CHAPTER III

Materials and Methods

This chapter describes the materials and methods for all experiments in this research. First, it introduces the preparation of surimi and methods for the development of fish protein gel including gel properties analysis such as gel strength, microstructure analysis, solid fraction and effect of temperature on protein. Thereafter, it describes the details of frying process and temperature measurement inside the gels during frying. Finally, it focuses on the methods to determine oil content, moisture content and structure visualization.

3.1 Development of Fish Protein Gel

3.1.1 Surimi Preparation

3.1.1.1 Threadfin Bream Surimi

Frozen threadfin bream (*Nemipterus* spp.) surimi was purchased from Sea Royal Marine, Co LTD, Thailand. Surimi was sealed in polyethylene bag and kept in a walk-in freezer at -18 °C until used.

3.1.1.2 Cod Surimi

Cod (*Gadus morhua*) fillets were purchased from Frosts Fish LTD, Reading, UK. The fillets were skinned, rinsed with clean water, blended in a Lab Micronizer (Waring Commercial), washed by the ratio of water: minced fish at 3:1 (v/w) and dewatered by a basket centrifuge. The washed sample was mixed with 6% sugar and

0.2% tetrasodium pyrophosphate and stored in a walk-in freezer at –18 °C as a frozen surimi sample.

3.1.2 Gel Preparation

The frozen surimi was thawed at 4°C until its temperature reached 0 °C and mixed with 2.5% salt by using a Lab Micronizer (Waring Commercial). The sol obtained was stuffed into stainless-steel cylinders of 2.5 cm. inner diameter and 2.5 cm. length. Surimi gels were prepared by heat setting at 35, 40, 45 and 50 °C for 0 to 300 minutes and at 60 and 80 °C for 0 to 60 minutes in a water bath (Grant Y28, type VFP). Then, the gels were cooked at 90°C for 20 minutes in a water bath (Grant Y28, type VFP), and cooled in ice water. The obtained surimi gels were stored at 4°C for 24 hours before analysed.

3.1.3 Measurement of Gel Strength

Gel strengths were measured by a TA-XT2 Texture Analyser (Stable Micro Systems Ltd., Surrey, UK.). All the cooked gels were compressed to 1.5 cm at the speed of 1.1 mm/second using a cylindrical shaped probe of 2.5 cm diameter. The changes in applied force were recorded. The gel strength was obtained from the peak force multiplied by the compression distance at the peak.

3.1.4 Microstructure Analysis

Microstructural analysis was carried out to examine the bulk and void inside the gels by using Scanning Electron Microscopy (SEM, LEO 1450VP, LEO Electron Microscopy Ltd., UK.).

Surimi gels were cut into 0.5 x 0.5 x 0.5 cm^3, using a surgical blade and subsequently, fixed for 2 hours in a mixture of 5 ml of 25% glutaraldehyde solution, 15 ml of 0.2 M sodium cacodylate, 20 ml of distilled water and 1 ml of 1% calcium

chloride·$6H_2O$ (analytical reagents, Fisher scientific U.K. limited, Loughborough, U.K.). Subsequently, the samples were soaked in 0.1 M sodium cacodylate for 2 times, each at 5 minutes and immersed in the solution of 1% osmium tetroxide in 0.1 M sodium cacodylate for 1 hour. After that, the samples were soaked in 0.1 M sodium cacodylate for 2 times, each at 5 minutes. Thereafter, the samples were dried for 2 times, each at 10 minutes in series of 70%, 90% and 100% acetone (analytical reagent, Fisher scientific U.K. limited, Loughborough, U.K.). After attaching to aluminium stubs, the samples were dried at 60 °C for 1 hour, then coated with approximately 20 nm of gold using a S150B Edwards sputter coater (Edwards High Vacuum, Crawley, U.K.).

