# INVESTIGATION OF COD SURIMI MADE ACCORDING TO THE pH-SHIFT PROCESS OR CONVENTIONAL WASHING AS CANDIDATE OF FOOD MATERIAL FOR 3D PRINTING

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## ABSTRACT

Developing countries generate over 60% of global fish trade and understanding how byproduct utilisation and 3D food printing impact the seafood industry in Indonesia and globally is critical. It is important to be forward-thinking and that starts with the research we are conducting. Byproduct utilisation can occur through surimi processing and in this study, we tested both conventional and pH shift surimi processing. With the implementation of 3D food printing there can be reduction in food waste and utilisation of seafood byproducts. This research is the first of its kind evaluating how printability of surimi paste and cooked gels in star form are affected by different surimi processing methods, source and quality of byproduct starting material, the addition of salt, and cold storage.

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# 1 INTRODUCTION

## 1.1 Background

Indonesia is a large country in Asia with a long coastline, and it serves as a significant fish resource in Southeast Asia for surimi production. There are 14 industrial surimi processing companies in Indonesia mainly located in Java with a total production in 2016 ranging from 38,000 -73,500 MT (MMAF, 2017). Surimi products from Indonesia are mostly exported to Korea, Japan, Singapore, and Taiwan (Guenneugues & Ianelli, 2014). Challenges in the surimi industry are continuously changing due to the variability of different fish stocks around the world. Global surimi production was about 800,000 MT in 2015 and the demand continues. Furthermore, surimi consumption continues to rise in developing countries (Endoo & Yongsawatdigul, 2014). Southeast Asia is the largest fish resource for surimi production, accounting for approximately 60% of the global surimi production. Key species used in the surimi production include Pacific whiting (*Merluccius productus*), Yellow stripe trevally (*Selaroides leptolepis*), small pelagic sardines (*Sardinops sagax* and *S. neopilchardus*), threadfin bream (*Nemipterus bleekeri*) and red tilapia (*Oreochromis mossambicus*) as well as tropical species (Arfat & Benjakul , 2012) (Bentis, Zotos, & Petridis, 2005) (Kaewudom, Benjakul , & Kijroongrojana, 2013).

Seafood product demand is increasing due to global population growth, and an ongoing fish supply decline has led to a great interest in using underutilised seafood byproducts, created through processing (Gehring, et al., 2011). Byproducts can be difficult to process but can be significant sources of protein and other high-value added ingredients. Surimi is a typical intermediate fish product and can be made from such byproducts. The utilisation of byproducts to make surimi can be problematic due to the presence of heme proteins and myoglobin, polyunsaturated fatty acids and lipids, and difficulties in removing impurities (e.g., bones, scales, connective tissues, and so on). Colour problems, lipid oxidation and poor yields can result from conventional surimi processing and protein isolation processing (Kristinsson & Liang, 2006) (Halldorsdottir, et al., 2011). The pH-shift process involves an acid and/or alkaline solubilisation followed by isoelectric precipitation (Hultin, et al., 2001). Compared to conventional surimi processing, the pH-shift process can result in greater recovery and improved quality of fish proteins, particularly from under and is recognized as a promising method for the recovery of proteins from underutilised seafood raw materials. The pH-shift method relative to the simple washing method is more effective in removing pro-oxidants and impurities in low-value and complex seafood byproduct raw materials.

It is particularly important to apply improved processing techniques to low value products to obtain fish products with good functionality, yield, colour, and stability. Matís as an Icelandic Food and Biotech R&D institute has performed limited work regarding the isolation of fish proteins from low value and complex seafood from raw materials in Icelandic fish species (unpublished), comparing conventional surimi material to surimi material made from the pH shift method. Market preferences have shifted from converting low value byproducts into high value fish ingredients that can be sold on various fish markets globally and applications of improved processing methods will be critical. The quality-driven product demand from low value starting raw materials is global and has prevailed in certain countries such as the USA, EU, and especially China.

## 1.2 3D Food Printing

Additive manufacturing (AM), also known as 3D printing, was invented in 1980, and since then, has received attention from industry, academia and the public due to its numerous

advantages (Wang, Zhang, Bhandari & Yang, 2017). The 3D printing process involves extruding material through a nozzle and the shape is determined through a model design software-program. The 3D printing of food affords various advantages: utilisation of byproducts and reduction in food waste, consumer personalisation, creation of shapes and textures never possible before, convenience, food security, help with chewing difficulties in the elderly (European Commission, 2014), as well as promotion of healthy foods, such as fish (Kim, Bae & Park, 2017) (Pallottino, et al., 2016).

Additionally, 3D food printing can serve as a way to test textures and functionality of foods on a small scale before industry scale-up. Various shapes and designs are possible, and the properties of the final form will vary (Godoi, Prakash & Bhandari, 2016). The 3D food printer is the microwave of the future and forward- thinking developments using the 3D food printer can result in the successful utilisation of fish byproducts (rest raw materials). Additionally, as other parts of the world will be using 3D food printers, working on seafood products that can not only be consumed by Indonesians but by global buyers and users of 3D food printers can satisfy a market demand now and into the future. Right now, Indonesia provides commodity products to the global industry that are being food printed today (Unknown, 2018). Health conditions are common in Indonesia such as heart disease and obesity, and 3D food printing is expected to be a way that will allow for controlled food portions and delivery of proper nutrition. Countries globally are printing seafood products for consumers.

In recent years, 3D printing has been widely applied in many food fields including chocolate (Hao, et al., 2010), meat products (Lipton, Cutler, Nigl, Cohen & Lipson, 2015) and mashed potatoes (Liu, Zhang, Bhandari & Yang, 2017). Investigating the use of 3D printing to produce cookies from a mixture of sugars, starch and mashed potato without the need of further post-processing (cooking) has been reported by Sun et al., (2015). To date, research regarding the utilisation of 3D food printing for fish applications is limited. In only one study, fish surimi gel from silver carp with different levels of NaCl addition were tested as potential material for 3D food printing applications (Wang, Zhang, Bhandari &Yang, 2017). Research of pre-processing methods and additions of ingredients of raw fish materials could serve to advance the utilisation of byproducts and fish proteins isolated from low-value raw materials. Developing countries generate over 60% of the global fish trade and understanding how food printing and byproduct utilisation impact the seafood industry in Indonesia and globally is critical. It is important to be forward-thinking and that starts with the research we are conducting.

As the market share of healthy and consumer centric food products increases globally, the demand for sustainable, high protein seafood products will continue to rise. Consumers are seeking convenience and easy to prepare seafood products. As a strategy to utilise byproduct mince and test the functional properties of the surimi with cold storage, we made surimi through two different processing methods and tested their printability in a 3D food printer, using the first commercial 3D food printer of its kind in Scandinavia. We hypothesize that the pH-shift process will result in functional proteins from raw fish byproduct starting material. It is hypothesized that the isolated proteins produced with the pH-shift process will have good gel-forming abilities, colour, and stability, potentially more so than the raw material produced with conventional surimi processing (Kristinsson, Lanier, Halldorsdottir, Geirsdottir, & Park, 2014). It is also hypothesized that fish byproducts can be transformed into valuable ready to print seafood products.

## 1.3 Overall objectives

The key objectives of this study were two-fold. First, we wanted to increase knowledge of fish surimi processing, comparing the effects of starting raw materials and different processing steps on production of surimi (conventional method/washed and protein isolates via the pH-shift process) and estimate the storage stability of the final surimi product after thawing and refrigeration. Secondly, we wanted to investigate the highly novel application of 3D food printing with surimi products made from conventionally washed and pH shift derived fish proteins. The printability of fish surimi prepared by two methods with the addition of additives (salt), steam cooking, and subsequent analytical testing of the 3D printed surimi gels has yet to be investigated. The knowledge, data and expertise gained through the project will be transferable to the fish processors in Indonesia to turn low value by-product into high value-added fish ingredients that can be sold on various fish markets globally in surimi forms and that can also be utilised as 3D food printers as a ready to cook product into the future.

## 1.4 Specific objectives

1) To use cod low-value mince as a candidate for increased higher value raw intermediate materials. The cod low-value mince will serve as a comparative starting material to Indonesian white fish (White Croacker, Threadfin Bream and Lizard Fish).
2) To optimize surimi gel forms from cod-by products through application of washing or the pH-shift method, pH adjustment, and NaCl addition.
3) To determine how the quality and storage ability of surimi products are influenced by the different surimi processing methods and starting material.
4) To investigate the printability, gel properties after printing, steam cooking and the physical and chemical properties of the heat-set surimi gels.

# 2 LITERATURE REVIEW

## 2.1 Surimi

Fish is usually low in saturated fats, carbohydrates, and cholesterol and provides not only high-value protein but also a wide range of essential micronutrients, including various vitamins, minerals, and polyunsaturated omega-3 fatty acids (FAO, 2012). Fish can be an effective food source for addressing food and nutritional security around the globe. To combat overfishing as well as improve the value and utilisation of byproducts from processing, surimi can be produced and serve as an ingredient in final food applications and seafood analogue products such as imitation crab sticks. This is due to the functional properties, nutritional added value, and stability with freezing (Akil, Bryant, & Jiddaw, 2008). Surimi consists of recovered protein (mainly myofibrillar protein) obtained through washing and fish protein solubilisation steps and recovery of fish muscle through dewatering, followed by the addition of cryprotectants. Surimi can be obtained from processing of deboned fish meat (Kong, et al., 2016) in addition to lower quality product containing higher concentrations of blood, skin, bone, etc. Lower quality mince is a candidate for surimi processing and methods of processing in addition to the starting material will affect the quality. Quality parameters affected include colour, stability, protein functionality, lipid oxidation, and microbial.

### *2.1.1 Protein components of surimi*

Myosin is a critical, dominant protein in fish muscle composing (~55-60% of the total myofibrillar proteins) (Lanier, Yongsawatdigul, & Rondanelli, 2014). Another key protein, actin composes 15-30% of the total myofibrillar proteins. Myosin is considered a major component of fish muscle responsible for the functional properties of fish muscle tissue, including gelation and water-binding. In the post-mortem stage, actin binds to myosin forming actomyosin. Myosin is 470 kDa (Bechtel, 1986), with a structure that is both fibrous (long, extended shape) and globular (spherical shaped) in structure.

Stroma is the insoluble connective tissue in fish, primarily consisting of collagen. During the washing process in surimi production, collagen in addition to myofibrillar proteins is retained. The refining process will remove and reduce the level of connective tissue in the final surimi. Stroma proteins are relatively low compared to the levels of myofibrillar in fish (Lanier, Yongsawatdigul, & Rondanelli, 2014). Sarcoplasmic proteins are involved in muscle metabolism and in surimi gelation. They contain many enzymes and are less susceptible to denaturation at higher temperatures (67.7$^{o}$C and 85.8$^{o}$C) compared to myosin (30-35$^{o}$C) (Tadpichayangkoon, Park & Thawornhinsombut, 2010). Gelation of fish protein can be enhanced by the presence of sarcoplasmic proteins in specific concentrations and processing conditions (Yongsawatdigul & Piyadhammaviboon, 2007).

Hemoglobin and myoglobin, known as heme proteins, from fish muscle tissue contribute to pigmentation in fish mince. Heme proteins can denature during surimi processing and bind to myofibrillar proteins, thus imparting colour to the final surimi raw material. Denaturation involves the unfolding of proteins, consisting of polypeptides. When a protein denatures, reactive protein sites become exposed altering the behaviour of a protein in different processing conditions. Heme proteins contain ferric iron, an important component of lipid oxidation reactions. Optimal surimi gelling is obtained with the reduction of lipids and heme proteins in the starting raw material (Lanier, Yongsawatdigul, & Rondanelli, 2014).

### *2.1.2 Production of fish protein ingredients*

In the first stage of surimi processing, fish are processed into a mince. Prior to mincing, fish are headed, gutted, and bones removed. In the second stage, fish mince goes through a refining and washing process. Washing removes water soluble, sarcoplasmic proteins as well as heme proteins, lipids, and other unwanted soluble fish muscle components. It is imperative that the wash water be cold for the best quality surimi (Park, Graves, Draves, & Yongsawatdigul, 2014). The ratio of water to fish material can range depending on starting material and processing restrictions. Quality surimi can result with as little as a 2:1 ratio of water to fish mince. The addition of salt to wash water can result in a greater removal of sarcoplasmic proteins and thus better gelation properties from surimi. (Lin & Park, 1996) (DeWitt, Lin, & Ismond, 2014).

After the washing and refining stages and before freezing, cryoprotectants are added to the surimi. These ingredients can maintain the functional properties of the proteins with frozen storage. Freezing can induce denaturation and aggregation of proteins, so it is important to add cryoprotectants (sucrose, sorbitol). Other additives include sodium tripolyphosphate and tetrasodium pyrophosphate, serving as metal chelators as well as buffering agents. These ingredients can help in raising the pH for the surimi for functional purposes (Alvares, Fernandez, & Canet , 2010) (Jin, et al., 2010).

Two methods of processing can be used to obtain surimi: conventional washing and fish protein isolation. With proper, conventional washing denaturation and protein damage are minimised. The fish protein isolate (FPI) process take the proteins through a denaturation process through pH step adjustments. In this process, acid or alkaline pH and isoelectric protein precipitation, extraction followed by isoelectric precipitation are followed (U.S Patent No. 6,136,959, 2000). The process can give greater yields than conventional surimi processing and better reduces the level of unwanted compounds, including lipids, heme, bone, connective tissue, etc. Surimi made with the pH shift process can contain more sarcoplasmic proteins compared to conventional surimi processing. However, the pH shift process surimi has been shown to have equal or greater gelling properties to conventional surimi (Nolsoe & Undeland, 2009).

In the pH shift process (Fig.1), the mince is solubilised in water through acidic or alkaline pH adjustment. The ratio of fish muscle to water as well as the pH adjustments will depend on the raw material and species of fish. After the first pH adjustment, the homogenate is centrifuged. The centrifugation results in 3 phases, a lipid (oil) fraction, a soluble protein phase, and a sediment (containing bone, skin, collagen, and connective tissue, etc.). The solubilised proteins are then precipitated out of solution through an isoelectric pH adjustment ranging from pH 5.2-5.5. The recovered FPI can then be adjusted to a neutral pH, followed by the addition of cyroprotectants, and freezing (Kristinsson, Lanier, Halldorsdottir, Geirsdottir, & Park, 2014).

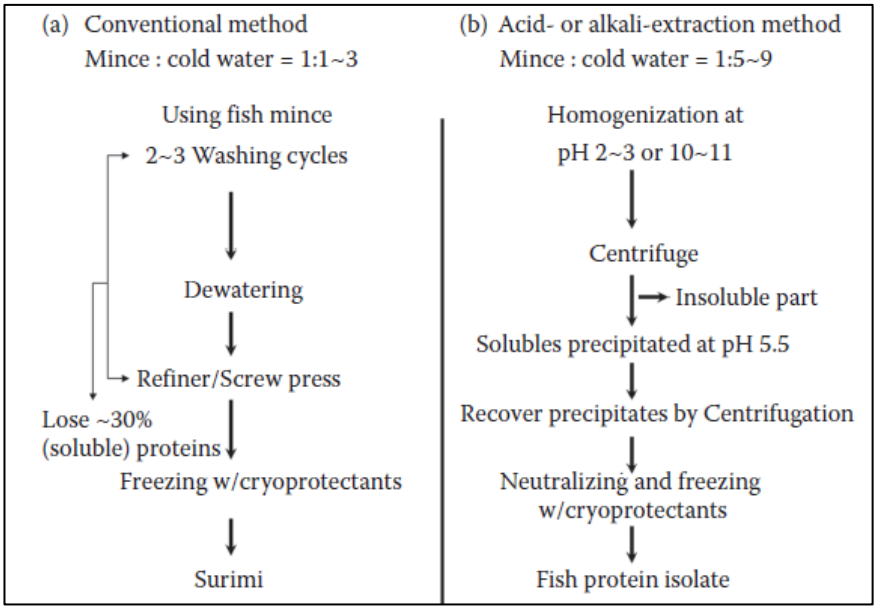



Figure 1. Schematic processing steps to produce conventional surimi (a) and protein isolates via the pH-shift process (b). (Adapted from JW Park. *Proceeding of 62nd American Meat Science Association Reciprocal Meat Conference*. pp.56-63, 2009.)

## 2.2 Factors affecting surimi quality

### *2.2.1 Species and seasonality*

White fish species (like cod) relative to dark muscled, pelagic fish (mackerel, sardines, salmon) do not contain high concentrations of heme proteins and polyunsaturated fatty acids. Starting raw materials from dark muscled fish are susceptible to lipid oxidation and rancidity. Further processing steps are required for these species, such as a need for more washing (Guenneugues & Ianelli, 2014) (Tokunaga & Nishioka, 1988). Optimal surimi is made from fish during the feeding period in which fish muscle is lower in moisture and pH. It is more difficult to dewater fish harvested during and following the spawning season due to higher pH and water retention in the fish muscle. Therefore, addition of salt with washing and pH adjustment can be beneficial (Lee, 1986).

### *2.2.2 Harvest and processing of raw material for surimi production*

Harvest conditions and raw material quality can be controlled and can have the greatest impact on fish quality and surimi. Handling as well as on-board processing and storage conditions and delivery time to the processors are all factors that affect surimi quality (Lee, 1986). Proper processing of fish prior to mincing is very important in determining the quality of surimi. Processing times, temperatures, as well as heading and degutting and removal of contaminants, etc. can all ultimate be factors in surimi functionality. Processing times after rigor are important and physiological changes in the fish muscle occur and can also affect fish proteins (Pigott, 1986).

### *2.2.3 Surimi processing conditions*

Processing temperatures above 5ºC can induce proteolysis of myofibrillar proteins. This can result in a greater percentage of myofibrillar proteins being solubilised and lost in the wash

water, lower protein yields and poor quality of surimi gelation (Lin & Park, 1996). Washing is one of the most important steps in the removal of unwanted compounds and in concentrating myofibrillar proteins. Minced meat is comprised of approximately 65% percent myofibrillar proteins while the remaining percent is composed of sarcoplasmic protein, heme proteins, blood, lipids, etc (Lin & Park, 1996). To emphasise, washing is critical for optimal colour, organoleptic properties (odour, taste), texture and stability of the surimi. Addition of 0.5% NaCl to the wash water can improve the solubility and removal of sarcoplasmic proteins. The pH of the solution with the pH shift process can be highly influential is achieving maximum solubility of the myofibrillar proteins (Thawornchinsombut & Park, Roles of pH in solubility and conformational changes of Pacific whiting muscle proteins, 2004).

