-PAGE, protein solubility, and DSC were closely related with the results of coagulation method.

INTRODUCTION

Products which do not require further cooking must be processed to adequate temperatures to destroy all harmful microorganisms before storage, shipping and marketing[1]. It is well recognized around the world that the issue of food safety is becoming one of the more intense scientifically, technically and institutionally debated topics today. Microbial concerns that are sometimes associated with sea foods on various viruses, such as hepatitis and Norwalk, or microbial pathogens[2]. The combined threat of food-borne illness from pathogens such as *Salmonella*, *Escherichia coli* 0157:H7, and *Listeria monocytogens* demand effective testing methods to insure that these microorganisms do not enter the food supply. To reduce the risk of infections from heat-labial pathogenic microorganisms, heating regulation have been established for the meat processing industry by United States Department of Food Safety and Investigation Service[3].

Heat treatment of processed products is important not only for reducing the risk of infection from heat-labile pathogens but also for improving the shelf life,

and produce of a palatable product[4]. For meat products, several research efforts have been focused on development of new and/or improved methods for assessing the extent of heat treatments (maximum internal temperature) of various meat products[5]. However, similar research on processed fish and fishery products has been negligible. Additionally, there is no official method available for determining the final cooking temperature of processed marine products. Recently Uddin *et al.*[6] successfully determined the previous processing temperature (up to 67°C) of blue marlin meat as a marine fish and that is scarce information about that vast resource. Marine products have an ever-growing role as a human food source, therefore, it becomes important to achieve adequate heat treatment during processing. From that point of view, the MIT of saury meat, which was heated at different time/temperature combinations, was assessed

EXPERIMENTAL

Fish meat: Frozen whole saury *Cololabis saira* was obtained from Shiogama, Miyagi Prefectures, Japan. Prior to keeping at –28°C, the fish were frozen at –40°C in the boat shortly after being caught. The fish were more or less of uniform size (30 cm) and average body weight was 160 g.

Heat treatment: Prior to pre-heat treatment, the fish were thawed overnight at 5°C and ground meat was prepared with Kitchen Aid FMI-KSSS grinder (Kitchen Aid Inc., Michigan, USA) through a grinder plate with 4.7 mm holes. 80 g of ground meat was wrapped with polyvinylidene chloride film and immersed in a stirred water bath. Samples were heated at 5°C intervals between 50 and 70°C. The temperature was monitored by using a recorder (Thermodac EF, Model 5020A, Eto Denki Co., Tokyo) with thermocouples (copper-constantan) inserted through the side edge of the meat blocks to its approximate geometric center. Samples were maintained at each temperature for either 0, 5, 15 or 30 min, then removed and immersed in ice-cold water for rapid cooling for about 30 min. All heated samples were packed in different polyethylene bag and stored at –40°C until tested.

Preparation of Filtrate: Proteins were extracted from 50 g of each heated meat with a 0.9% NaCl solution and were homogenated (12,000 rpm) for one min using a homogenizer (Model HG3O, Hitachi Co.), then allowed to stand for 20 min, followed by centrifugation (5,500 g for 20 min). The supernatant was filtered in accordance with the standard Food Safety and Inspection Service "Coagulation Test" procedure[7]. Visually clear filtrate was used for coagulation test and SDS-PAGE.

The pH values of the primary extracts and of the filtrates before and after filtration treatment were determined by using a pH meter (Model HM-3 V, Toa Co.). Protein concentration was determined by the biuret procedure of Gornall *et al.*[8] Crystalline bovine serum albumin (Sigma Chemical Co., St. Louis, MO) was used for preparation of the standard curve.

Determination of coagulation temperature: The coagulation temperatures of the filtrates were determined by the USDA, Food Safety and Inspection

Service (FSIS) "Coagulation Test" procedure (USDA-FSIS, 1986). The determination for each sample was replicated three times.

