

## THE EFFECTS OF DIFFERENT RAW MATERIALS AND GEL FORMING CONDITIONS ON THE QUALITY OF FISH CAKES FROM MINCED REDFISH

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### ABSTRACT

Nowadays, fish cakes are not only popular products in Asian markets but also consumed widely in many countries in the world. Shortage of fresh fish for producing fish cakes has been a great concern of producers. This study aimed to evaluate quality of fish cakes prepared from fresh redfish mince (January 2016), 1-month old frozen redfish mince (December 2015) and 6-month-old frozen redfish mince (July 2015) and in forming conditions of chilled temperature (0 – 4 °C) and room temperature (18 - 20°C) during 4 weeks under chilled storage. The sampling points were 0, 2 and 4 weeks. Results indicated that there were no significant differences in sensory scores, pH, breaking force, hardness of fish cakes made from fresh redfish mince compared to those made from frozen redfish mince. Shear force of fish cake made from fresh mince was higher than that from frozen mince. The different forming conditions did not cause changes in colour, pH, and lipid compounds of fish cakes (with an exception for TBARS value). Forming at 18 – 20 °C brought higher breaking force and shear force of fish cakes than those formed at 0 – 4 °C. Breaking force and hardness increased after second week of storage. Changes in FFA during 4 weeks under chilled storage were not great for all fish cake groups. TBARS showed a marked increase after cooking and during chilled storage. PV of fish cakes increased significantly after cooking and up to 2 weeks of storage before it dropped at the fourth week. Increase in phospholipid of fish cakes from frozen mince was faster than those from fresh mince throughout the storage time. Generally, one-month and six-month frozen redfish mince can be used to produce fish cake of a quality equivalent to that of fresh mince. Forming at 18 - 20 °C brought some advantages for improving the textural properties of fish cake.

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**LIST OF ABBREVIATIONS**

ASEAN	Association of Southeast Asian Nations
F1M	1 Month Old Frozen Material
F6M	6-Month-Old Frozen Material
FFA	Free Fatty Acid
FPC	Fish Protein Concentrate
FPH	Fish Protein Hydrolysate
FR	Fresh Material
MDA	Malondialdehyde
PV	Peroxide Values
SD	Standard Deviation
SIA	Seafish Industry Authority
TBARS	Thiobarbituric Acid Reactive Substances
TMA	Trimethylamine
VASEP	Vietnam Association of Seafood Exporters and Producers

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## 1 INTRODUCTION

Fish is a very good source of nutrient for humans. In the fish processing industry, yield of fillet is commonly around 30 - 50% of total fish mass, hence the rest raw material constitutes a significant proportion of the catch. Rest raw material includes fillet cuts, head, backbone, skin, viscera, roe and trimmings. The offcuts account for approximately 8 - 17% of the whole fish (Dumay, 2006). The Norwegian cod fisheries utilized only 15.5% of the total by-products for human consumption (Rustad, 2003). In Vietnam, the offcuts are mainly utilised in the low value products such as fish meal and animal feed (VASEP, 2014). Furthermore, in developed countries such as Iceland and France, it has been used to produce value-added products such as fish protein concentrate (FPC), fish protein hydrolysate (FPH), surimi, fish cakes, soup, liver oil, omega-3 oil, canned products and sauce products (Anais, Raul, & Jean-Pascal, 2013; Arason, 2003).

Fish cakes are a popular product among Asian communities. Thais consume about 12,000 tons and Singaporeans consume about 70 tons of fish cake per day (Park 2005a, cited by (Kok, 2005). Fish cakes can be prepared from one or more kinds of fish, with or without starch, condiments and permitted colouring and should contain not less than 40% fish (Agri-Food & Veterinary Authority of Singapore, 2014).