## 2.3 3D Food Printing

3D printing, also known as "additive manufacturing ", is the process of extruding food material out of a cartridge through a defined size nozzle. The material is extruded layer upon layer in a chosen shape (Pinna, et al., 2016). To properly print a food or ingredient, the printer must be calibrated/optimised for that ingredient and this involves the height of the nozzle, printing speed and force, and proper extrusion rate with a given nozzle size (Pallottino, et al., 2016). The biggest challenges in 3D food printing with fish is that the connective tissue must be removed, and a certain nozzle size can be used depending on the fish raw material. The beginning raw materials, pre-processing, and mix of ingredients can affect the overall printability and quality of the food form. The food material to be printed should be as homogenous as possible and flowable and with printing should remain in the shape/food form designated prior to printing (Godoi, Prakash, & Bhandari, 2016).

### *2.3.1 Considerations and applications*

When setting out to print a food, such as seafood, key factors should be considered: a) printability and maintenance of shape post-printing and b) application and post processing of printed material.

**a) Printability**

Various parameters can affect printability including formulation and preparation, temperature, texture, ingredients, the rheological and viscous properties of the ingredients and food. The printability would translate to the ability of the printer, with optimised printing parameters, to extrude a food material into a well-defined structure. The food material should be extrudable without excessive air pockets, such that a stable food form is printed with minimal gaps in in well defined, stable layers. Food safety is also a consideration and like with any food, particularly raw, safe handling practices should be practiced similar to manual handling. Regarding applications and post processing, the material should have the same applications (Lipton, et al., 2010).

**b) Application and post processing of printed material**

The printed food form should uphold shape and height, generated by printing layer upon layer, with chosen cooking parameters. Once cooked, the product should have typical rheological properties expected of the food product itself. This involves detailed research and planning and starts with the raw materials and ingredients themselves. To emphasise, the printing itself will not impact the flavour of the food. The properties of the food material must be considered for proper formulation relating to the physical, mechanical, and rheological properties (Lipton, Cutler, Nigl, Cohen, & Lipson, 2015).

# 3 MATERIALS AND METHODS

## 3.1 Surimi sample preparation

Fresh cod (*Gardus morhua*) byproduct mince was obtained from a local fish processing company (batch 1) and a fish store (batch 2) in Reykjavik, Iceland. The refrigerated mince was transported to Matís in coolers containing flake ice (1:1 ratio). Surimi was prepared with two different processing methods in a cold (refrigerated) room (Fig. 2): (1) conventional washing and by the (2) pH-shift process. To make conventional lab surimi for each batch, 30 kg of fish mince was washed with cold distilled water (4$^{o}$C) using a water/mince ratio of 3:1 (w/w). The mixture was stirred gently for 10 min in cold water (4$^{o}$C) and washed mince was filtered with a strainer/screen. Washing was performed again, adding water back to make a 3:1 (w/w) water to mince ratio, with an addition of 0.5% salt to the wash water. The mixture was then dewatered through cheese cloth lining a screen. The temperature of the mixture was kept below 5$^{o}$C during processing.

For the pH shift method, the fish mince from cod byproduct was first washed (3:1) similar to the first wash in making conventional surimi. This was an additional step added at the beginning of the process that is not required but will make the starting material similar to that of the conventional surimi and also remove impurities and blood. The main steps in the processing were according to Undeland, Kelleher and Hultin (2002) with revision. Fish mince was homogenized with 3:1 (w/w) volumes of cold distilled water for 1 min. The homogenate was then adjusted to pH 11 through gradual addition of 2 N NaOH until a pH of 11 (alkaline pH-shift processing) was reached. The mixture was then strained to remove insoluble proteins (particularly connective tissue). Water was then added again, adding water back to achieve a water to mince ratio of 3:1. The pH 11 adjusted solution was then adjusted to pH 5.5 with the gradual addition of 2N HCL to precipitate out the proteins. The fish protein precipitate was then dewatered through cheese cloth.

The fish protein recovered by conventional washing and pH-shift processing was blended with the cryoprotectant mixture (4% sucrose, 4% sorbitol, 0,3% sodium tripolyphosphate and 0,5% sodium bicarbonate (for the pH shift material). The additives were chopped into the fish protein at low speed (1400 revolutions/ min) for 1 min and 30 seconds. The resulting surimi paste was frozen in amounts of 500 g in polyethylene bags at -25$^{o}$C for 7 days. For the cold storage study, the surimi paste was thawed and immediately transferred to the refrigerator for cold storage. The surimi paste was analysed and used to make surimi gels through 3D printing regularly at day 0, 4 and 7 days of cold storage.

## 3.2 Preparation of surimi for 3D Printing process

On day 0, cod surimi was partially thawed in the cold room (5 $^{o}$C) and tested for moisture to ensure approximately 80% moisture. Moisture adjustment was not required as the material prior to freezing was squeezed to 85% moisture. Salt (NaCl) was added in 1,5% and 3% levels. The final pH of both treatments was adjusted to 7.5 by the dropwise, gradual addition of NaOH. As a control treatment, a surimi batch without salt was made as well. To mix the salt and NaOH with the surimi, chopping was applied at low speed (1200 revolutions/ min) for 3 bouts of 45 seconds.

The surimi material was printed through the optimum nozzle size (4 mm) and material was extruded onto wax paper on top of a flat glass plate. The shape of extrusion was a star shape (50 mm in diameter and 30 mm in height). The printing process occurred at ambient

temperature (25$^{o}$C) and optimal printing parameters were calibrated prior to printing according to Natural Machines software. The samples were steam cooked for 20 min at 90$^{o}$C. The samples cooled (refrigerated) overnight for optimal setting. Texture analysis was performed the following day at room temperature.

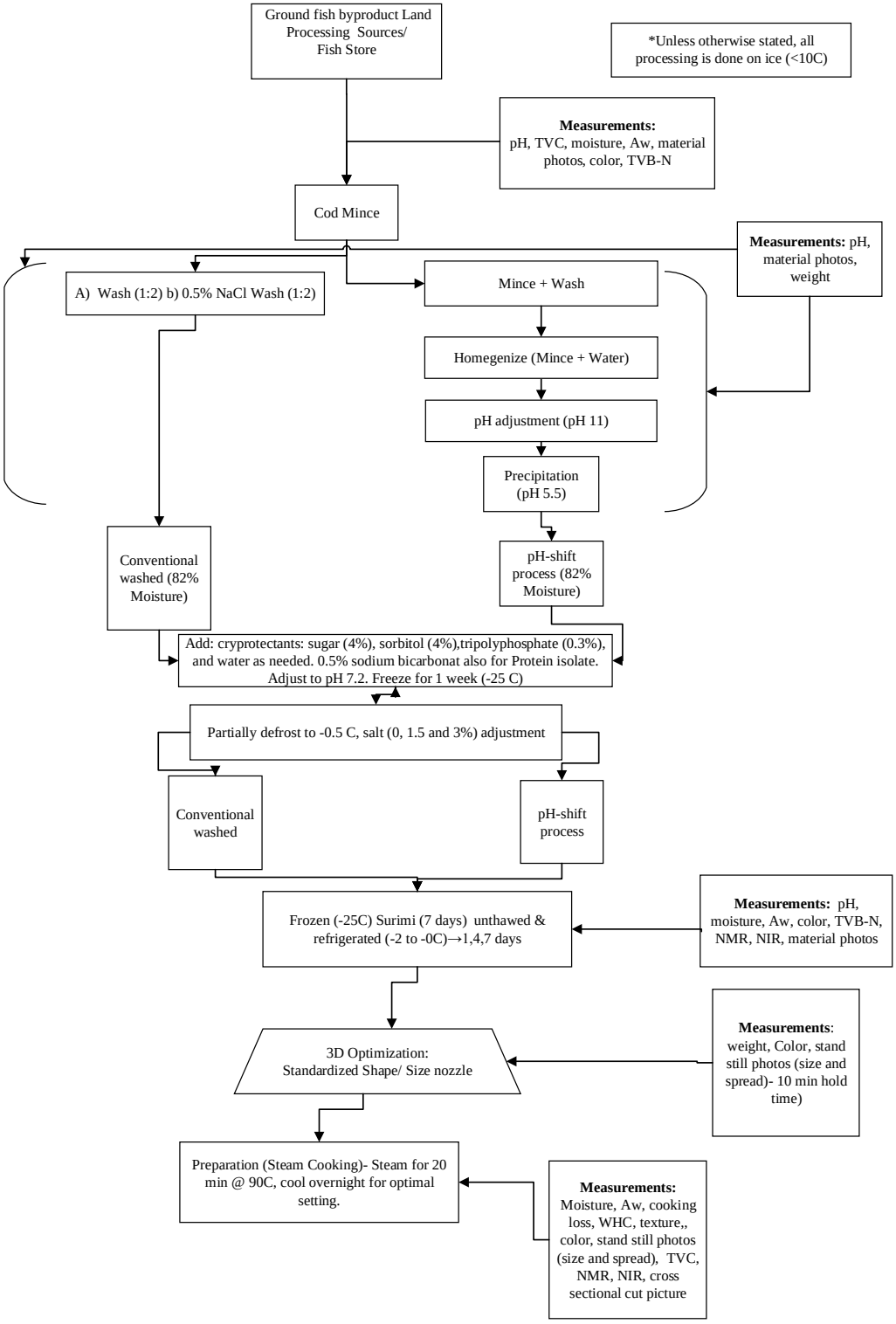



Figure 2. Originally proposed experimental design, preparation of cod surimi and 3D printing

## 3.3 Testing of material properties

### *3.3.1 pH Value*

Value of pH was determined by adding 90 ml of distilled water to 10 g of the test sample and then homogenised for 1 min (AOAC, Official methods of analysis of AOAC international (16th ed.), 1999). The pH of the suspension was measured with a glass electrode pH meter.

### *3.3.2 Total Viable Count (TVC)*

At each sampling point, 20 grams of sample were aseptically transferred to stomacher bags with 180 g of buffer solution and homogenised for 1 min in stomacher. Appropriate decimal dilutions from the homogenised samples were prepared and plated to enumerate the following microorganisms. Plates were incubated at 30$^{o}$C for 48 hours. Microbiological data were expressed as the number of colony-forming units per gram of fish (CFU $g^{-1}$).

### *3.3.3 Moisture content and water activity determination*

Moisture content of the surimi was determined using the oven method. Samples were dried in a drying oven at 105$^{o}$C until a constant weight was achieved (24 h). Weighing was performed using a digital balance, and then moisture content values were calculated. The tests were triplicated, and the results are expressed as (%). Water activity (Aw) was determined using an Aqulab Pawkit (Labo-Scientifica, Parma, Italy).

$$\text{Moisture (\%)} = \frac{pre-dry\ weight\ (g) - after-dry\ weight\ (g)}{pre-dry\ weight\ (g)} \text{ x } 100$$

### *3.3.4 Water Holding Capacity (WHC)*

Water Holding capacity was carried out based on a method proposed by Borresen (Eide et al. 1982). Gel samples were cut into thin slices, and approximately 2 g of the samples were weighed and placed into the bottom of glass centrifuge tubes. Samples were centrifuged at 1500 rpm for 5 minutes in special sample glass from plexi-glass. Gels were weighed again after centrifugation. **$WHC = W_2/W_1 \times 100\%$,** where **$W_1$** is the initial weight of gels, g; **$W_2$** is the final weight of gels, g. Three replicates of the measurements were taken.

### *3.3.5 Cooking loss*

The liquid on the surface of each gel was wiped gently to remove excess moisture around the cooked samples. The samples were weighted before ($G_1$) and after heating (90 $^{o}$C, 20 min) ($G_2$). Cooking loss (CL) was represented as the loss of liquid:

$$CL\% = (G_1 - G_2)/ G_1 \text{ x } 100.$$

### *3.3.6 Colour measurement (L*a*b*)*

Colour measurements of the surimi samples were assessed using a Chromameter CR-300. After calibration using a reference plate supplied by the manufacturer. Hunter *L*, *a* and *b* values were recorded in six replications at the surface of surimi samples. L represents lightness and varies from 0 (dark/black) to 100 (white). The magnitude of *a* and *b* both vary between -300 and 299, which describe green *(-a)* to magenta *(+a)*, and blue *(+b)* to yellow *(-b)*, respectively. Whiteness of cod surimi was calculated by the following equation (Tahergorabi, Sivanandan, Beamer, Matak, & Jaczynski, 2012) (Tahergorabi, Sivanandan, & Jaczynski, 2012b).

$$\text{Whiteness} = 100 - [(100 - L^{*})^2 + a^{*2} + b^{*2}]^{½}$$

### *3.3.7 Total volatile basic nitrogen (TVB-N)*

The total volatile base nitrogen (TVB-N) value was performed using MgO as described by (Sallam, 2007) and expressed as mg N/100 g of sample. A 10 g sample of cod surimi was weighed and the soaked in 100 mL of distilled water for 30 min, followed by a filtration step. Then, 5 mL of filtrate and 5 ml of MgO (10g/L) were distilled using a Kjehdal apparatus, with 5 mL distilled water used as the control. The distillate was collected in 10 mL of boric acid aqueous solution (20g/L) with the addition of a mixed indicator produced from the dissolution of 0.1 g methyl red and 0.1 g of methylene blue in 100 mL of ethanol. The absorption liquid was immediately titrated with a 0.01-mol/L hydrochloric acid solution and their concentration expressed as N mg/100 g sample.

### *3.3.8 Texture profile analysis (TPA) of 3D printed surimi gel*

Texture properties were measured using a TA-XT plus Texture Analyzer (Texture Technologies Corps., Scarsdale, NY, USA). Texture tests were carried out at room temperature (25 °C) on samples (diameter 50.0 mm; height 12.0 mm). Texture profile analysis (TPA) was performed as described by Bourne (2002). Probes of 100 mm in diameter (SMS-P/100) and 10 mm in height axially compressed the sample to 65% strain of the original height (25 N load cell). A total of 5 samples were measured for each treatment. From the first compression curve, hardness was recorded.

### *3.3.9 Low-field Nuclear Magnetic Resonance (NMR) measurements*

A low field Bruker mg 20 benchtop NMR analyser (Bruker Optics GmbH, Rheinstetten, Germany) with 20 MHz and 0.47 T magnetic field was used for the proton relaxation analysis. Small pieces were cut from the samples and placed in 10 mm sample tubes. Three replicates were made from each sample group and all measurements were performed at ambient temperature. The transverse relaxation times ($T_2$) were measured with a Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence (Carr & Purcell, 1954) (Meiboom & Gill, 1958), with an interpulse spacing $\tau$ of 250 μs, a recycle delay of 10 s, 16 scans, and the number of collected echoes was 8100, while no dummy shots were used.

The NMR data was collected with the Bruker Minispec software and successively maximum normalized to allow comparison of samples with different size and water content. This was done by giving the strongest echo signal a value of 100 and scaling the following echo signals accordingly. The transversal relaxation data was then fitted to a multi-exponential curve by using the Low-field NMR toolbox for Matlab (The Mathworks Inc. Natric, MA), as described by (Pedersen, Bro, & Engelsen, 2002). Residual analysis of the exponential fittings was used to assess the number of proton populations in each sample. Principal component analysis (PCA) was performed in Unscrambler X (CAMO, Trondheim, Norway) to assess the multivariate effects from the conventional washing vs. pH-shift processing, salting, cooking and storage on the NMR relaxation responses. All parameters were normalised with the inverse of their standard deviation to allow comparison of variables of different units and ranges.

### *3.3.10 NIR analysis*

Measurements were done on cod surimi paste and gel from different NaCl concentrations using the FT-NIR reflectance measurement. Samples were analysed over the wavelength range from 800 to 2500 nm using Bruker Multi-Purpose Analyser (MPA) system with a fiber probe (Bruker Optics, Rheinstetten, Germany). The fiber probe measures over an area of 0.50 $cm^2$. For each sample, five spectra were collected and the average spectrum of these five spectra was used for analysing. All samples were measured in triplicate. OPUS spectroscopy software

(v.6.5 Bruker Optics, Rheinstetten, Germany) was used for spectral acquisition, instrumental control and data treatment (Karlsdottir, Arason, Kristinsson, & Sveinsdottir, 2014).

### 3.4 Statistical Analysis

The experiments were triplicated (n=3). Data were subjected to one-way analysis of variance (ANOVA) using SPSS 22 software. A significant difference ($p < 0.05$) between mean values of samples was determined and differences between treatments were tested using Duncan's test (Freud & Wilson , 1997).

# 4 RESULTS

## 4.1 The effect of different methods of processing surimi on surimi paste properties

The changes in physicochemical characteristics of cod surimi paste prepared from two different methods were determined and compared after freezing and with cold storage, printing and cooking. For each designated time point in cold storage, the cod surimi was prepared with different concentrations of NaCl (0%, 1.5% and 3% w/w) and the paste was adjusted to pH 7.5. The pH range of 7.2 – 7.5 has been found to be optimal for gelation of fish muscle (Shaowei, Nozawa, & Seki, 2001). The pH, moisture content, water activity, TVB-N, color and TVC in the surimi (after frozen for 7 days, thawed, and stored in refrigerated temperature) were measured. These measurements were taken both before the addition of cryoprotectants and before printing and are summarized in Table 1.

### *4.1.1 Raw material properties*

The initial values of TVB-N in the cod byproduct mince from the processing facility (1st batch) and the fish store (2nd batch) were 7.59 and 19.56 mg/100 g, respectively. The TVB-N values in the cod mince from the fish store was higher but the value was below the recommended spoilage limit, 35 mg N/100 g (European Commision , 2005). The raw material whiteness of the cod mince from the processor and the fish store were 54.7 and 57.0, respectively. Raw material obtained from the fish store exhibited a higher whiteness value. Moreover, total viable psychrotrophic counts (TVC) in raw material obtained from fish store (6.34 CFU $^{g-1}$) were higher than in cod surimi from the local fish processing company (5.43 CFU $^{g-1}$) (Table 1).

Table 1. Quality parameters of byproduct cod mince

| Sample of cod mince | TVB-N [mg/100g sample] | Color | | | TVC [log CFU/g] |
|---|---|---|---|---|---|
| | | *L** | *a** | *b** | |
| 1st Batch | 7.59±0.52 | 54.7±0.8 | 0.5±0.4 | `-1.5±0.9 | 5.43 |
| 2nd Batch | 19.56±2.24 | 57.0±0.7 | `-1.3±0.4 | `-1.4±0.4 | 6.34 |

*values are given as means ± SD from triplicate determinations.