The prepared samples were examined with SEM at an accelerating potential of 20 kV, using magnifications ranging from 500 to 10,000x. Finally, the scanned images were collected digitally using IScan 2000-1.2.0 software (Fletcher Callin Ltd., U.K.).

3.1.5 Volume Fraction of Solid and Voidage Fraction

Surimi gels were dehydrated by soaking for 2 times, each at 20 minutes in series of 50, 70, 90 and 100% acetone. After that, they were left in fume cupboard for 2 hours to allow the solvent residue to evaporate. For density determination, the dried gels were weighted, thereafter, they were dipped into measuring cylinder containing a volume of hexane (Sigma Chemicals, Poole, UK.). The volume of the gels was obtained from a size measurement. The volume of the gel-solid was obtained from the replaced hexane volume and the volume fraction of solid was obtained from $\rho_{gel} / \rho_{gel\text{-}solid}$ where gel represents the gel solid plus void. ρ_{gel} was calculated using the volume based on gel and air and $\rho_{gel\text{-}solid}$ was calculated using the volume based on solid only.

The voidage fraction was obtained from the reciprocal of the volume fraction of solid.

3.1.6 Differential Scanning Calorimetry (DSC)

DSC measurement was performed on a Perkin-Elmer DSC 7 with TAC7/DX thermal analyzer controller. The instrument was calibrated using indium (m.p. 156.6 °C, ΔH fusion 6.8 cal/g). 10-20 mg of sample was weighed into a standard 40 μl aluminium pan, hermetically sealed and heated from 20 °C to 90 °C at 10 °C/minute. An empty pan was used as reference.

3.1.7 Fitting Method

The competitive-consecutive first order reaction for gel texture (B) and Arrhenius model were fitted and calculated by using Mathcad Program (Mathcad 2001i Professional, Mathsoft Engineering & Education Inc., U.K.).

3.2 Frying

3.2.1 Sample Preparation

The frozen surimi was thawed at 4°C until the temperature reached 0 °C and mixed with 2.5% salt by using a Lab Micronizer (Waring Commercial). The sol obtained was stuffed into stainless-steel cylinders of 2.5 cm inner diameter and 2.5 cm length. Samples at different gel strengths were prepared by varying heat setting conditions as follows: (1) for the gel strength of 1,000 g.cm, the sol was cooked in a water bath at 90 °C for 20 minutes (Grant Y28, type VFP); (2) for the gel strength of 1,700 g.cm, the sol was set at 50 °C for 15 minutes, then, cooked at 90 °C for 20 minutes; (3) for the gel strength of 2,300 g.cm, the sol was set at 40 °C for 30 minutes then cooked at 90 °C for 20 minutes. After cooking, they were cooled in ice water, packed in a polyethylene bag and stored at 4°C for 24 hours before frying.

3.2.2 Frying Process

The oil chosen for this experiment was sunflower oil (Marks and Spencer plc, London, UK.). Frying was carried out in an electric deep fryer (De'longhi, Type: F350WA), which was thermostatically controlled to maintain the set frying temperature within ± 2 °C using an ADP 15 control system (Mantracourt Electronic Ltd., Chesterfield, UK.).The fryer was filled with 1.5 l of sunflower oil, which was preheated to 180°C for 30 minutes before frying. Samples with three different gel strengths were held in the proper position using a wire-mesh structure shown in Figure 3.1. This arrangement can prevent the gels from floating during frying. After frying at 180°C for 3 minutes, samples were removed from the fryer and soaked in petroleum ether (GPR grade, BDH, Poole, UK.) for 1 second to remove the surface oil.

3.2.3 Temperature Inside Surimi Gel

The temperature gradients inside the gel during cooking and frying were recorded with thermocouples (0.5 mm diameter fast response thermocouple, Labfacility Ltd., Teddington, UK.). Thermocouples were inserted either at approximately 5 mm below the surface of the pieces, along the axis, to record the temperature beneath the surface, or at the center of cylinder, along the axis, to record the core temperature. The thermocouple was attached to a digital thermometer (model 765TC, Labfacility Ltd., Teddington, UK.).