As with neighbouring countries, fish cakes are a popular traditional product in Vietnam. Before 2008, fish cake production in Vietnam was mostly conducted on a small-scale. Since 2008, some Vietnamese enterprises have accessed international markets and exported fish cake products successfully. In 2012, fish cake became one of the top four exported seafood commodities. It was mainly exported to South Korea, ASEAN, Japan and Europe (VASEP, 2012). In the first half of 2015, the export value of fish cake and surimi exceeded 120 million USD (Ngoc, 2015). In Vietnam, only fresh fish is commonly used in the manufacture of fish cake. Hence, shortage of raw material for production is a concern for the producers (Nguyen Trang, 2013). It is therefore important to evaluate whether it is possible to use fish offcuts to make high quality fish cake. Furthermore, it would be useful to know if offcuts after freezing could be suitable to produce fish cake of a quality equivalent to the one made from fresh fish.

Many types of fish can be used to produce fish cakes, including fatty and lean, marine and freshwater species. Fish cake production includes basic steps such as selection of raw materials, washing, cutting and chopping, mixing several ingredients together, grinding, gel-forming, shaping and cooking. Freshness and composition of the raw material and conditions of grinding and gel-forming are the main factors that determine the characteristics of fish cake (Nguyen, Do, & Nguyen, 2006). Moreover, the characteristics of fish cake involve specific texture properties such as sponginess, hardness, cohesiveness and elasticity which are based on the gel-forming of muscle proteins in fish (Muoi & Nguyen, 2005; Yoo, 2011). Gel strength can be controlled by time and temperature of the gel formation step (Kok, 2005).

The overall goal of this study is to evaluate the effects of the different minced redfish offcuts material and gel forming methods on the quality of fish cakes. Additionally, stability of fish cakes during chilled storage will be studied. Firstly, the objective of the study was to evaluate the influence of the raw material conditions (fresh vs. frozen) on quality of fish cakes. Secondly, the aim was to evaluate the effect of different gel-forming conditions (temperature and time) on texture and physio-chemical properties of fish cakes. Finally, the stability of the fish cakes from minced redfish offcuts during chilling storage was investigated. Quality indicators measured include drip loss (frozen samples), cooking yield, proximate composition (water content, total lipid content, phospholipid content), physical properties (colour, texture), level of

lipid deterioration (peroxide value (PV), thiobarbituric acid reactive substances (TBARS), free fatty acid (FFA) level), and sensory evaluation and pH analysis were conducted.

## 2 LITERATURE REVIEW

### 2.1 Golden redfish (*Sebastes marinus*)

In Iceland, golden redfish (Figure 1) are caught all year round but the quality of fish is highest in late winter. Bottom trawl is the most popular method for redfish fishing. The waters West and South West of Iceland are the richest in the fish, where ocean temperature is at 3 - 8 °C. Golden redfish is present mostly at a depth of 100 - 400 m. Golden redfish are long-lived, slow-growing and late maturing fish species. The size of mature male is 31 - 34 cm whereas the size of mature female is 35 - 37 cm. Typical weight in landings of the redfish is 0.5 to 1.5 kg. The total catches of golden redfish in Icelandic waters were around 150,000 tonnes from about 1955 to 1988, but in recent years, the catches of golden redfish have declined to about 40,000 tonnes a year (FAO, 2013; Nghi & Sigurdsson, 2002). Redfish is one of the most important commercial species in Iceland, sold largely as fresh or frozen fillets or whole, frozen at sea or ashore. In 2012, the export values of golden redfish reached 66% for products frozen at sea, 24% for fresh and chilled and 10% land-frozen products. Main markets for golden redfish from Icelandic waters are Germany, Japan, China and South Korea (Iceland Seafood International, 2014). Therefore, a huge amount of redfish mince from this fish processing industry could be utilized to produce added value products.



**Figure 1: Golden redfish**

The average compositions of redfish includes 78% of water, 19% of protein, 3% of fat, 1.4% of minerals and 52% of edible portion (Sa & Pe, 2005). Additionally, the another result was reported that the proximate composition of the fresh redfish is 80% moisture, 1.9% minerals, 2.2% fat, 15.95% protein (Ayinsa & Maalekuu, 2013). The difference in proximate composition of fish muscle depends on species, season, sex, spawn cycle and environment (FAO, 2002). Other reports about chemical composition of redfish have been negligible to date.