### *4.1.2 Effect of different methods of processing surimi on surimi paste properties*

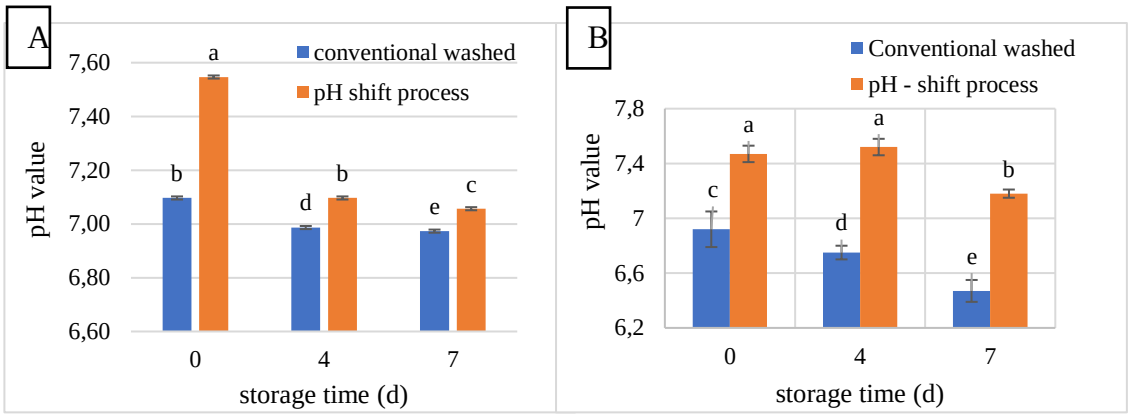
The values of pH, moisture, water content, TVB-N, color and TPC with the means ± SD are given in Table 2. The values of pH (Fig. 3) in the surimi paste in both batches decreased during the refrigerated storage (samples after freezing for seven days, thawed and mixed with cryoprotectants). In batch 1, the pH significantly decreased from day 0 to day 4 was higher in the pH shift treatment than the conventionally washed surimi. From day 4 to 7, the percentage change in pH was lower than the earlier days of storage in day 0 and day 4. Similarly, in batch 2, there was an overall decrease in pH among both prepared surimi treatments with refrigerated storage. The pH values significantly decreased in the conventionally washed surimi. A drop in pH was seen in the pH shift process surimi from day 4 to day 7. A steady decrease in pH was observed from day 0 to day 4 and from day 4 to day 7. A large drop in pH was seen in the pH shift process surimi from day 4 to day 7. A steady decline, in pH was observed from day 0 to day 7 in the conventionally washed, thawed surimi. Compared to the processing methods, the initial pH values in surimi, after thawing, from the pH shift process were higher than the surimi paste made from conventional washed, pH 7.47 – 7.55 and 6.92 – 7.10, respectively. On the day of processing the surimi was adjusted to approximately 7.2 for both treatments. The initial

higher value of the pH shift process on day 0 can likely be attributed to the sodium bicarbonate. Due to innate changes in the pH of the surimi with storage, the treatments were adjusted to 7.5 prior to printing.

Table 2. Effects of different washing methods on surimi paste properties

| | Day 0 | | Day 4 | | Day 7 | |
|---|---|---|---|---|---|---|
| **Quality parameter (1st batch)** | Conventional washed | pH - shift process | Conventional washed | pH - shift process | Conventional washed | pH - shift process |
| surimi pH | 7.10±0.01b | 7.55±0.01a | 6.99±0.01d | 7.10±0.01b | 6.97±0.01e | 7.06±0.01c |
| moisture content (%) | 79.33±0.01f | 79.81±0.01c | 79.47±0.01d | 79.89±0.01a | 79.45±0.01e | 79.85±0.01b |
| water activity (Aw) | 0.989±0.000a | 0.983±0.004a | 0.984±0.004a | 0.986±0.006a | 0.988±0.000a | 0.984±0.005a |
| TVB-N [mg/100 g sampel ] | 3.06±0.14 | 3.63±0.05 | 4.52±0.14 | 3.72±0.37 | 5.12±1.03 | 4.62±0.09 |
| *L** | 52.6±1.8c | 61.1±1.8b | 44.3±0.3c | 60.0±0.5a | 45.0±0.7c | 60.1±0.6a |
| *a** | -0.7±0.1 | -0.9±0.1 | -1.7±0.3 | -2.5±0.1 | -2.9±0.1 | -2.9±0.1 |
| *b** | 2.5±0.6 | 3.1±0.6 | -5.0±0.2 | -2.8±0.2 | -4.9±0.2 | 2.7±0.4 |
| TVC [log (CFU/g)] | 5.415 | 4.763 | 6.204 | 4.799 | 7.23 | 5.748 |
| **Quality parameter (2nd batch)** | Conventional washed | pH - shift process | Conventional washed | pH - shift process | Conventional washed | pH - shift process |
| surimi pH | 6.92±0.13c | 7.47±0.06a | 6.75±0.05d | 7.52±0.06a | 6.47±0.08e | 7.18±0.03b |
| moisture content (%) | 77.91±0.06b | 78.41±0.25a | 77.23±0.15c | 77.84±0.02b | 77.40±0.15c | 76.94±0.03d |
| water activity (Aw) | 0.984±0.002a | 0.983±0.008a | 0.982±0.003a | 0.984±0.001a | 0.986±0.002a | 0.987±0.006a |
| TVB-N [mg/100 g sampel ] | 5.64±0.00 | 6.00±0.51 | 9.62±1.50 | 7.87±0.35 | 11.27±1.92 | 11.57±0.21 |
| *L** | 51.3±1.3e | 68.2±0.2a | 52.3±0.5e | 63.2±0.9c | 53.2±0.4d | 66.1±0.5b |
| *a** | -1.9±0.3 | -3.6±0.0 | -2.3±0.3 | -3.4±0.3 | 2.0±0.2 | -2.6±0.1 |
| *b** | -2.2±1.0 | -3.8±0.2 | -4.8±0.5 | -3.8±0.3 | -6.0±0.3 | -4.9±0.3 |
| TPC [log (CFU/g)] | 6.255 | 5.623 | 6.633 | 6.322 | 7.531 | 7.255 |

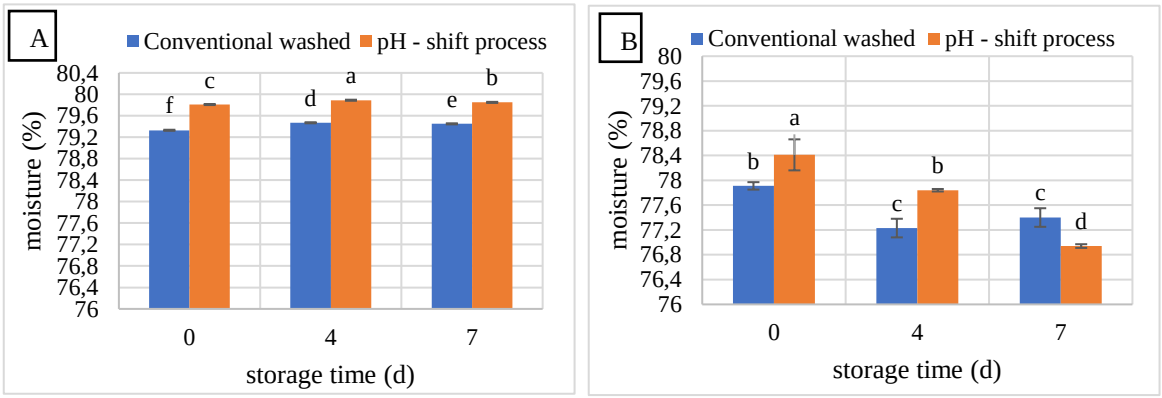
The errors bars indicate the standard deviation obtained from a total of three analyses. Means in the same row followed with the same letters are equals (P>0.05).




*Mean values ± SD (n=3). Different letters (a-e) indicate significant differences ($p<0.05$) between washing methods within the group of cod surimi prepared from batch 1 (A) and batch 2 (B).

Figure 3. pH value of surimi paste during refrigerated storage measured from batch 1 (A) and batch 2 (B) with different washing methods

Moisture content is another important factor influencing the formation of the surimi gel matrix. As can be seen in Table 2, paste made from cod mince washed methods during storage time showed significant differences in water content in both treatments (Fig. 4). Higher values of moisture contents obtained from the pH shift process increased during storage time. The cod surimi from batch 2 had lower moisture content and Aw during the storage time until day 4, but higher at the last day of storage time compare to data from batch 1 and significantly change during refrigerated store ($P<0.05$). The Aw value was not affected by washing methods and storage time ($P>0.05$). Aw value in both treatments were shown increased in the 2nd batch after refrigerated storage.

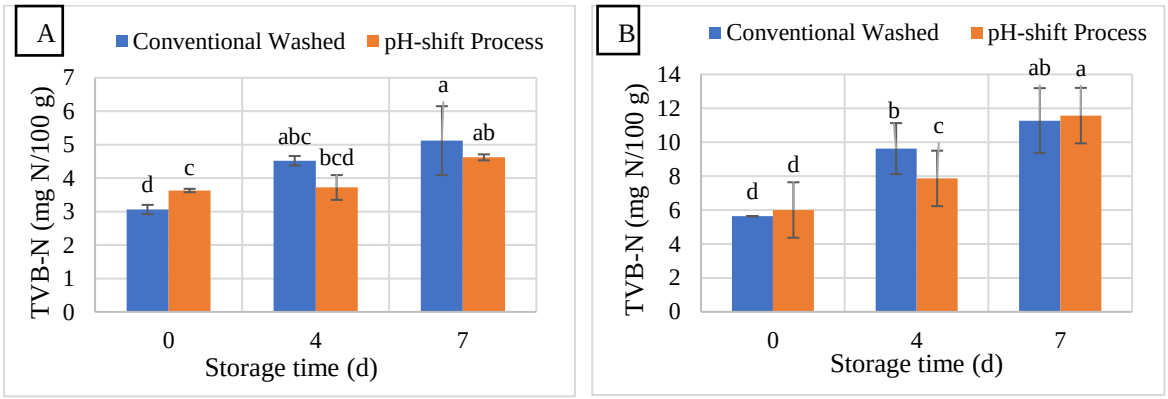



*Mean values ± SD (n=3). Different letters (a-e) indicate significant differences ($p<0.05$) between washing methods within the group of cod surimi prepared from batch 1 (A) and batch 2 (B).

Figure 4. Moisture content of surimi paste during refrigerated storage measured from batch 1 (A) and batch 2 (B) with different washing methods

During storage time (Fig. 5), the TVB-N production was affected by washing methods during refrigerated storage. Moreover, the TVB-N values of cod surimi from the pH-shift process increased at a slower rate compared with the cod surimi made with the conventional washing processes in both batches. There was a significant difference in TVB-N values showed among the treatments during refrigerated store ($P<0.05$). However, TVB-N values was overall

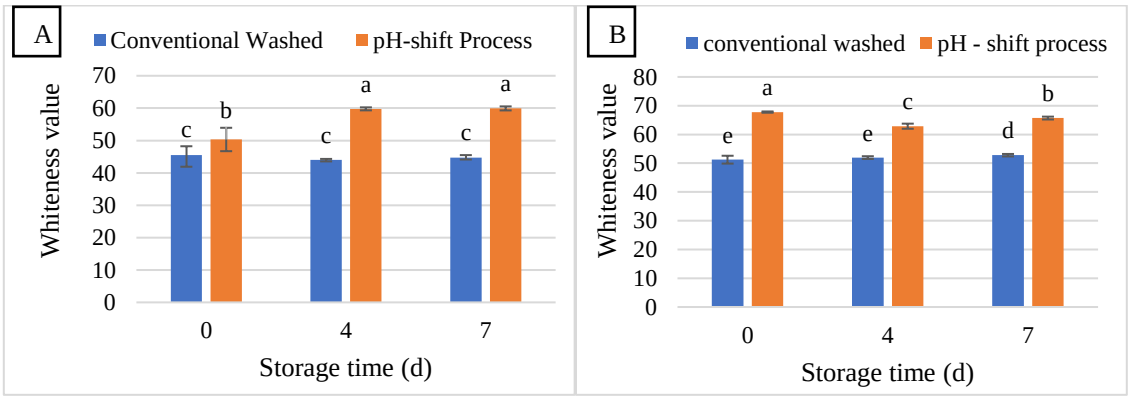
significantly higher in cod surimi made from the cod byproduct mince from the fish store than those of cod surimi byproduct mince made from the processor.




*Mean values ± SD (n=3). Different letters (a-e) indicate significant differences ($p<0.05$) between washing methods within the group of cod surimi prepared from batch 1 (A) and batch 2 (B).

Figure 5. TVB-N value of surimi paste during refrigerated storage measured from batch 1 (A) and batch 2 (B) with different washing methods

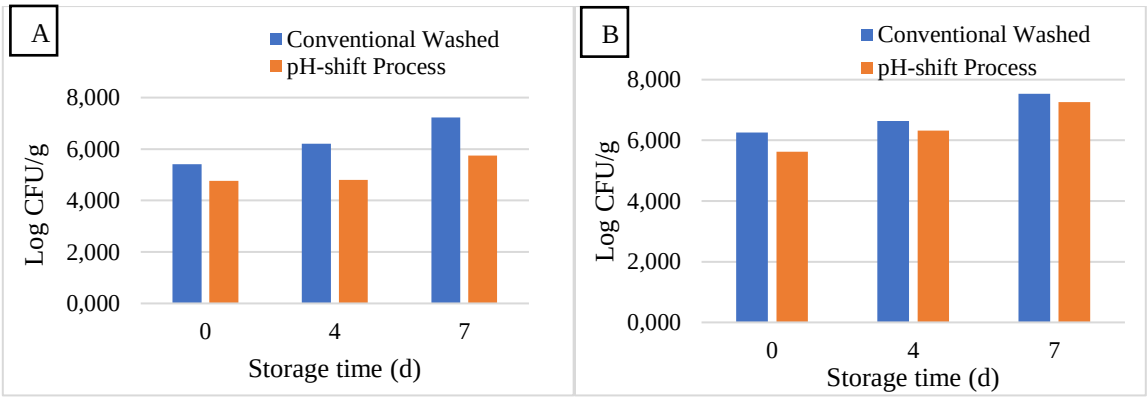
Whiteness results of cod surimi paste during refrigerated storage are presented in Table 2 and Figure 6. Cod surimi paste made from the pH shift process had higher lightness ($L^*$) in both batches ($P<0.05$). Redness ($a^*$) and yellowness ($b^*$) were significantly lower in cod surimi paste by conventional washing in both batches. After seven days of refrigerated store, the whiteness was increased significantly in both batches generally. Among surimi paste produced from two different washing processes, cod surimi paste made by conventional washing tended to be more stable in colour during the storage period.




*Mean values ± SD (n=3). Different letters (a-e) indicate significant differences ($p<0.05$) between washing methods within the group of cod surimi prepared from batch 1 (A) and batch 2 (B).

Figure 6. Whiteness value of surimi paste during refrigerated storage measured from batch 1 (A) and batch 2 (B) with different washing methods

The total viable counts (TVC) in both batches increased throughout seven days of refrigerated storage (Figure 7). Generally, cod surimi by the conventional washing method had more total bacteria than in the surimi produced by the pH-shift process, especially when the storage time increased. Moreover, total bacterial counts observed in batch 2 and were higher (5.623 – 7.531 log colony-forming units CFU/g)) due to the freshness of the byproduct mince and processing method.




*Mean values ± SD (n=3). Different letters (a-e) indicate significant differences (p<0.05) between washing methods within the group of cod surimi prepared from batch 1 (A) and batch 2 (B).

Figure 7. TV count of surimi paste during refrigerated storage measured from batch 1 (A) and batch 2 (B) with different washing methods.

### *4.1.3 Effect of different processing surimi methods and concentration of salt on colour of surimi paste*

The colour ($L^*$, $a^*$ and $b^*$ values) of cod surimi paste after salt addition and refrigerated storage was measured in both batches. As shown in Table 3 (Fig. 8), the highest value for whiteness at day 0 after thawing of the surimi was for the samples from pH-shift process (66.4) which contained 1.5% of salt, which is significantly different from samples made from conventional washed surimi (P<0.05). No significant difference (P>0.05) was seen for whiteness of samples containing 1.5% and 3% at day 0 and day 7 made from pH-shift processed. Lightness (L*) values all increased significantly (P<0.05) with salt addition in cod surimi made by conventional washing, where as a and b-values decreased during storage time. However, the L* value from cod surimi made from the pH-shift process decreased when the added salt concentrations increased after refrigerated store at day 4 and 7.

Table 3. Effects of different methods on processing and concentration of salt on colour of surimi paste in batch 1.

| Storage time (d) | Whiteness | Conventional washed | | | pH-shift process | | |
|---|---|---|---|---|---|---|---|
| | | 0% | 1.50% | 3% | 0% | 1.50% | 3% |
| 0 | *L** | 54.4±1.2 | 61.4±1.1 | 60.7±0.3 | 62.5±0.6 | 66.6±0.9 | 66.4±0.9 |
| | *a** | 1.8±0.2 | 1.2±1.3 | 1.4±0.1 | -2.9±0.2 | -2.8±0.2 | -2.6±0.1 |
| | *b** | 1.3±0.3 | -0.8±0.8 | -1.0±0.2 | -2.5±0.3 | -2.7±0.7 | -2.5±0.4 |
| 4 | *L** | 53.7±0.9 | 54.3±1.3 | 65.8±0.2 | 63.7±0.5 | 54.3±1.0 | 57.3±0.6 |
| | *a** | 1.9±0.2 | -1.7±0.2 | -3.4±0.1 | -2.5±0.2 | -3.0±0.2 | -1.9±0.2 |
| | *b** | 1.6±0.4 | 2.6±0.4 | -4.3±0.3 | -1.3±0.4 | -3.7±0.4 | -2.9±0.4 |
| 7 | *L** | 56.7±1.0 | 56.3±0.7 | 58.7±1.0 | 66.3±0.8 | 65.4±0.6 | 65.5±0.4 |
| | *a** | 1.9±0.1 | 1.9±0.2 | 1.4±0.1 | -2.7±0.1 | -3.5±0.2 | -3.1±0.1 |
| | *b** | 1.6±0.2 | 2.4±0.4 | 1.2±0.4 | -1.3±0.4 | -4.9±0.4 | -3.8±0.3 |

The errors bars indicate the standard deviation. Means in the same row followed with the same letters are equals (P>0.05).