Sodium Dodecylsulfate Polyacrylamide Gel Electrophores (SDS-PAGE): SDS-PAGE was performed by using the method of Laemmli[9]. The amount of protein applied was 25 μg per lane. Electrophoresis was carried out on a Biocraft vertical gel electrophoresis apparatus using 12.5 % polyacrylamide gels at a constant current of 10 mA with 0.05 M Tris-0.384 M glycine-0.1 % SDS (pH 8.3) as a running buffer. A molecular weight standard mixture (Sigma Chem. Co.) composed of bovine serum albumin (66 kDa), egg albumin (45 kDa), glyceraldehyde-3-phosphate dehydrogenase (36 kDa), bovine carbonic anhydrase (29 kDa), bovine pancreas trypsinogen (24 kDa), soybean trypsin inhibitor (20 kDa), and bovine milk α -lactalbumin (14.2 kDa) was used. Proteins were stained with Coomassie Brilliant Blue R250 (Wako Pure Chemical Industries, Ltd.), and scanned at 580 nm with a dual wavelength flying spot scanning densitometer (Type CS-9300 PC, Shimadzu Corp.).

Differential Scanning Calorimetry (DSC): Differential scanning calorimetry of saury meat was performed on a Perkin-Elmer Model DSC-7 (Perkin-Elmer Corp.) over 30–90°C range at heating rate of 5°C/min. Nitrogen was used as the purge gas. Sample weight ranged from 13 to 15 mg and the samples were hermetically sealed in aluminum pans to avoid moisture loss during heating. An empty pan was used as the reference. The determination of each sample was repeated three times.

RESULTS AND DISCUSSION

The coagulation test claims that MIT can be estimated by observing coagulation (protein precipitation) during reheating of a filtered extract of heated fish meat[6] and also revealed the sample preparation methods (block, ground and homogenized meat) and holding times had no significant effect on the MIT up to a certain temperature level[5, 6]. Several researchers reported that the coagulation temperature of the protein filtrate extracted with 0.9% NaCl was more closely correlated with the actual cooking temperatures of heated fish meat[10], and other meat products. Therefore, 0.9% NaCl was used for extraction of proteins from heated saury ground meat.

Table 1 shows the coagulation temperature of proteins extracted from saury ground meat. The coagulation temperatures of filtrate from the samples of 0, 5, 15 and 30 min holding times at 50, 55, 60, and 65°C corresponded fairly well with the actual heat treatments. However, the relationship between preheating temperatures and coagulation temperatures was rather poor when preheated temperatures were higher than 60°C. This could be because of lower extractability of soluble proteins from saury ground meat as a highly fatty fish but the results are quite comparable with the results of heated blue marlin[6] and hake meat[13]. No coagulum was observed from filtrates prepared from 70°C heated meat and this might be due to the insufficient concentration of soluble proteins which could not produce a visible coagulum. Similar observations were also reported in fish meat by Huang *et al.*[14] and others[15]. It should be noted that there are an inverse

relationship between protein solubility and higher temperature, additionally the soluble extracted proteins are responsible for coagulation. At 70°C heated meat most of the protein could lose its solubility. However, the best correspondence between MIT and coagulation temperature of the filtrates was obtained in 55 and 60°C heated meats which are in good agreement with Doesburg and Papendrof[13] observed in hake (*Merluccius capensis)* meat.

Weakness in this coagulation method includes the difficulty of obtaining a clear filtered solution without removal of most of the protein, and the subjective assessment of the coagulation point as the temperature is increased. However, in this study, successfully prepared a visually clear filtrate which was applied for determining coagulation temperature, by using together with centrifugation, filter paper, and celite pad. To minimize the subjectiveness of the coagulation test, protein concentration and their SDS-PAGE were also evaluated.

The biuret protein method has been used to determine the relationship between protein content of the filtrate and MIT in this study. Soluble protein content in

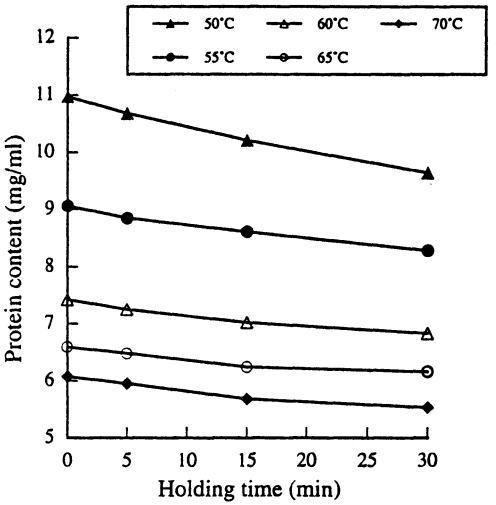

Fig. 1. Changes in protein content of the filtrates prepared from saury ground meat heated at various temperatures with holding times.