3.2.4 Oil Content

3.2.4.1 Determination of Surface Oil

Surface oil was determined by washing the sample for 1 second in 150 ml of petroleum ether. The washing solution was transferred to a 250 ml round bottom flask that was under vacuum of 200 to 300 mm Hg, and evaporated at 45 °C using a rotary evaporator (Rotavapor RE 111, Buchi, Flawil/Schweiz, Switzerland). The extracted oil was dried in a convective hot air oven at 105 °C (Prime Oven 100 110s, Gallenkamp, Loughborough, U.K.) to constant weight, cooled in a desiccator and weighed. The amount of the surface oil was calculated as follows:

$$surface\,oil\,(\%) = \frac{extracted\,oil\,(g)}{dried\,sample\,(g)} \times 100$$

3.2.4.2 Determination of Structural Oil

The standard oil content in a food material was determined by solvent extraction using the sohxlet technique (AOAC., 1995).

After surface oil extraction, the samples were finely cut and dried at 105 °C for 8-12 hours in a convective hot air oven. The dried mass was ground (Braun aromatic KSM2 grinder, Braun, Barcelona, Spain) and placed inside single-thickness cellulose extraction thimbles (28 x 80 mm, Whatman International Ltd., Maidstone, U.K.). A clean, dry round bottom flask (250 ml) was weighed and 150 ml of PE were added to the flask. Extraction was carried out for 4 hours using a Soxhlet apparatus (neck size 24/29 and socket size 34/35, Quickfit-BDH, Poole, U.K.). After extraction, the solvent was removed on a rotary evaporator and the extracted oil was

dried to constant weight in a convective hot air oven at 105 °C, cooled in a desiccator and weighed. The amount of structural oil was calculated as follows:

$$structural\ oil\ (\%) = \frac{Soxhlet - extracted\ oil\ (g)}{dried\ sample\ (g)} \times 100$$

3.2.5 Moisture Content

Moisture content of surimi gels was carried out by AOAC method (1990).

3.2.6 Measurement of Gel Strength

3.2.6.1 Initial Gel Strength

The initial gel strength of surimi gels were tested as described in 3.1.3.

3.2.6.2 Strength of Crust

The strength of crust was measured by a TA-XT2 Texture Analyser (Stable Micro Systems Ltd., Surrey, UK.) with a puncture element, 2 mm diameter probe, at the speed of 1.1 mm/second and a travel distance 1.5 cm. The strength was obtained from the peak force multiplied by the compression distance at the peak.

3.2.7 Structure Visualization

3.2.7.1 Structure Change during Frying

Pictures of the surimi gels were taken with a digital still camera (model DSC-P2, SONY Cyber-Shot, SONY Corporation, Japan) after frying at 180°C for 30, 60, 90, 120, 150 and 180 seconds to show crust formation and internal gel structure development.

3.2.7.2 Microstructure Analysis

Microstructural analysis of fried gels were carried out to examine the crust surface of fried gels by using SEM.

The fried gels were cut into 0.5 x 0.5 x 0.5 cm^3 using a surgical blade and subsequently, defatted by immersing in acetone (analytical reagent, Fisher scientific U.K. limited, Loughborough, U.K.) for 12 hours. After attaching to aluminium stubs, the samples were coated with gold and examined with SEM using magnifications ranging from 35 to 500x as described in 3.1.4.

3.2.8 Statistical Analysis

Statistical analysis of the data was performed by using SPSS 8.0 for window (SPSS Inc., Illinois, USA). Differences among means were analysed by a LSD range test at a significance level of $p \leq 0.05$.

3.2.9 Solution of the Mathematical Model

The mathematical model for heat transfer during frying of surimi gel was programmed and solved using Mathcad Program (Mathcad 2001i Professional, Mathsoft Engineering & Education Inc., U.K.).