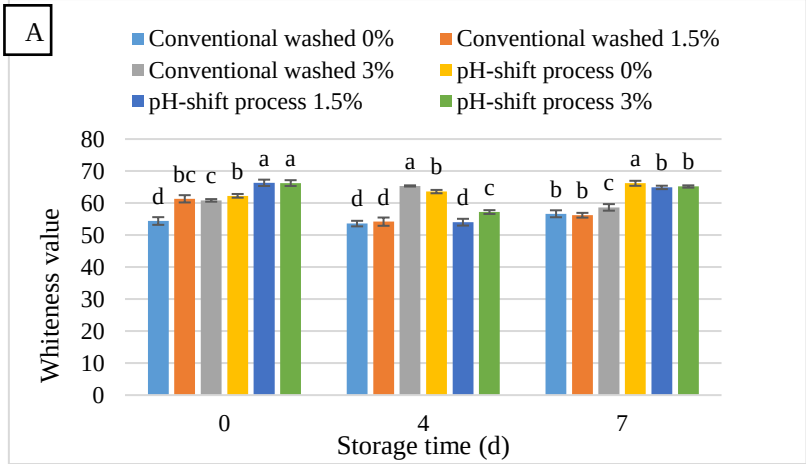



Figure 8. Effects of different methods on processing and concentration of salt on color of surimi paste in batch 1

In cod surimi paste made from the pH-shift process in batch 2, the addition of salt resulted in no change in whiteness value relative to cod surimi made from the conventional washed process (Figure 9). However, final storage of the whiteness value of cod surimi in both treatments were considerably higher ($P<0.05$). Moreover, addition of salt concentration to surimi that had been stored caused the whiteness of cod surimi made from conventional washed surimi to increase slightly.

Table 4. Effects of different methods on processing and concentration of salt on colour of surimi paste in batch 2.

| Storage time (d) | Whiteness | Conventional washed | | | pH-shift process | | |
|---|---|---|---|---|---|---|---|
| | | 0% | 1.50% | 3% | 0% | 1.50% | 3% |
| 0 | *L** | 57.3±0.7 | 62.5±1.0 | 59.6±0.7 | 65.6±0.3 | 66.2±0.4 | 66.5±0.5 |
| | *a** | -1.9±0.2 | -1.9±0.1 | -1.8±0.2 | -3.0±0.1 | -3.1±0.2 | -3.2±0.1 |
| | *b** | -2.1±0.3 | -1.6±0.3 | -2.3±0.4 | -2.9±0.4 | -3.3±0.2 | -3.6±0.3 |
| 4 | *L** | 58.1±1.1 | 58.6±0.9 | 59.7±1.0 | 69.3±0.4 | 68.8±0.4 | 68.8±0.9 |
| | *a** | -1.6±0.2 | -2.0±0.0 | -1.5±0.2 | -3.1±0.1 | -3.6±0.1 | -3.0±0.3 |
| | *b** | -2.6±0.4 | -2.7±0.3 | -2.6±0.6 | -2.3±0.2 | -3.7±0.2 | -3.3±0.3 |
| 7 | *L** | 61.8±0.6 | 61.5±0.6 | 61.1±0.8 | 71.6±0.3 | 71.9±0.6 | 71.0±0.5 |
| | *a** | -2.2±0.1 | -2.3±0.1 | -2.0±0.1 | -2.5±0.1 | -2.9±0.1 | -3.3±0.1 |
| | *b** | -1.7±1.6 | -2.5±0.5 | -2.7±0.4 | -1.9±0.2 | -2.8±0.4 | -3.6±0.3 |

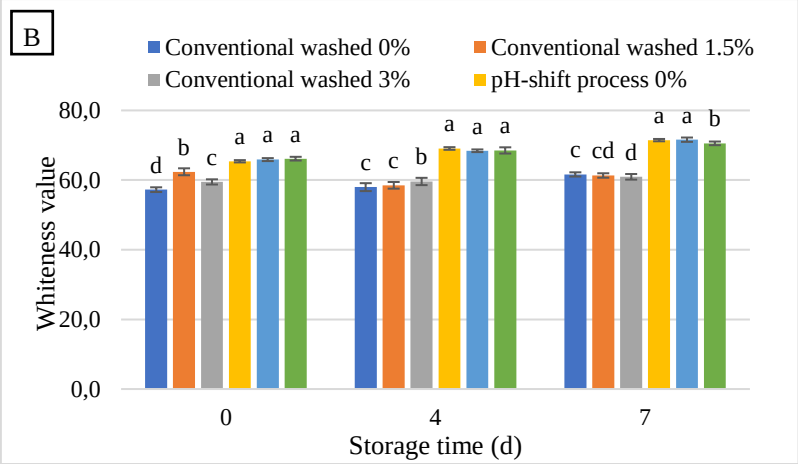



Figure 9. Effects of different methods on processing and concentration of salt on color of surimi paste in batch 2.

## 4.2 The effect of different methods of processing surimi on surimi gels properties

### *4.2.1 Effect of different methods on cod surimi gels and concentration of salt on moisture content and water activity*

Table 5 shows the moisture content and water activity (Aw) of cod surimi gels from the 2nd batch only ($P<0.05$). There was a significant decrease of moisture content and value of water activity with the salt addition in both cod surimi sample. Conventionally washed cod surimi samples were significantly lower compare to pH-shift process. Higher salt content was enough to unfold the proteins and expose the reactive groups, which were able to form more bonds by themselves (Cando, Herranz, Borderias, & Moreno, 2016).

Table 5. Effect of different methods on washing surimi and concentration of salt on moisture content and water activity (Aw).

| Quality Paramaters | Storage time (d) | Conventional washed | | | pH-shift process | | |
|---|---|---|---|---|---|---|---|
| | | 0% | 1.50% | 3% | 0% | 1.50% | 3% |
| Moisture content (%) | 0 | 77.55±0.19b | 76.63±0.09c | 77.63±0.21d | 78.82±0.22a | 78.21±0.13a | 76.78±0.48c |
| | 4 | 76.80±0.97abc | 75.83±0.28bc | 75.55±0.08c | 77.69±0.23a | 77.11±0.05ab | 77.35±0.03a |
| | 7 | 78.10±0.19a | 76.98±0.41b | 75.87±0.01c | 78.41±0.09a | 77.12±0.03b | 75.97±0.01c |
| Water activity (Aw) | 0 | 0.984±0.004a | 0.977±0.001b | 0.966±0.002c | 0.983±0.003a | 0.974±0.000b | 0.961±0.002c |
| | 4 | 0.986±0.005a | 0.976±0.002b | 0.964±0.003c | 0.986±0.001a | 0.977±0.003b | 0.967±0.004c |
| | 7 | 0.982±0.005a | 0.972±0.006ab | 0.964±0.001b | 0.982±0.003a | 0.972±0.006ab | 0.963±0.001b |

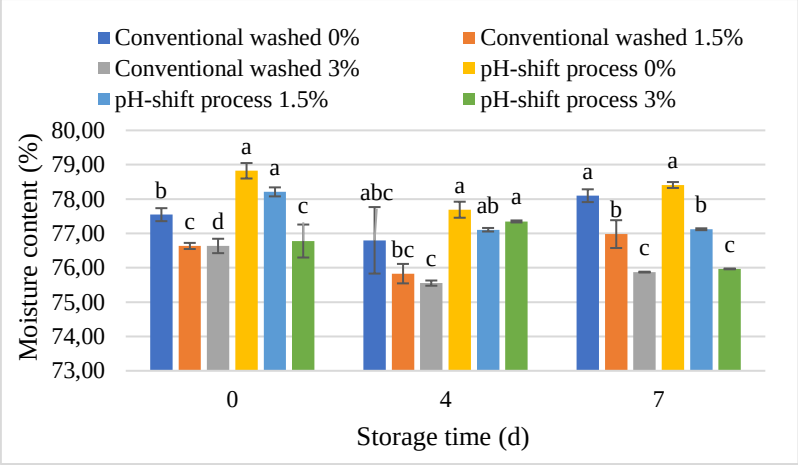



Figure 10. Effect of different methods on washing surimi and concentration of salt on moisture content and water activity (Aw).

### 4.2.2 *Effect of different methods on processing surimi, storage, and concentration of salt on cooking loss and water holding capacity*

Cooking loss of surimi gels is considered the reduction of moisture and indicates changes in protein interaction during the cooking process. In Batch 1 (Table 6), the conventionally washed surimi and pH shift treatments with cooking experienced a small percentage of cooking loss (±1-2% approx.), this applies for all treatements at 0, 4, 7 days of storage (Fig 11). No significant change in cooking loss at day 0 for all treatments after steaming at $90^0$C for 20 min occured (P>0.05). However, cooking loss values decreased in the pH shift treatments with increasing salt concentration.

Table 6. Effect of different methods on processing surimi and concentration of salt on cooking loss in batch 1.

| Quality Paramaters | Storage time (d) | Conventional washed | | | pH-shift process | | |
|---|---|---|---|---|---|---|---|
| | | 0% | 1.50% | 3% | 0% | 1.50% | 3% |
| Cooking loss (%) | 0 | 1.70±1.48 | 1.41±2.13 | 0.89±0.94 | 1.35±0.76 | 0.43±0.44 | 0.23±0.16 |
| | 4 | 2.35±0.47b | 5.60±0.59a | 2.32±2.88b | 1.88±1.12bc | 2.83±0.57b | 0.69±0.27c |
| | 7 | 1.62±0.88a | 1.60±0.94a | 1.77±0.55ab | 0.48±0.30b | 0.70±0.19a | 0.62±0.22b |

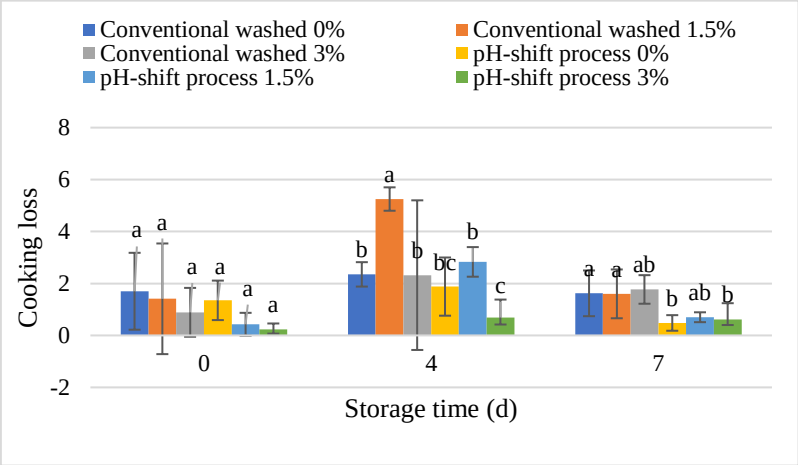



Figure 11. Effect of different methods on processing surimi and concentration of salt on cooking loss in batch 1.

In Batch 2 (Table 7), cooking losses followed a different trend and this is likely due to the freshness of the material. With storage and decrease of pH, the functionality of the proteins may decrease to a greater extent. Cooking loss was minimal on day 0 in the conventional treatment group, and with storage cooking loss increased to 9.49% in water loss in the 0% salt treatment group ($P<0.05$) in the day 7 material. The addition and increase in salt helped in the functionality. In the pH shift group there was also an increase in cooking loss with cold storage and salt concentration. It was observed that there was less cooking loss in the pH shift group from day 0 and 4 relative to the conventional treatment group ($P<0.05$). This could be due to a greater degredation of the myofibrillar proteins with greater decreasess in conventional surimi relative to the pH shift process.

Table 7. Effect of different methods on processing surimi and concentration of salt on cooking loss in batch 2.

| Quality Paramaters | Storage time (d) | Conventional washed | | | pH-shift process | | |
|---|---|---|---|---|---|---|---|
| | | 0% | 1.50% | 3% | 0% | 1.50% | 3% |
| Cooking loss (%) | 0 | 2.13±1.09a | 1.41±2.13b | 0.89±0.94ab | -1.55±0.47c | -1.84±1.80cd | -3.04±1.11d |
| | 4 | 5.73±2.31a | 5.25±0.45b | 2.32±2.88bc | 0.27±1.31c | -1.85±1.38e | -1.50±1.40de |
| | 7 | 9.49±0.94a | 1.60±0.94cd | 1.77±0.55cd | 6.47±4.17bc | 8.44±4.01ab | 3.37±3.15d |

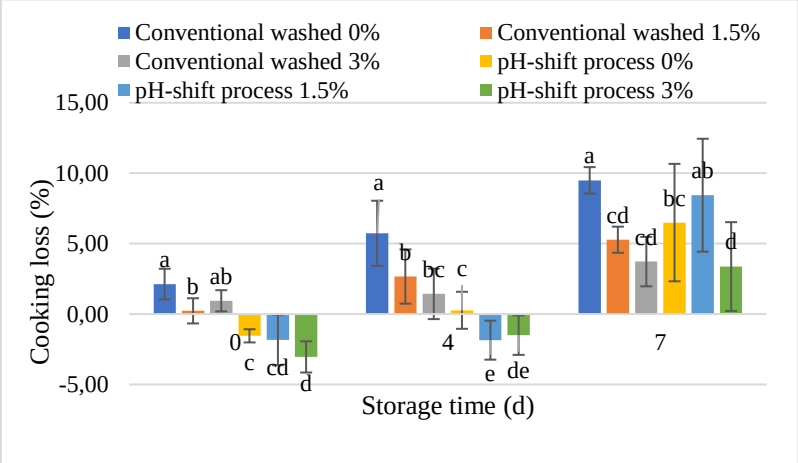



Figure 12. Effect of different methods on processing surimi and concentration of salt on cooking loss in batch 2.

Water holding capacity (WHC) of surimi gels can be defined as the ability of a protein gel to retain water. WHC tended to increase slightly with increasing addition of salts in conventionally washed surimi treatments during refrigerated storage ($P<0.05$). This indicates an enhanced capacity of gel network to retain water. As shown in Fig 13., WHC of cod surimi gels obtained by pH-shift process showed lower WHC especially with the addition of salt. The lowest WHC observed in gels samples obtained using pH shift process at day 7 might be related to partial denaturation of myofibrillar proteins and exposure of hydrophobic sites which could reduce WHC of the gel network.

Table 8. Effect of different methods on processing surimi and concentration of salt on WHC in batch 2.

| Quality Paramaters | Storage time (d) | Conventional washed | | | pH-shift process | | |
|---|---|---|---|---|---|---|---|
| | | 0% | 1.50% | 3% | 0% | 1.50% | 3% |
| WHC (%) | 0 | 84.3±1.54bc | 91.7±0.79ab | 92.5±1.29a | 92.7±0.20ab | 85.4±1.47abc | 82.1±0.00c |
| | 4 | 79.0±2.95a | 85.8±1.03ab | 88.0±3.11a | 84.9±2.7ab | 81.7±7.04ab | 79.6±0.18b |
| | 7 | 81.8±1.55b | 92.0±0.74a | 92.3±0.83a | 79.1±1.34c | 70.7±1.04e | 74.4±1.52d |

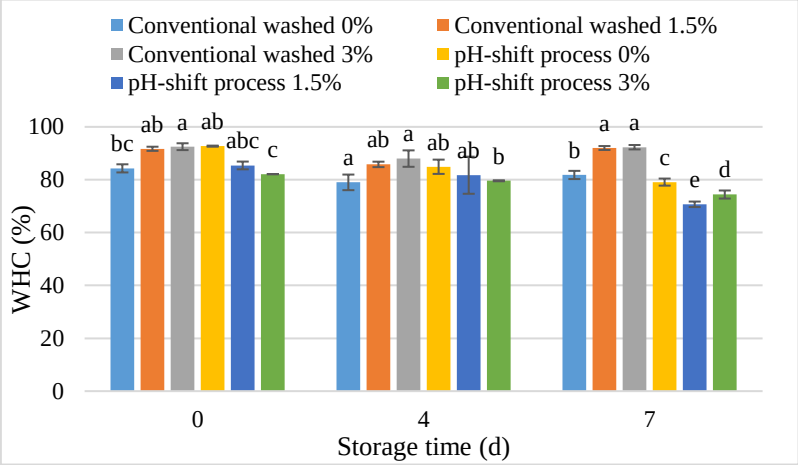



Figure 13. Effect of different methods on processing surimi and concentration of salt on WHC in batch 2.

### *4.2.3 Effect of different methods on processing surimi and concentration of salt on colour of surimi gels*

Proper colour is considered a critical quality attribute for surimi-based products due to strongly affected the consumer acceptance. Surimi is made by washing deboned fish mince with water to remove myoglobin, fat, and other impurities; thus, increasing lightness (L*) and decreasing redness (a*) and yellowness (b*) (Tahergorabi R. , Sivanandan, Beamer, Matak, & Jaczynski, 2012). The colours of the cod surimi gels from batch 1 presented in Table 9. Different washing processes had produced differences in whiteness during heat treatment (Fig.14) ($P<0.05$). As the salt concentration increased, the gel whiteness values showed an increasing tendency ($P<0.05$), except for the cod surimi made from conventional washed at day 4. Treatment with conventional washing along with the increasing of salt concentration produced significantly ($P<0.05$) higher whiteness nearly in all cod surimi gels when compared to the pH shift process group. Lightness (*L**) and yellowness (*b**) were higher in treatments of conventionally washed, while the redness (*a**) was higher in treatments of pH-shift process.