0.9%.saline solution gradually decreased when the MIT increased and the major decrease occurred between 50°C and 60°C (Fig. 1). Also there was an inverse relationship between soluble protein content and MIT. These results are closely correlated with the results of coagulation method (Table-1). Decrease rate of soluble protein was slow after 60°C heated samples because the major part of soluble proteins already solubilized between 50 and 60°C. Similar protein decreasing rates were also reported in meat products by Parsons and Patterson[16] and Townsend *et al.*[17, 18]. In all cases, when the protein content of the filtrate was lower, the coagulation temperature of the filtrate was higher. Comparable

observations were also reported in meat products by Townsend *et al.*[17, 18], Davis *et al.*[19] and Popescu and Din[20]. The pH values were also measured in both primary extracts and filtrates, but there were no significant changes (data not shown).

TABLE 1
COAGULATION TEMPERATURE OF PROTEINS EXTRACTED FROM SAURY GROUND MEAT WHICH WAS HEATED AT 50, 55, 60, 65 AND 70°C WITH DIFFERENT HOLDING TIMES

Holding time (min)	Minimum and maximum coagulation temperature after heat treatment at				
	50°C	55°C	60°C	65°C	70°C
0	48.1–48.2	53.1–53.4	60.4–60.7	65.7-66.0	No coagulation*
5	48.1–48.3	53.3–53.4	60.8–61.2	66.2–66.6	No coagulation*
15	48.5–48.8	53.6–54.0	61.5–61.6	66.8–66.9	No coagulation*
30	49.0–49.3	54.3–54.6	61.8–62.0	67.0–67.1	No coagulation*

*No coagulum observed up to 80°C.

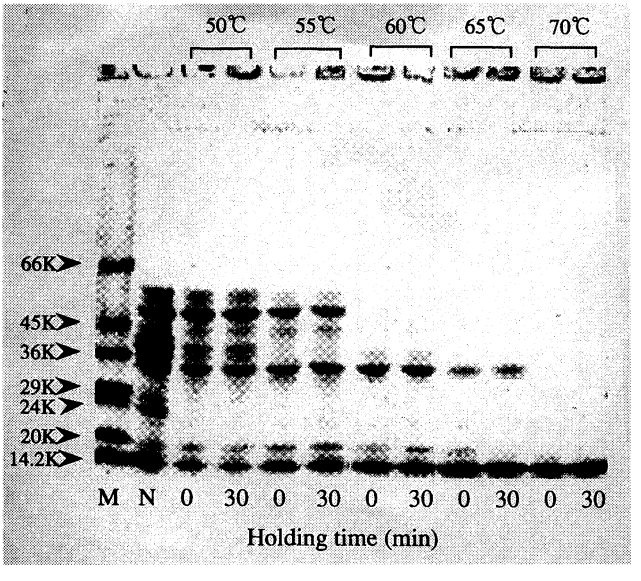

Fig. 2. SDS-PAGE patterns of saury muscle proteins extracted by 0.9% NaCl heated at different temperatures with holding times. (M: molecular weight marker; N: meat without heating).