Table 9. Effect of different methods on processing surimi and concentration of salt on colour of surimi gels in batch 1.

| Storage time (d) | Whiteness | Conventional washed | | | pH-shift process | | |
|---|---|---|---|---|---|---|---|
| | | 0% | 1.50% | 3% | 0% | 1.50% | 3% |
| 0 | *L** | 70.2±1.2 | 74.8±1.1 | 74.4±0.9 | 56.1±0.7 | 64.7±0.4 | 63.8±0.9 |
| | *a** | -3.9±0.2 | -3.7±0.2 | -3.9±0.2 | -2.2±0.1 | -2.5±0.1 | -2.6±0.1 |
| | *b** | 0.2±0.5 | 0.87±0.4 | 0.9±0.2 | -2.7±0.3 | -0.8±0.5 | -1.9±0.3 |
| 4 | *L** | 70.0±0.9 | 74.2±0.5 | 75.2±0.6 | 55.3±0.9 | 631±0.3 | 66.0±1.5 |
| | *a** | -4.1±0.1 | -3.7±0.1 | -3.1±0.1 | -2.2±0.5 | -2.7±0.2 | -2.4±0.4 |
| | *b** | 1.5±0.2 | 2.4±0.1 | 1.6±0.5 | -2.4±0.7 | -1.1±0.5 | 0.5±0.4 |
| 7 | *L** | 72.3±0.6 | 74.7±0.5 | 74.8±0.6 | 59.0±0.5 | 62.9±1.0 | 63.4±0.5 |
| | *a** | -4.0±0.1 | -3.7±0.1 | -3.5±0.1 | -2.2±0.2 | -1.9±0.1 | -1.6±0.1 |
| | *b** | 1.1±0.2 | 1.6±0.4 | 1.5±0.2 | -2.6±0.3 | -1.1±0.3 | -0.8±0.2 |

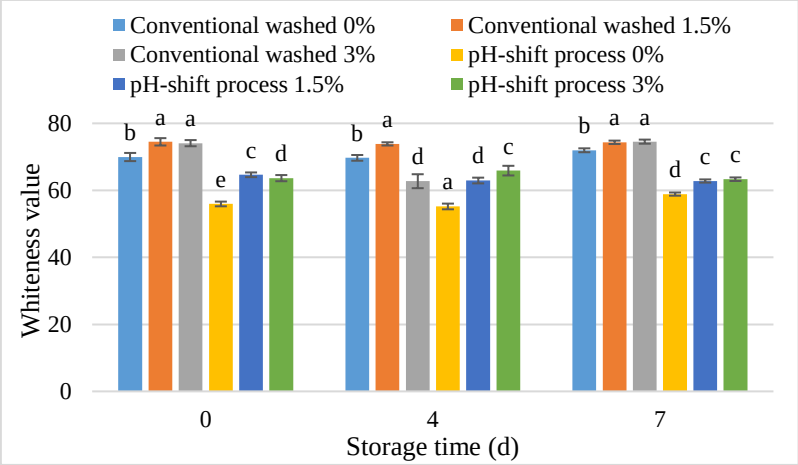



Figure 14. Effect of different methods on processing surimi and concentration of salt on colour of surimi gels in batch 1.

The highest whiteness of gel was found in cod surimi treated by conventional washed process especially with the highest concentration of salt ($P<0.05$; Table 2). Whiteness of surimi gels from conventionally washed group significantly increased with the addition of salt ($P<0.05$; Table 10). Compared to with the cod surimi paste, the whiteness value of cod surimi gels by the conventional washed methods was higher. This result might be due to heat process that had an effect on whiteness of cod surimi when considered in the different washing treatments (Fig. 15).

Table 10. Effect of different methods on processing surimi and concentration of salt on colour of surimi gels in batch 2.

| Storage time (d) | Whiteness | Conventional washed | | | pH-shift process | | |
|---|---|---|---|---|---|---|---|
| | | 0% | 1.50% | 3% | 0% | 1.50% | 3% |
| 0 | *L** | 67.3±0.4 | 72.1±0.7 | 70.6±0.7 | 61.4±0.5 | 66.6±0.4 | 65.4±0.8 |
| | *a** | -4.0±0.1 | -3.5±0.1 | `-3.5±0.1 | -3.6±0.2 | -4.1±0.1 | -4.3±0.2 |
| | *b** | -1.7±0.2 | -1.0±0.3 | `-1.6±0.3 | -4.1±0.4 | -2.7±0.4 | -3.4±0.4 |
| 4 | *L** | 65.7±0.6 | 68.0±0.8 | 69.5±0.5 | 60.4±0.4 | 64.8±0.6 | 67.2±0.5 |
| | *a** | -3.7±0.2 | -3.5±0.1 | `-3.5±0.1 | -4.1±0.2 | -4.2±0.1 | -4.2±0.1 |
| | *b** | -1.8±1.1 | -1.8±0.2 | `-1.8±0.2 | -4.1±0.4 | -2.5±0.2 | -2.6±0.4 |
| 7 | *L** | 68.7±0.6 | 71.2±0.6 | 71.9±0.6 | 64.4±0.6 | 66.9±0.3 | 68.9±0.7 |
| | *a** | -3.7±0.1 | -3.5±0.1 | `-3.5±0.1 | -3.9±0.3 | -3.9±0.1 | -4.1±0.1 |
| | *b** | -1.3±0.2 | -0.8±0.4 | `-1.2±0.4 | -2.7±0.4 | -2.8±0.5 | -1.9±0.3 |

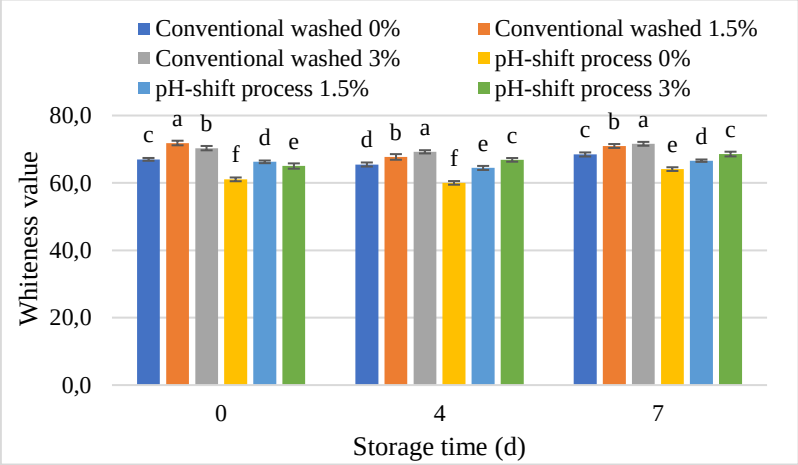



Figure 15. Effect of different methods on processing surimi and concentration of salt on colour of surimi gels in batch 2.

### *4.2.4 Effect of different methods on processing surimi and concentration of salt on texture profile of surimi gels*

In Batch 1 (Fig.16), from day 0 to day 7, there was an increase in hardness of the printed gel, which can be an indicator of gel strength in the conventionally washed material with 0, 1.5, and 3% salt ($P<0.05$). The highest amount of hardness was measured for gels made of conventionally washed with 3% salt (16.00 kg) and pH-shift process with 3% of salt (12.64 kg) after refrigerated storage for 7 days. The greater increases were seen in the conventionally washed groups which contained a greater amount of salt ($p<0.05$). On day 7, there was an observed decrease in hardness in the conventional surimi 0% salt printed surimi gels. This is likely due to the inability of the proteins to form a gel relative to the myofibrillar proteins that have enhanced functionality with additions of salt. In the pH shift treatment, there was similar hardness trend amongst the treatments relative to the conventional washed surimi of all salt concentrations. With increasing salt concentration there were increases in hardness observed similar to the conventionally washed surimi. The conventional surimi had the greatest hardness relative to the other treatment groups.

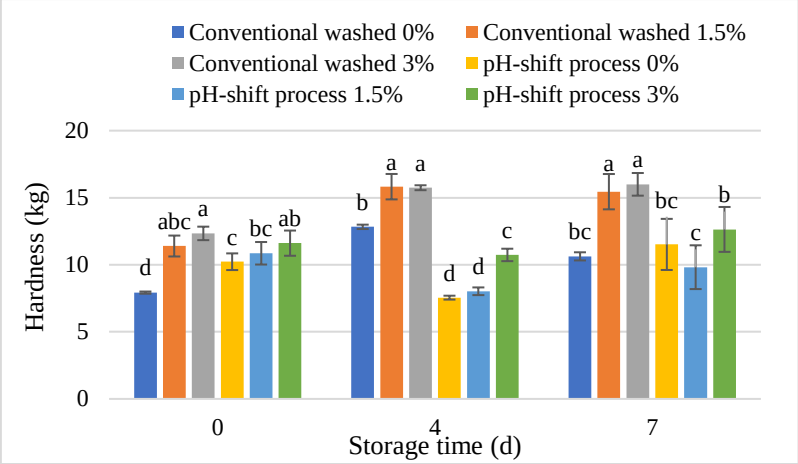



Figure 16. Effect of processing methods and concentration of salt on hardness* of surimi gels during storage time in 1st batch. Data are given as mean values ± standard deviation (SD, n=3). Small bars of the top of the data bars indicate SD.

Batch 2 trends followed the same trends and batch 1 (Fig.17). However, Batch 1 resulted in harder gels. The exception to the trend in gel hardness was on day 7, in which the surimi gel in the pH shift treatment in batch 2 did not print well (in shape definition, formula texture, or in height). This is likely due to cross linking or degradation and significant aggregation with storage due to lower quality starting material.

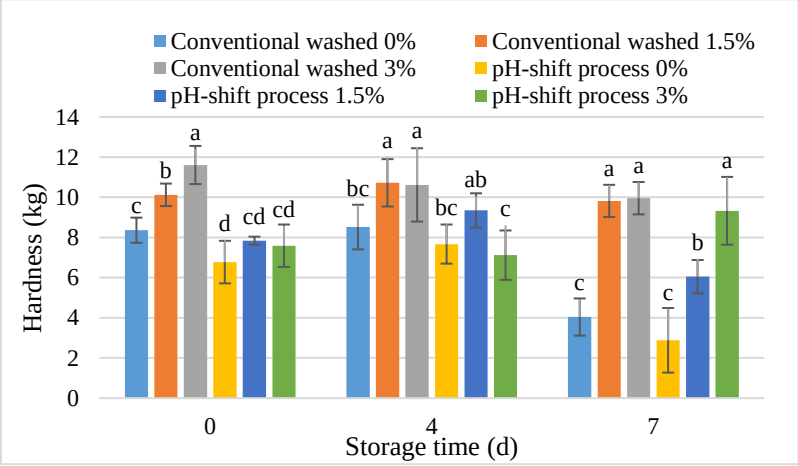



Figure 17. Effect of processing methods and concentration of salt on hardness* of surimi gels during storage time in 2nd batch. Data are given as mean values ± standard deviation (SD, n=3). Small bars of the top of the data bars indicate SD.

## 4.3 Printability and Application

### *4.3.1 Printability of cod surimi pastes*

Representative pictures of printed cod surimi paste are shown in Fig 18. In batch 1, a clear distinction between conventionally washed (CW) obtained surimi and the pH-shift (PS) surimi can be identified. Cod surimi paste made by conventional washing was easy to print, gave softer texture and there was a slight variation in prints when salt was added into the surimi, specifically more spreading. On comparing with cod surimi paste by the pH-shift process, printed surimi stars showed more gaps between deposited layers. Samples were sticky, and the material did not fully keep its shape after printing. In batch 2, Figure 19, the pH shift stars

were more viscous in texture and it is evident that there is more definition in the extruded surimi.

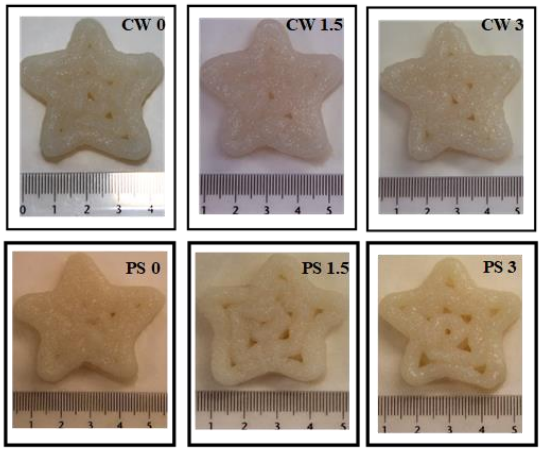



Figure 18. Object printed of cod surimi paste made of conventional washed (CW) and pH-shift process (PS) and salt addition (0%, 1.5% and 3% in batch 1).

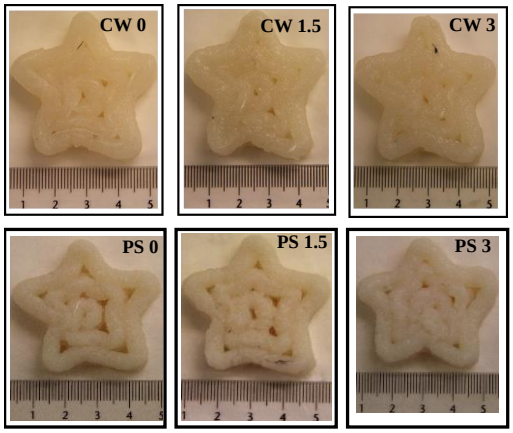



Figure 19. Object printed of cod surimi paste made of conventional washed (CW) and pH-shift process (PS) and salt addition (0%, 1.5% and 3% in batch 2).

Using low quality starting material in batch 2 and with storage, printability was significantly different from other printed forms amongst both treatments for the PS surimi. The printability of the PS surimi after refrigerated storage for 7 days was poor (Fig. 20). A continuous flow of surimi paste through the nozzle was prevented by the texture of aggregated and/or cross-linked proteins. With salt addition, there was some improvement in printability due to the salt opening the protein structure such that the surimi paste was more flowable and homogenous in texture. According to the above evidence, it probably explained that a starting material plays a key role for surimi 3D printed. Because the PS surimi did not demonstrate a printable sample, it is not surprising that the WHC value also lower than other treatments (Fig. 13).

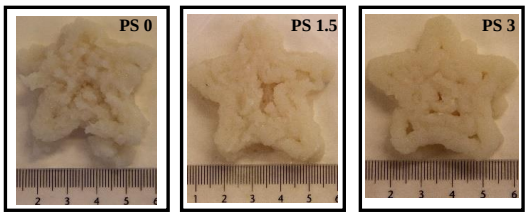
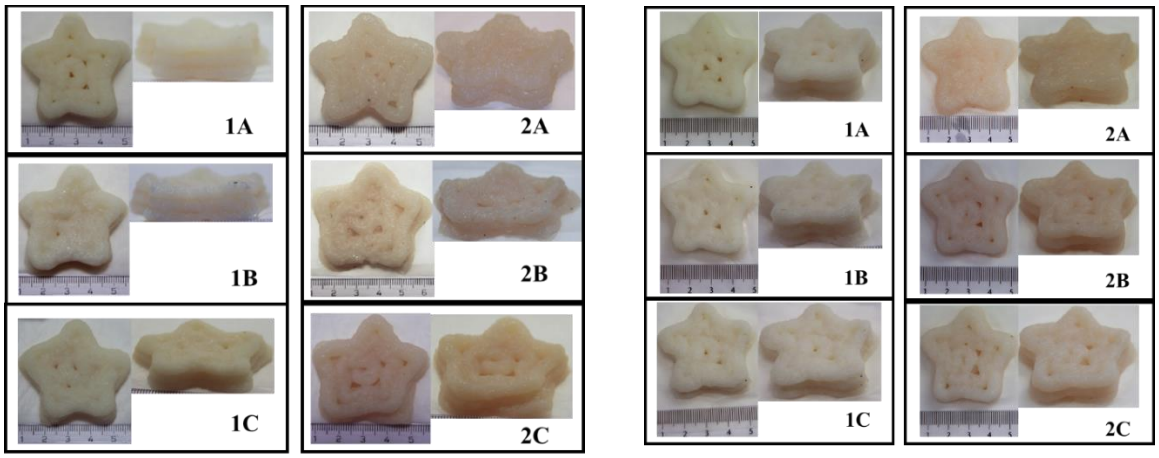


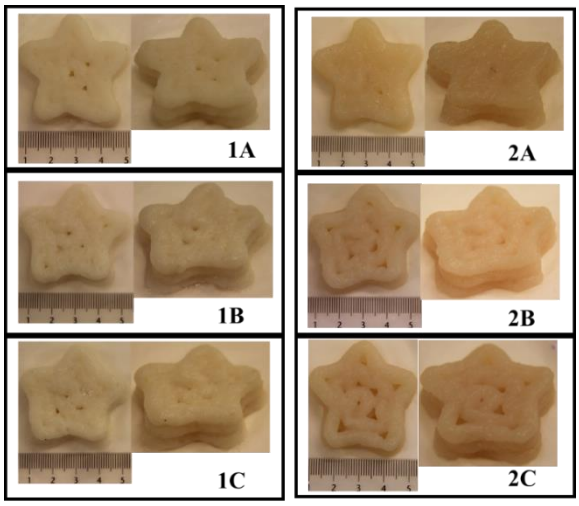

Figure 20. Object printed of cod surimi paste made of pH-shift process (PS) with salt addition (0%, 1.5% and 3%) at day 7 in batch 2.

### *4.3.2 Cooking application of surimi pastes*

After printing, there was a 30-minute setting time followed by cooking in a steam oven at 90°C for 20 minutes. A setting time can improve the gel strength with cooking (Kim, Park, & Yoon, 2005). The cooking of the star surimi paste is different relative to conventional surimi gels. When making traditional surimi gels, surimi paste is stuffed into tubes or casing that is sealed off and then is placed in a water bath. Due to the shape and nature of the 3D printed star gel, conventional surimi cooking was not applied. The surimi gels for the various treatments (Fig. 21) are shown. It is evident that by day 7 in the pH shift treatment, 0% salt, the surimi paste was barely printable. Amongst the treatments, there are differences in definition, height, colour, and width of the stars. True acceptability of these surimi gels for shape, definition and appeal, colour, taste, smell, etc. would need to undergo a consumer panel analysis and more texture analysis as well. The steam oven cooking method was indeed applicable to the cooking of the surimi star gels as they were indeed able to maintain shape and form strong gels. Other cooking applications should be tested in the future for not only a star shape and cooking time, but setting time, and parameters should also be investigated further.

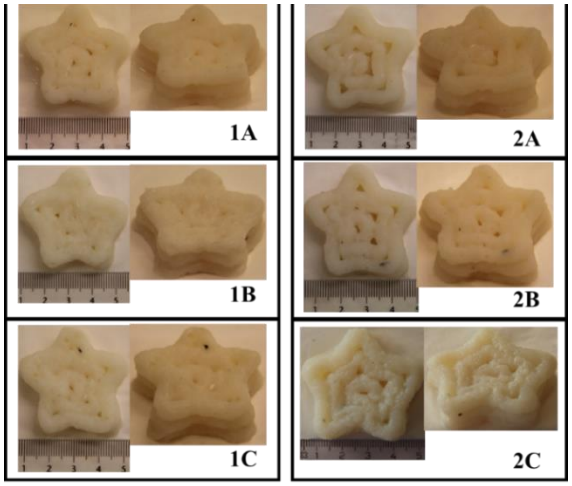



**Day 0** **Day 4**

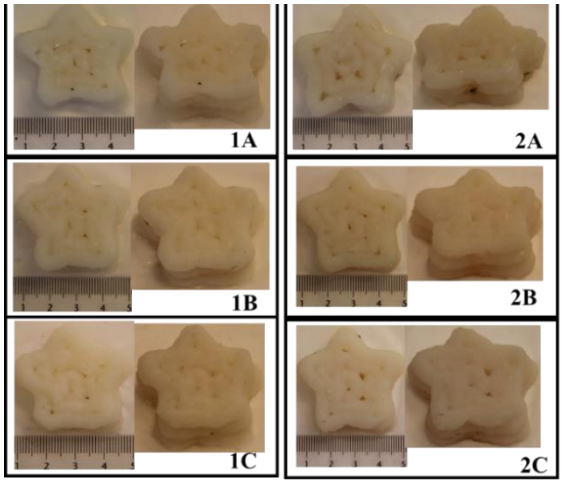



**Day 7**

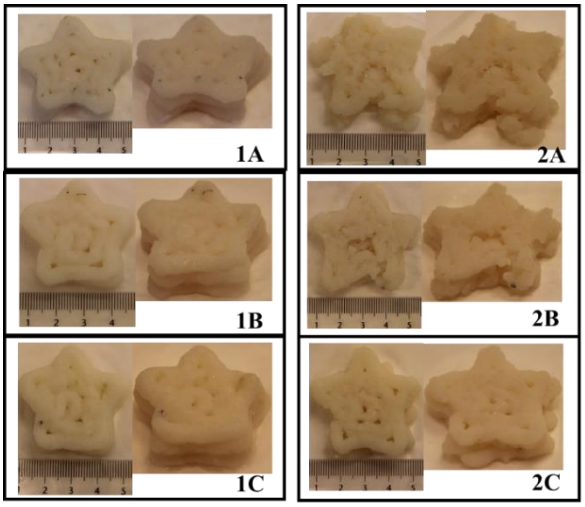
Figure 21. Effect of different methods on processing surimi and addition of different levels of salt on geometrical shapes of 3D printed cod surimi gel samples in batch 1 (1A = conventionally washed 0%, 1B = conventionally washed 1.5%, 1C = conventionally washed 3%; 2A = pH-shift process 0%, 2B = pH-shift process 1.5%, 2C = pH-shift process 3%).




**Day 0**




**Day 4**




**Day 7**

Figure 22. Effect of different methods on processing surimi and addition of different levels of salt on geometrical shapes of 3D printed cod surimi gel samples in batch 2 (1A = conventionally washed 0%, 1B = conventionally washed 1.5%, 1C = conventionally washed 3%; 2A = pH-shift process 0%, 2B = pH-shift process 1.5%, 2C = pH-shift process 3%)

The chosen cooking parameters were not only for optimal gelling of the surimi but were also implemented for food safety. The cooking method was effective in reducing the TVC count to safe levels for food consumption. TVC of the raw surimi from both treatments were $4.2x10^6$ – $2.1x10^7$ CFU/g for cod surimi made from pH-shift process and $1.8x10^7$ - $3.4x10^8$ CFU/g for cod surimi made from conventional process. This number was higher than other reported values. Shie and Park (Shie & Park , 1999) reported that APC of surimi seafood was $2x10^6$ CFU/g. Season, sources, grades and processing procedures can result in differences in the microbial quality of surimi. Almost all (99%) of TVC was destroyed by the initial cooking, after steamed heat at 90 $^oC$ for 20 min (Table 11). Therefore, we expected the TVC to be zero after 20 min of steaming, since the product need to be ready to eat. The time required to destroy pathogenic microorganisms in food products is mainly dependent on the initial microbial load, composition of the microbial population, and the target F-value (Rippen & Hackney, 1992).

Table 11. TVC values for surimi paste before and after cooking.

| | Day 0 | | Day 4 | | Day 7 | |
|---|---|---|---|---|---|---|
| | Conventional washed | pH - shift process | Conventional washed | pH - shift process | Conventional washed | pH - shift process |
| Before cook | $4.2x10^6$ | $1.8x10^7$ | $4.3x10^7$ | $2.1x10^7$ | $3.4x10^8$ | $1.8x10^7$ |
| After cook | <10 | <10 | <100 | $2x10^2$ | <100 | <100 |

*Storage time at 0, 4 and 7 days without added salt for the pH-shift and conventional surimi (composite samples at 30°C)

## 4.4 The effect of starting raw material in surimi paste and methods of processing surimi

### *4.4.1 NIR analysis of surimi paste and surimi gels*

Near infrared (NIR) reflectance spectra of the samples were obtained from the various samples of both raw material batches. All spectra were maximum normalised, and baseline corrected (Figure 23). Characteristic peaks for the stretching of the O-H bonds in the water molecules present were observed at 10,000 $cm^{-1}$, as well as at 7,000 $cm^{-1}$. Effects of lipid O-H stretching was furthermore observed at approximately 8,300 $cm^{-1}$, as well as around 4,400 $cm^{-1}$, and the effects of stretching and bending of various proteins in the range from 4,000 to 4,300 $cm^{-1}$.

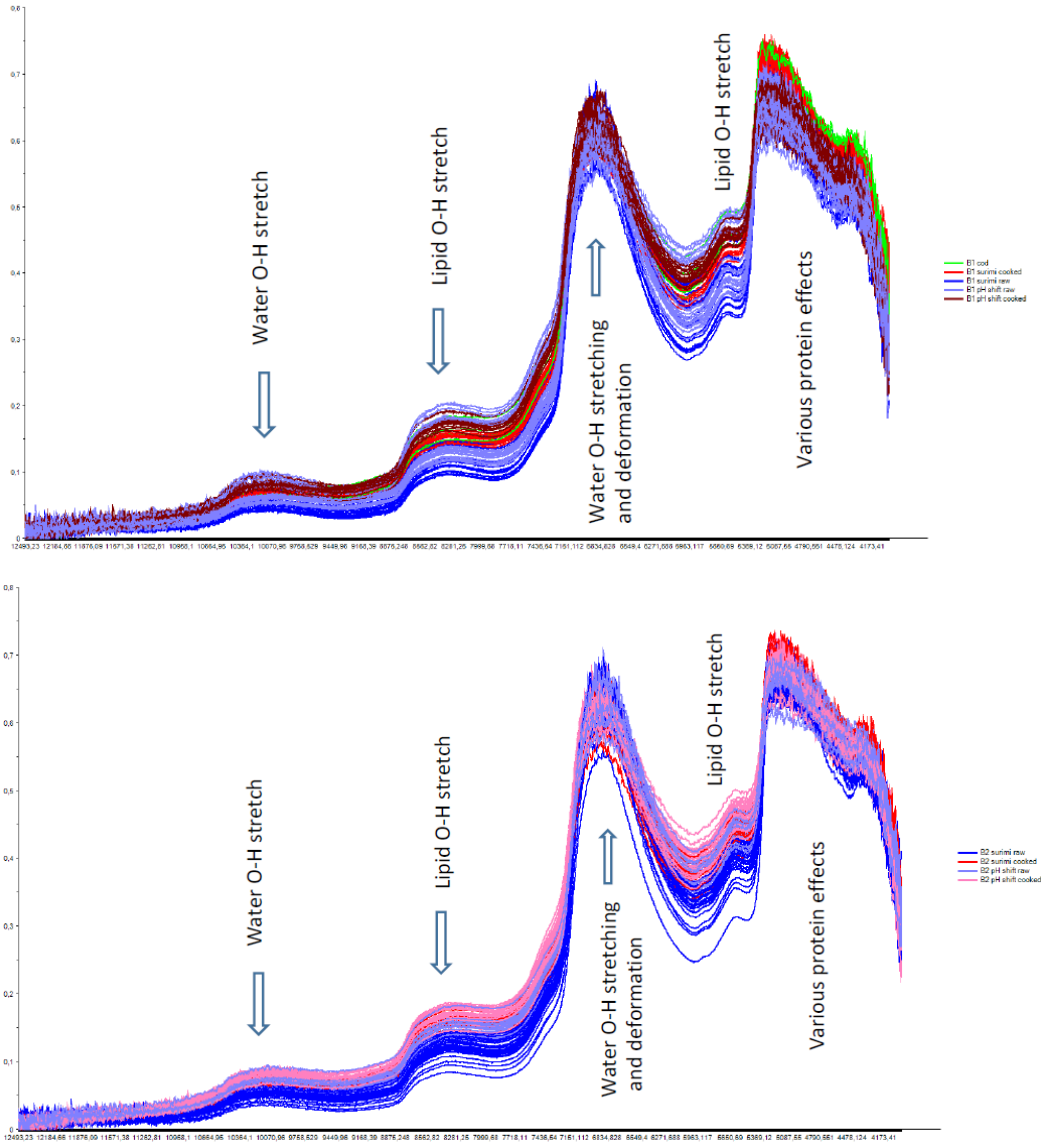



Figure 23. NIR spectra of samples from batch 1 (top) and batch 2 (bottom). All spectra were maximum normalised and baseline corrected prior to further analysis. The blue colours indicate the raw, uncooked samples, while red/pink colours represent cooked samples.

### *4.4.2 Principal component analysis of NIR results*

A principal component analysis (PCA) was then performed to study the similarities and differences between the samples in further detail (Fig. 24). The analysis revealed a clear distinction between the materials used in the two batches. Furthermore, the wide distribution of the samples in batch 1, indicated more variation within the samples of this batch. Since a clear distinction between the two batches were observed it was decided to analyse them further separately (Fig. 25).

The analysis showed that the samples could be distinguished by their NIR spectra, both with regards to their production processes (surimi vs. pH shift), and whether they were raw or cooked. Analysis of the spectral loadings (not shown) indicated that these changes were mostly reflected in changes in the earlier mentioned water peaks, especially the dominating peak at approximately 7,000 $cm^{-1}$, as well as by structural changes in the protein peak spectral range (4,000-4,300 $cm^{-1}$). This indicates that the processing and cooking processes had an effect on

the state of both proteins and water in the samples. However, analysis by other methods is needed to answer in what way these parameters were affected by the production and/or heating processes. Therefore, low field NMR transversal relaxation time analysis was also applied to shed further light on the effects of the treatments on protein denaturation and water distribution within the samples.

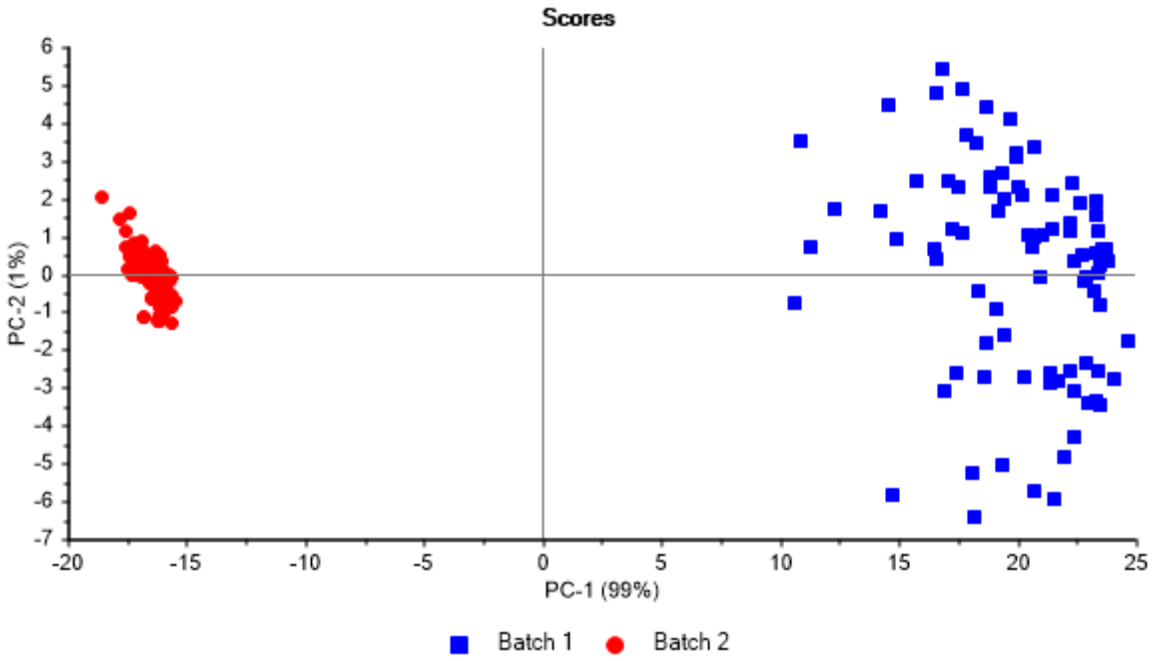
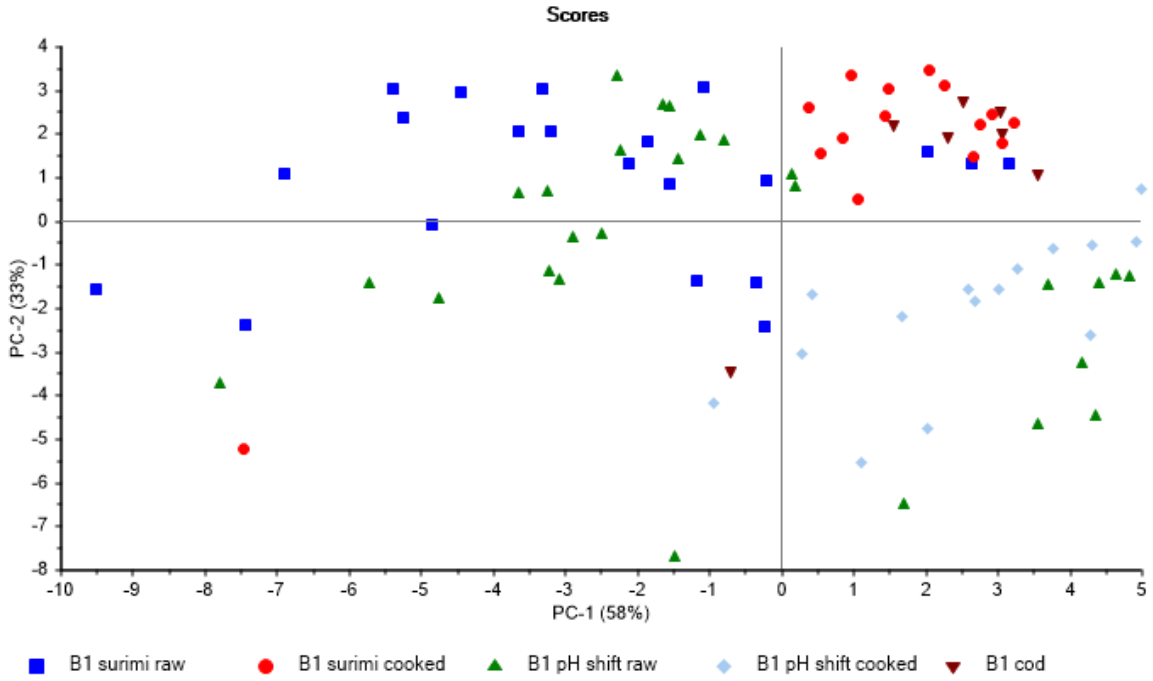



Figure 24. Principal component analysis (PCA) scores of the NIR spectral data from all samples. PC1 and 2 describe 100% of the variation between samples. Samples from batch 1 are marked with a blue box, while samples from batch 2 were marked with a red circle.



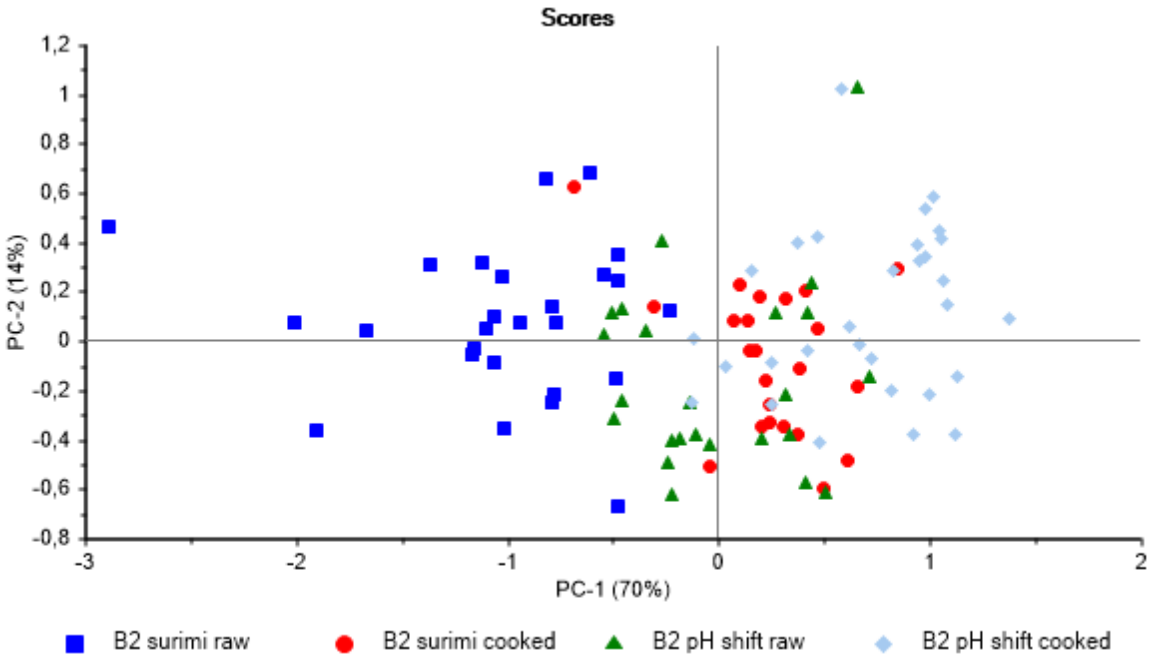



Figure 25. Separate PCA score analysis for batch 1 (top) and batch (2). Raw surimi was denoted with a blue box, cooked surimi with red circles, raw pH shift treated samples with a green triangle, and cooked pH shift treated samples with a grey diamond. Cod samples were also compared to the samples in batch 1 (brown inverse triangle).