SDS-PAGE was employed in this study as a tool to accomplish electrophoretic separation of proteins solely on the basis of molecular weight, as well documented by Laemmli[9]. An advantage of using SDS-PAGE is to obtain constant electrophoretic mobilities of proteins, independent of isoelectric point and amino acid composition, which can be slightly modified during cooking[21]. Fig. 2 shows SDS-PAGE patterns of the proteins extracted from heated saury ground meat. In

fresh meat, a wide variety of protein bands was observed with molecular weight estimated between 19 and 53 kDa. Most of the protein bands disappeared when the meat was heated at 60°C. Remaining protein bands also gradually disappeared with higher time/temperature heating combinations. These observations are in good agreement with those reported by previous workers[6, 14, 15, 21] in different fish and other vertebrate meats. Saury meat heated at 60°C for 30 min possessed only two bands but the intensity of those decreased, and one of them disappeared at 65°C with increasing holding time. One protein band with molecular weight of around 33 kDa still remained at 65°C until 30 min holding time, and this protein could be responsible for producing coagulum at 65°C. However, all proteins bands disappeared at 70°C, and these results are quite comparable to the results obtained by coagulation method. Similar observations were also reported in fish meat by Huang *et al.*[14]. The disappearance of all protein bands detected by the present electrophoretic method indicates that the saury meat was heated above 65°C and using SDS-PAGE confirms that the coagulation method used in this study offers an excellent means to accurately determine MIT.

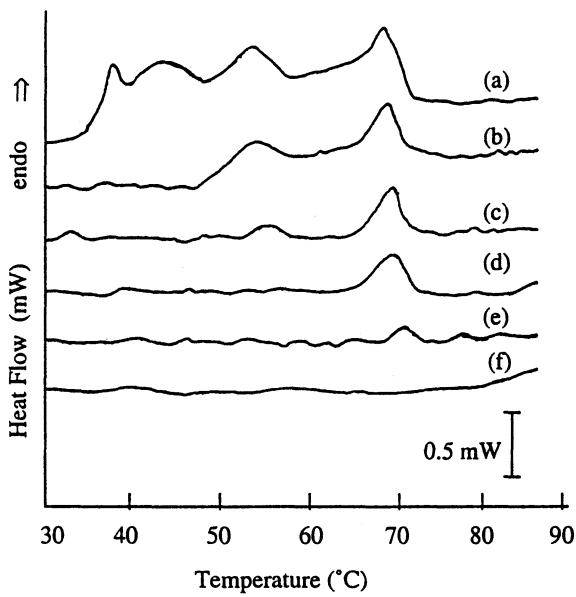

Fig. 3. DSC thermograms of raw and previously heated saury meat: (a) raw meat, (b) 50, (c) 55, (d) 60, (e) 65 and (f) 70°C. Scanning rate: 5°C/min.

Saury raw meat had four characteristic endothermic peaks in DSC thermogram within the temperature range of 30 to 90°C (Fig. 3). These peak maxima were at 37, 46, 55.2 and 69.8°C. Several researchers[22–26] reported that most of the fish meat had three main characteristic endothermic peaks in DSC thermogram in same temperature range being composed of myosin, sarcoplasmic proteins or actomyosin complex and actin. However, saury meat showed a small endothermic

peak at 37°C. This small peak was observed in fish habitat at relatively low temperature and defined as low temperature endothermic peak (LTEP). The LTEP of sharks was considered to be caused by the heat denaturation of myofibrillar proteins[27]. Hastings *et al.*[24] suggested that the LTEP of cod protein around 35°C might correspond to the first transition of myosin and collagen. However, other investigators[21, 26, 28] concluded that this LTEP over the temperature range of 36–39°C corresponds to the transition temperature of their respective myosin tail. It should be noted that saury is considered to be a cold water fish.

DSC thermograms obtained from meat that were heat-treated up to 70°C correspond to their protein denaturation during heating (Fig. 3). As the temperature of heat treatment increased, peaks gradually disappeared and the thermogram of meat previously heated at 70°C had no peaks because the sample was irreversibly and completely denatured during the earlier heat treatment. These results are comparable to those observed in meat products by Ellekjaer[29] and Parson and Patterson[16]. The disappearance of all peaks in DSC thermograms indicates that saury meat was heated above 70°C. From the results described above, it was concluded that MIT up to 70°C of previously heated saury meat could be successfully determined by the coagulation method, which was confirmed by SDS-PAGE, protein solubility and DSC techniques. Although this study was performed on saury meat, it could be valid as a general technique for determining the MIT of other heat treated marine products.

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