## 4.5 The effect of different methods of processing surimi on water distribution in surimi paste and surimi gels

Due to technical problems occurring during the assessment of batch 1, only the results from batch 2 are discussed in this report. Low field NMR relaxation time analysis revealed 2-3 water populations in the 3D printed samples on Day 0 (Fig. 26). These were a dominating fast relaxing component $T_{21}$, corresponding to intracellular water or water entrapped in pores in the matrix, a smaller intermediate population $T_{22}$, mainly corresponding to extracellular water, and finally a small amount of free water ($T_{23}$). Generally, in the raw conventionally washed samples, no effects were observed on the relaxation times when the salt concentration was increased to 1.5%, compared to the unsalted product. However, when the salt concentration was increased to 3% a significant decrease in the most restricted water population ($A_{21}$) was observed, coupled with a corresponding increase in the intermediate population $A_{22}$ instead. However, the least restricted water population remained constant for all salt concentrations.

The salt concentration had, on the other hand, a significant increasing effect on all relaxation times of the pH-shift processed samples. This effect indicated that increased salt made the pores swell, due to repellent electrostatic forces which arise when the $Cl^-$ ions connect to the myofibrillar proteins. This was coupled with small changes in the water distribution, similar to those observed in the conventionally washed samples.

Cooking, on the other hand, had a decreasing effect on the $T_{21}$ relaxation time and its corresponding apparent population ($A_{21}$), which can be correlated to protein denaturation during the cooking process and subsequent loss of water from the cells and pores to the extracellular space. This decreasing trend in the $T_{21}$ relaxation time was slightly stronger in the

pH shift processed samples than in the conventionally washed samples, indicating slightly more protein denaturation during cooking in the pH-shift processed samples. However, proportionally more water was lost from the restricted population $A_{21}$ to the more freely moving water population $A_{22}$ in the conventionally washed samples during cooking. Increasing the salt concentration to 3%, then seemed to have a protective effect towards this exchange of water between the $A_{21}$ and $A_{22}$ populations in the conventionally washed samples, while such effects were more subtle in the pH shift treated samples.

Similar trends in the relaxation parameters were observed after 4 (Fig.27) and 7 days (Fig. 28) of storage as well, with regards to effects of salt concentrations and cooking effects. Interestingly, the intermediate population $A_{22}$ with relaxation time $T_{22}$, seemed to be most affected by the processes. It should also be noted that the differences between the samples decreased with longer storage. On day 7 significant differences in the relaxation parameters were only observed in the $T_{21}$ relaxation time of the raw pH-shift samples due to salt concentration, as well as in the $T_{23}$ relaxation time of the cooked pH-shift samples. In both cases higher salt concentrations were associated with longer relaxation times, indicating that the salt concentrations used had a salting in effect, leading to increased water retention in the samples.

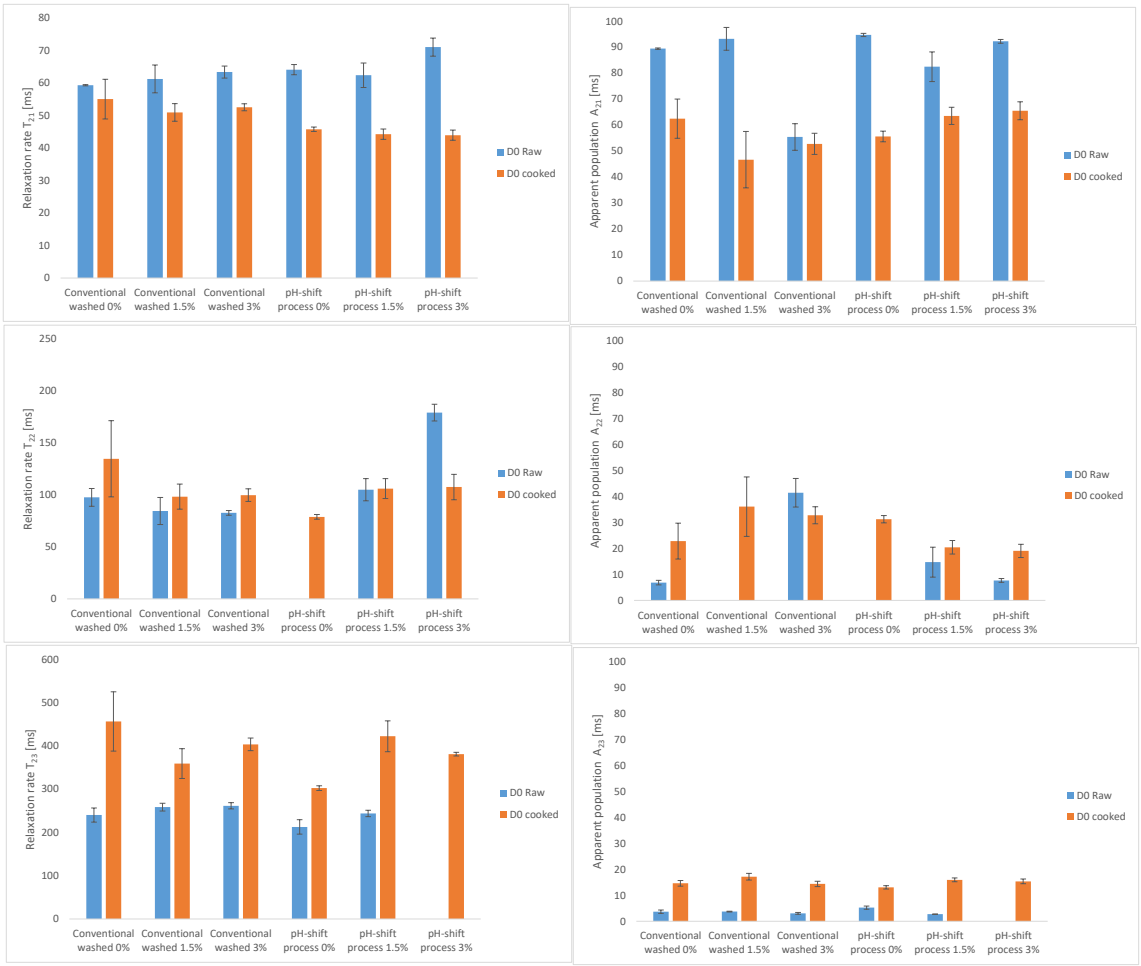



Figure 26. NMR parameters of batch 2 on day 0.

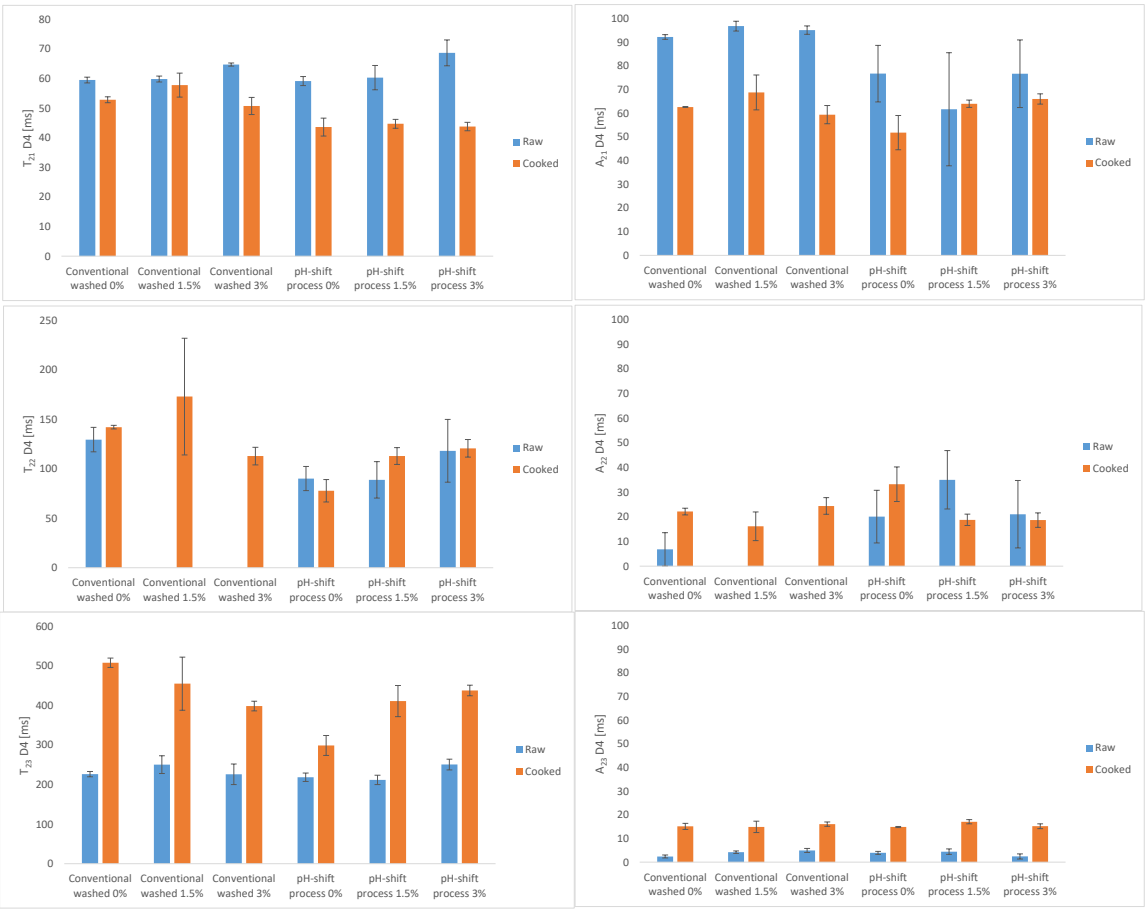



Figure 27. NMR parameters of batch 2 on day 4.

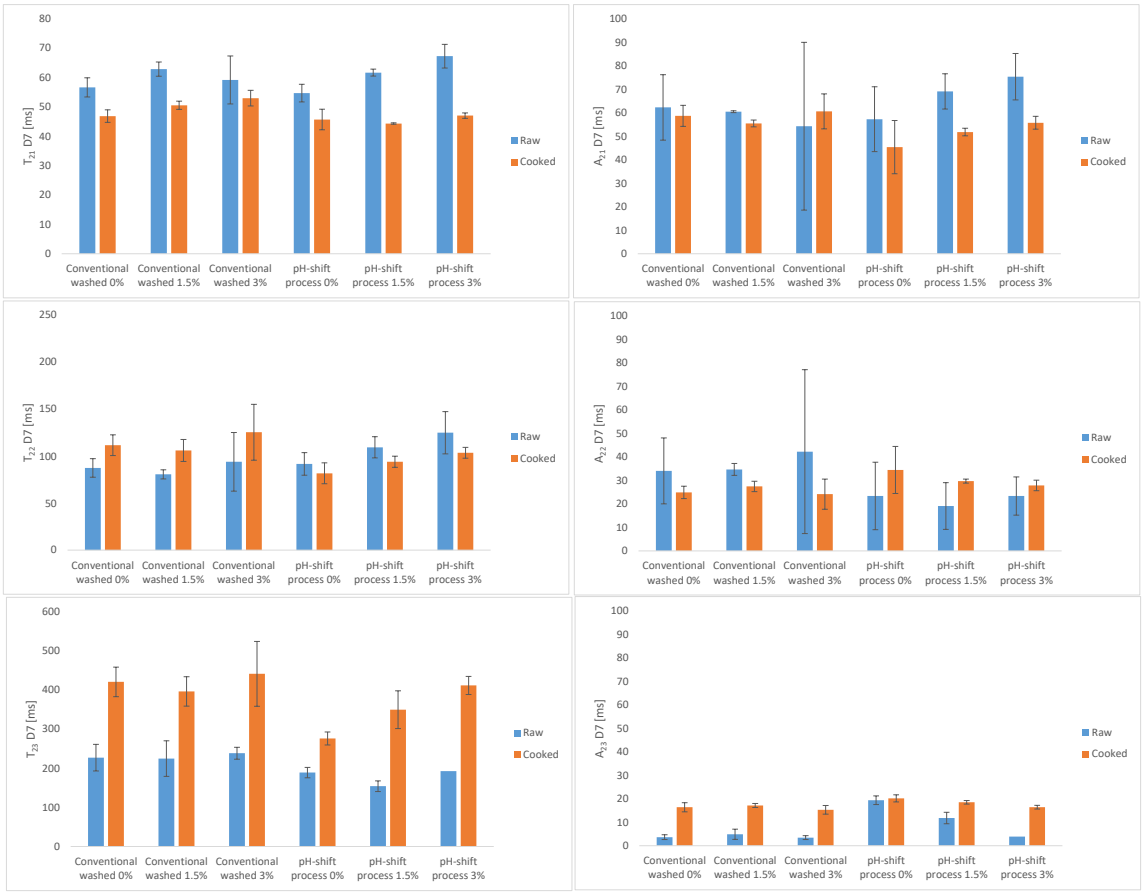



Figure 28. NMR parameters of batch 2 on day 7.

To get a better overview of the effects of the experimental design variables on the water distribution a principal component analysis (PCA) was performed on the NMR parameters (Fig. 29 and 30). The first 3 principal components (PCs) explained 70% of the sample variation in total. PC1 showed a clear distinction between the raw and cooked samples, since the raw samples were characterised with longer $T_{21}$ relaxation times, and more water associated with this population ($A_{21}$), while the cooked samples were characterised with longer $T_{23}$ and $A_{23}$ parameters. This indicates that water was expelled from the more restrained population to the more freely moving water populations during the cooking process, in agreement with the myofibrillar/cell denaturation and shrinkage observed during cooking.

An effect of storage time was seen in both PC1 and PC2 of the samples (marked with blue and red arrows for the raw and cooked samples, respectively). This effect was described with a decrease in $T_{22}$, indicating more restrictions of the intermediate water population, associated with a slight increase in the intermediate population $A_{22}$. Only small differences between the conventionally washed and pH-shift samples could be observed in the cooked samples, due to the storage effect. PC3 explaining 15% of the variation was associated with the effect of the salting on the water distribution. No clear associations were observed between the salt content and the individual NMR parameters, while the salt concentration still has a clear effect on the overall water distribution and characteristics. This emphasises the need to use multivariate analysis to assess the effects of processing and other treatments on NMR data.

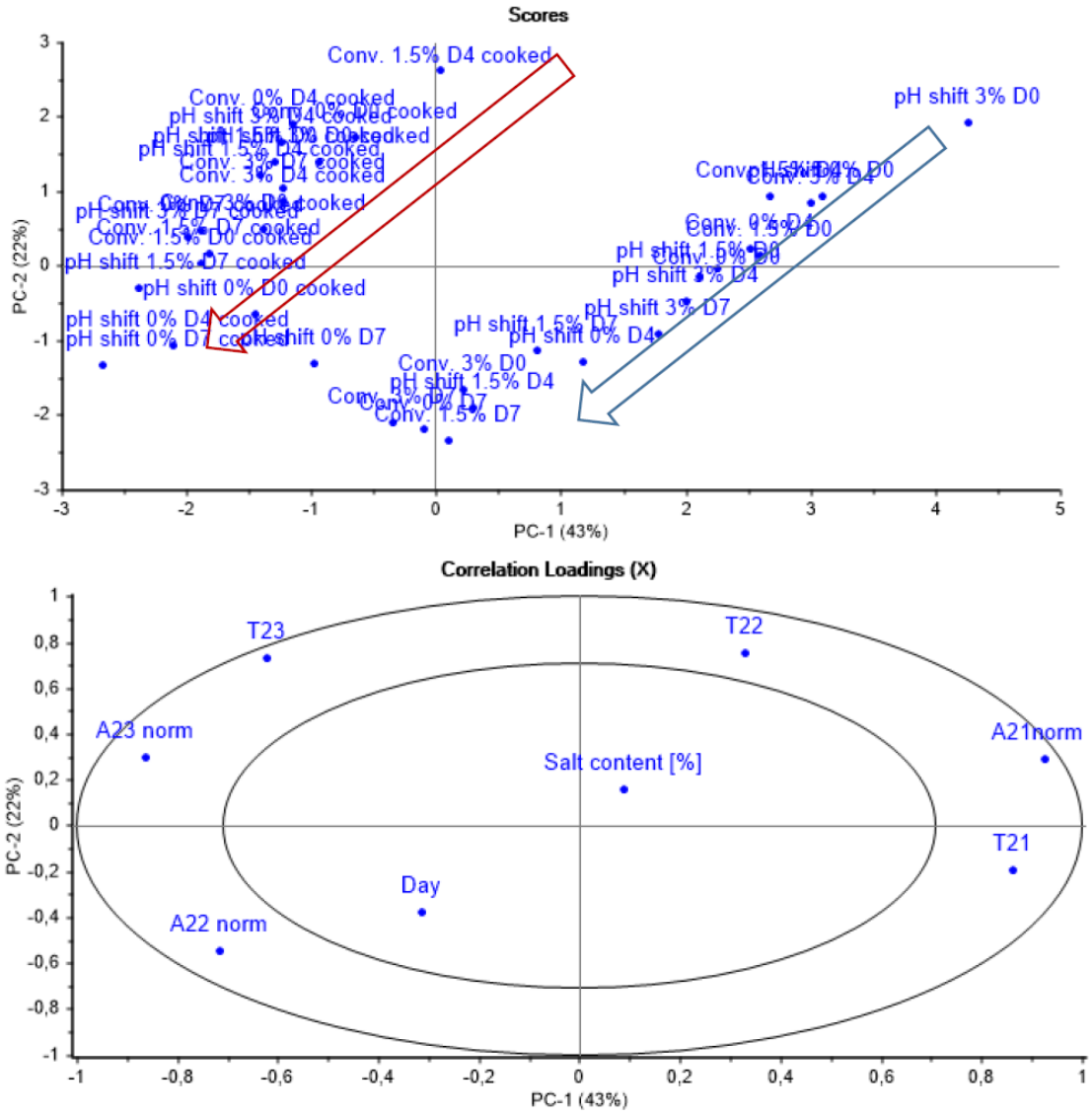



Figure 29. Principal component analysis of the NMR relaxation data. PC1 and PC2 explain 43% and 22% of the differences between samples, respectively. The blue arrow indicates the storage effect on the relaxation parameters in the raw samples, while the red arrow indicates the storage effect in the cooked samples.

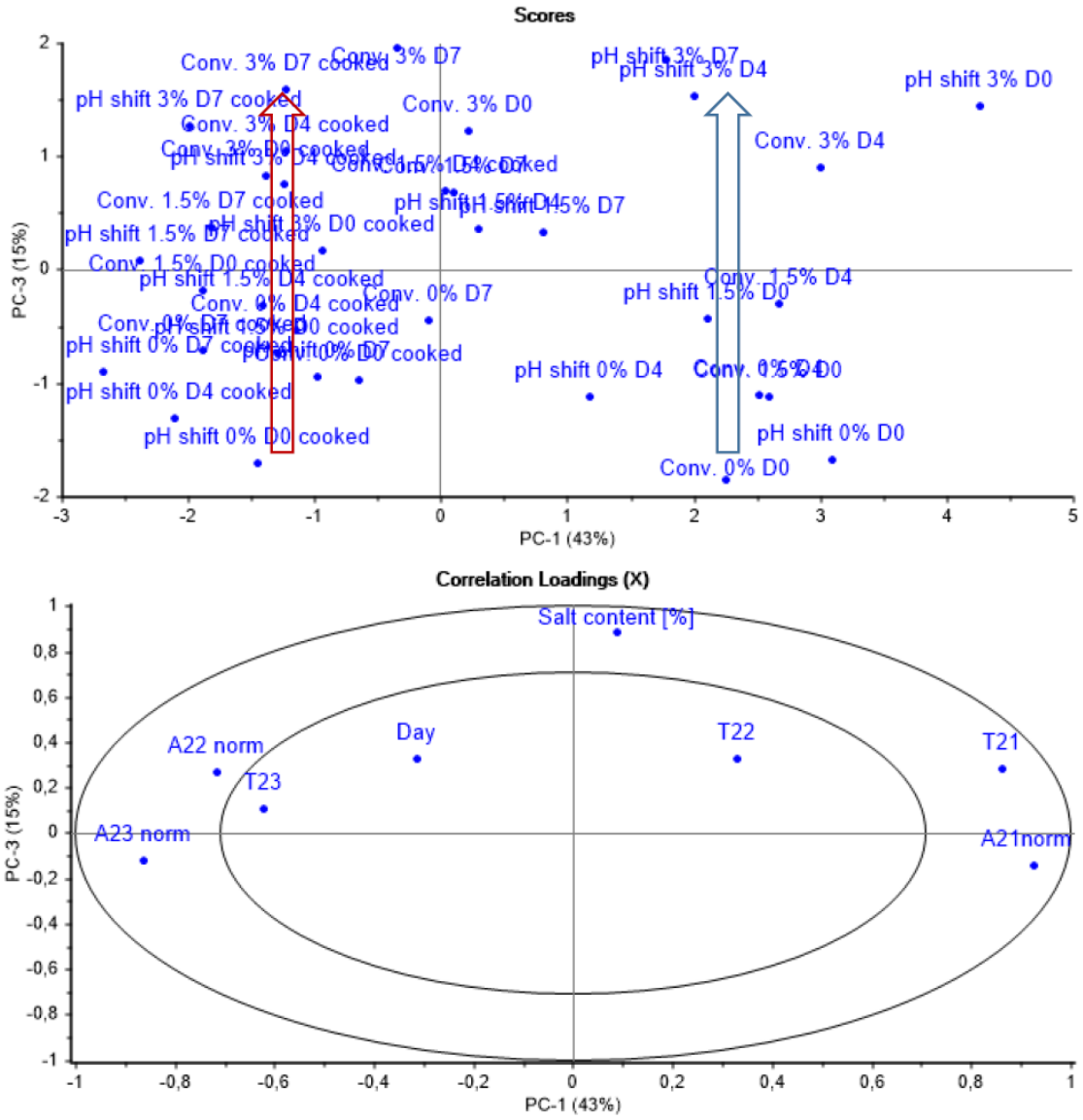



Figure 30. Principal component analysis of the NMR relaxation data. PC3 explains 15% of the differences between samples. The blue arrow indicates the salting effect on the relaxation parameters in the raw samples, while the red arrow indicates the salting effect in the cooked samples.

# 5 DISCUSSION

## 5.1 Raw material properties

Quality of surimi is affected by the period of handling fish between capture and processing. Maintaining the freshness of the fish with proper time/temperature during processing will retain the degradation of nucleotides (Huss, 1995). Degradation of the fish quality was influenced by reaction with enzymes and chemicals that lead to a decrease in the freshness of seafood products and activity of bacterial is responsible for the obvious spoilage (Gram & Huss, 2000). Shelf life is defined as the product still safe during storage and usually indicated by the level of Total volatile basic nitrogen (TVB-N) and trimethylamine (TMA) (Olafsdottir, et al., 1997). TVB-N production from cod byproduct as starting material for surimi products was minimal in batch 1, and higher value was obviously noticed in batch 2. A similar result of the initial value of TVB of cod loin was 10 mgN/100 g (Mai, et al., 2011). This means that raw material from fish store had longer holding period and temperature abused during processing that allowed the degradation of myofibrillar proteins in cod minced fish meat. Apparently, the difference resources of raw material may have an influence on the outcome of the study, resulting in different quality parameters of starting material. These results emphasise the importance of starting material with good quality to improve the printability of the 3D printed.

Minced fish meat consists of myofibrillar, blood, myoglobin, fat and sarcoplasmic proteins, which influence the final quality of surimi gels. Concentrating myofibrillar protein during washing could improve the gelling abilities and remove dark colour and odour in surimi (Park, Graves, Draves, & Yongsawatdigul, 2014). A higher TVC in batch 2 which could be related to poorer hygienic handling and longer period of holding time before processing, hence slightly allowing the bacterial growth rapidly. This result is similar to a previous study which reported that initial TVC of cod loin amounted to log 5.3 CFU $g^{-1}$ (Mai, et al., 2011).

## 5.2 Effect of different methods of processing surimi on surimi paste properties

### *5.2.1 Raw material changes with processing*

During the washing process, a slight change occurred in the colour of the mince, becoming lighter with both processing methods. This is to be expected with the different processing methods. In processing of fish for surimi, it has been found that the lightness for surimi paste from protein isolates from either the acid or alkali process were significantly lighter than the conventional surimi. Colour of cod surimi paste from the surimi pH processing methods were lighter and whiter. This was also observed via visual inspection. Others have also reported this difference in lightness amongst the treatments (Kristinsson & Liang , 2006), due to addition of alkaline process enabling the protein to produce better gelation, color, removing fat and giving flavour (Kristinsson, Lanier, Halldorsdottir, Geirsdottir, & Park, 2014). Differences of whiteness index in fish species may be related to the sarcoplasmic protein removal efficiency, metmyoglobin content, and level of muscle lipid composition (Chaijan, Benjakul, Visessanguan, & Faustman, 2004) which mostly depends on raw material and processing parameters.

TVBN values were reduced with both surimi methods. It is expected that the beginning TVBN values of the conventionally washed fish protein and the pH shift process isolate would be initially decreased due to removal of decomposed amino acids, amines (Nityananda, Khuntia, Raychaudhuri, & Ganguly, 2015). There was also a reduction in microbial counts as seen

between Table 1 and Table 2. It can also be concluded that both processing treatments improved the quality of the fish protein surimi material, particularly in Batch 2. Maximum values for TVBN is 25-35 mg of nitrogen per 100 grams (European Commision , 2005) and the starting byproduct mince in batch 2 could be considered low quality grade. The initial reduction of TVC on the day of processing was 4.60 and 5.20 for the pH shift process surimi and the conventionally washed byproduct mince, correspondingly. Compared to the incoming byproduct mince that was processed, log reductions in TVC were measured. In the first batch, for the pH shift process there was almost a 1 log reduction and in batch 2 (which was high in TVC to start with), there was half a log reduction. From a consumption and spoilage perspective, processing the byproduct raw material by conventional and pH shift process methods would be recommended for improved acceptability and shelf life of the surimi of byproduct mince and for consumer acceptability of the final product after printing.

### *5.2.2 Surimi paste properties at different washing methods*

In Batch 1 and 2, for both the conventionally washed and pH shift processed material, there was a decline in pH with refrigerated storage. This is to be expected with storage due to changes in fish muscle over time during cold storage (Park & Korhonen, 1990). This can be attributed to multiple factors including increases in lactic acid, organic acids, and hydrolysis of fish protein and lipids. Changes in the protein matrix were more evident based upon pH in the first batch between day 0 and 4 while in batch 2, changes were more evident between day 4 and 7. This is might be influenced by the different handling techniques and time elapsed from catching until processing. Additionally, the difference in initial pH value showed the age of the raw material when processed due to the autolytic post-mortem changes (Huss, 1995). Before printing, all the samples of cod surimi from different washing methods were adjusted to the same pH (7.5) after being mixed with cryoprotectants to produce a surimi with good functional properties (Kristinsson, Lanier, Halldorsdottir, Geirsdottir, & Park, 2014). pH is one of other factors that affects myofibrillar protein solubility (Thawornchinsombut & Park , 2004).

The initial water content of cod surimi from both batches before freeze was approximately 80%. It was important to study the natural losses in moisture with storage. It is not expected that the overall moisture would affect the gel strength of the printed cooked gels. As reported by (Kim, Park, & Yoon, 2005), a moisture level in the range of 75 to 81% moisture should not affect shear strain. TVB-N is mainly composed of ammonia, methylamine, dimethylamine and trimethylamine and can be a relative indicator of fish freshness and it can serve as a chemical measurement of fish quality and shelf life of seafood products (Fernandez, Aspe, & Roeckel, 2009). TVB-N values can be an indicator of amino acid degradation. The TVB-N values will also increase due to the microbial growth during storage (Olafsdottir, et al., 1998).

Washing is an essential step in removing dark muscle and other impurities which are thought to impede the gel strength and colour of the surimi (Ochiai, Ochiai, Hashimoto , & Wanatabe , 2001). A proper washing process is necessary in improving the color by concentrating myofibrillar proteins and removing sarcoplasmic proteins, blood, fat, and other nitrogenous compounds from the minced fish flesh. Thus, improving the texture, colour, and odour of surimi when these impurities are removed by washing. However, washing process usually results in higher protein loss, estimated around ~50% of total proteins and increased waste water disposal (Lin & Park, 1996) (Yang & Froning, 1992).

These analyses (Table 2) indicated that the samples were suitable for consumption ($<10^6$ CFU $g^{-1}$) from a microbiological standpoint for the entire duration of the experimental period. Fresh

food products usually contain a variety of microorganisms and the degree of contamination depends on the growth environments and methods of harvesting and handling. It has been known that surimi production involves a series of processing steps that can influence microbial count that leads to cross contamination during process. The spoilage bacterial species of a packaged product depend on its endogenous microbiota, the processing undergone, the type of packaging (MAP, vacuum, aerobic, etc.) and storage temperature (Mace, et al., 2014).

### *5.2.3 Effect of different processing surimi methods and concentration of salt on colour of surimi paste*

Protein recovery with pH-aided process can increase the functional properties from underutilised muscle protein resources. During the process the majority of unwanted components can be eliminated economically at a relative high recovery rate and may have improved the shelf-life over surimi (Undeland, Kelleher, & Hultin, 2002) (Kristinsson & Demir, 2003). pH shift process group had higher lightness than conventional surimi group for both batches. Kristinsson and Liang (2006) also reported higher L* values for croacker surimi paste made from alkali-aided isolate compared to the conventional process. Kristinsson and others (2005) reported, that catfish surimi made by conventionally washed had lower L* value, but higher redness (a*value) compared with surimi made from pH-shift process. It was noted that during the process, the higher L* value indicated the retention of connective tissue, and a slight increase in redness showed the more-co-precipitation of heme proteins (Kristinsson, Theodore, Demir, & Ingadottir, 2005).

## 5.3 The effect of different methods of processing surimi on surimi gels properties

### *5.3.1 Effect of different methods on cod surimi gels and concentration of salt on moisture content and water activity*

Moisture content showed higher in cod surimi gels made from pH-shift process compared to conventional surimi and its contribution to the texture of the surimi (Fig. 17). Hardness of protein gels will decrease with increasing moisture content (Lin, Huff, & Hsieh, 2000). Moisture content (%) of surimi depends on fish species, compositional properties by season, and other processing conditions (mincing, washing, dewatering, refining, and screw press). Generally, ranges of surimi contents between 72% and 75% after mixing with cryoprotectants (Park, Graves, Draves, & Yongsawatdigul, 2014). Moisture content plays a key role in gelation, by adjusting surimi to a fixed moisture concentration (such as 78%), could determine gelation properties (Poowakanjana & Park , 2014). Salt content also influence the protein solubility and behaves differently at different pH conditions. Salt also lowers water activity, and therefore, suppresses growth of foodborne pathogens (Tahergorabi, Beamer, Matak, & Jacynski, 2012).

### *5.3.2 Effect of different methods on processing surimi, storage, and concentration of salt on cooking loss and water holding capacity*

The low cook loss of all samples (less than 5%) indicates that the surimi has excellent water holding capacity. Cooking loss can be expected with storage of the surimi due to degradation of muscle proteins and reduction in physiological pH. It is expected that with decreases in pH during refrigeration of the surimi there can be reduced functionality of the proteins (Park & Korhonen, 1990). Myofibrillar protein denaturation, aggregation, and physiological changes due to proteolysis all play factors in pH lowering with cold storage in muscle meat. Increasing the concentration of chloride and sodium ions results in increases in ion protein interactions (Greiff, Aursand, Erikson , Josefsen , & Rustad, 2015). Sodium chloride can help disrupt the

ion linkages amongst the surimi proteins and can increase the solubility and dispersion. This is very important for forming a good gel matrix (Lanier, Yongsawatdigul, & Rondanelli, 2014).

Water holding capacity is an important attribute of muscle protein gels as it not only affects the economics of their production but also their quality. This is consistent with the recent report that surimi gels made with 1.5% of salt had given better gel network formation due to increasing elasticity with water bonding in the gel matrix (Wang, Zhang, Bhandari, & Yang, 2017). Sodium bicarbonate was an ingredient in addition to the sodium tripolyphosphate that was used to raise the pH of the protein precipitated at 5.5 during the pH shift process. Sodium bicarbonate like sodium tripolyphosphate can serve as a buffering agent. A greater buffering capacity in the pH shift surimi material may have enabled the protein to keep a better gelation functionality and water binding with storage regardless of salt addition. These results are similar to the result of (Davenport & Kristinsson ) (2011) which indicates the improvement of water holding capacity during cooking (cooking loss) of catfish protein isolate made from pH-shift processed over conventional processing.

### *5.3.3 Effect of different methods on processing surimi and concentration of salt on colour of surimi gels*

With cooking, there was an inverse relationship with whiteness relative to the surimi pastes before cooking. The conventional surimi gels were lighter than protein shift isolate stars. This relationship has been observed in other research conducted on both rockfish, whiting, and croaker fish species (Yongsawatdigul & Park, 2004) (Choi & Park, 2002). These changes were noticed in surimi that was first frozen for a week and that then was heat set (Kristinsson & Liang , 2006). Further investigation will need to be conducted on what colour is preferred by consumers upon cooking. Colour, in addition to other textural properites in addition to hardness, and organoleptic properties (odour and taste) will all help create a better picture as to the acceptability of the surimi gels made with washed fish or fish protein isolates.

### *5.3.4 Effect of different methods on processing surimi and concentration of salt on texture profile of surimi gels*

Salt (NaCl) at concentration of 1-3% is added during processing of seafood to enhance protein gelation, to achieve the desired texture, flavour as well as microbial safety. In batch 1, from an analytical texture perspective, the conventional washed surimi treatments groups with various salt concentrations performed better in relation to forming a gel; however, the actual acceptance of the surimi gels would need further investigation with consumer sensory group involvement. The pH shift surimi group did not require salt to form a good gel relative to the conventionally washed surimi, this could be advantageous in formulating surimi for ready to print applications as salt does not need to be added later and remixed and a reduction in salt could be better for overall health as well. During manufacturing of these products salt is required to extract myofibrillar proteins and consequently develop desired texture upon cooking. In surimi products, gelling forming-ability is enhanced by addition of salt to dissociate protein complexes (Matsukawa, Hirata, Kimura, & Arai, 1995).

Addition of salt on day 7 in the pH shift treatments in batch 2, likely helped unfold the proteins and gelling properties to an extent that the material could be successfully printed. It has been shown that adding salt to surimi can improve gel texture in FPI surimi (Perez-Mateos, Amato, & & Lanier, 2004). The addition of salt to surimi is a functional ingredient as its incorporation will cause the proteins to unfold (Matsukawa, Hirata, Kimura, & Arai, 1995). A recent report

(Wang, Zhang, Bhandari, & Yang , 2017) confirmed that surimi gels made with 1.5% of salt mixture can be used for 3D printing.

# 6 CONCLUSION

Clearly, the data points to surimi gel printability strongly relating to the processing method and beginning quality and composition of the raw material and source. Surimi made from two washing methods was successfully printed with increases in salt concentration and surimi cold storage. The conventionally made surimi processing seemed to benefit more from salt addition, while the pH shift process surimi did not respond as well to salt addition. For a ready to print raw surimi product, the pH shift surimi may be easier to work with. Microbial counts and TVBN levels increased with time in the cold stored surimi and were reduced with both processing methods, more so in the pH shift treatment. The gels were successfully cooked, and microbial levels were reduced to safe levels for consumption with a steam oven. With cooking, gels held their shape and in general their layers and height. There were appearance and colour differences before and after cooking of the surimi paste stars. The pH shift surimi paste 0% salt was barely printable on day 7 in batch 2, likely due to crosslinking and/or aggregation of proteins not enabling for the material to remain in as much of a homogenous, smooth flowing paste relative to the other treatments. It cannot be concluded that one surimi preparation method was better than the other. Based on the beginning raw material and storage, the treatments still resulted in fish surimi starts but quality of prints, taste, organoleptics, etc. must all be assessed in the future to fully evaluate the best surimi paste for a ready to make consumer application.

Based on the results, it can be concluded that the starting material should be preferably fresh and prepared by the processor and that by day 7, the cold stored surimi product should be ideally consumed. Further studies regarding freezing of the surimi longer than one week and effects on printing with cold storage also warrants investigation. This research is the first of its kind evaluating how printability of surimi paste and cooked gels in star form are affected by different surimi processing methods from raw byproduct fish (cod) mince, addition of salt, and cold storage of thawed surimi gel.

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