### 2.2 Fish mince

Rest raw materials of fish account for approximately three-quarters of total catch weight (Shahidi, 2006a). The rest raw materials such as head, backbones, trimmings (offcuts), skin and guts differ in composition and can be utilized for different purposes. Valuable components such as fish oil, proteins, collagen and gelatine, enzymes and minerals can be obtained from the rest raw material. These compounds can be extracted and purified with the application of complex technologies. As a result, new bioactive compounds can be extracted from marine rest raw material, previously considered as waste (Arason et al., 2009). The proportion of offcuts constitute around 8 - 17% of the whole fish and has been utilized to produce fish meal, fish oil, fish protein concentrate (FPC), fish protein hydrolysate (FPH), fish silage and pet food.

Furthermore, high quality mince can be used to make products such as fish fingers and fishcakes, surimi, soups and sauce products (Innovation Norway, 2014; Jouvenot, 2015).

Fish mince can be prepared from whole fillets, offcuts, V-cut, and fish frames. If fish mince is prepared from whole fish, processing the fish is headed and gutted first. In addition, it is necessary to remove a major part of the backbone from raw material before filleting. Most of microorganism and enzymes responsible for discoloration and spoilage of the mince are found in the viscera, gill, and blood along the backbone (Shahidi, 2006a). If fish mince is prepared from offcuts, V-cut or fillet trimming, the raw materials can be fed directly into a separator as well as the refiner (FAO, 2001). Fish frames such as backbone, tail and dorsal fins constitute a large proportion of the by-product obtained from the filleting industry. Separation of the fish flesh from these materials is difficult, although a considerable amount of fish mince can be recovered. However, mince from fish frames is of low quality compared to minced fillets or offcuts because of the presence of bones, parasites, bacterial contamination and off-colours from the skin. To utilize frame mince effectively, processing techniques should be developed. Washing frame meat or mince contaminated with kidney tissue can be more effective than removing the kidney tissue before deboning to reduce textural degradation and protein denaturation (FAO, 2001; Shahidi, 2006a).

Fish mince has advantages over surimi, which can enhance productivity and extend application of fish mince (Table 1). Fish mince has nutritional advantages as it contains water- soluble vitamins, minerals and lipids. The yield of fish mince is higher than the one of surimi because its water consumption is very low and no losses for nutritional compositions (Shahidi, 2006a). However, frozen stability of fish mince is poor because the high contents of active enzymes and their substrates, metals, lipids and trimethylamine can change easily during storage. The changes of lipid composition in mince from fatty fish often lead to the loss of quality of the mince that can be observed by measuring PV, FFA, TBARS and phospholipid values (Benjakul, Visessanguan, Thongkaew, & Tanaka, 2005; Boran, Karaçam, & Boran, 2006; Eide, Borresen, & Strom, 1982; Eymard et al., 2005; Jobling, Johansen, Foshaug, Burkow, & Jørgensen, 1998; Sobha, Harini, & Veeraiah, 2007)

**Table 1: Definition and characteristics of fish mince/ surimi (Shahidi, 2006b).**

Items	Mince	Surimi
Definition	* Deboned fish flesh separated from fillets or fish frames that have not been washed	* Deboned fish flesh that has been washed with cold water and mixed with cryoprotectants
Advantages	<ul style="list-style-type: none"> <li>* High yield</li> <li>* High nutritional properties</li> <li>* High functional properties</li> <li>* Simple processing</li> <li>* Low water consumption</li> </ul>	<ul style="list-style-type: none"> <li>* Good frozen stability</li> <li>* High functional properties</li> </ul>
Disadvantages	* Poor frozen stability	<ul style="list-style-type: none"> <li>* Low yield</li> <li>* Complicated processing</li> <li>* High water consumption</li> </ul>

To decrease the disadvantageous changes of mince during frozen storage, fish mince can be washed and mixed with cryoprotectants (Arason, 2003; Eide et al., 1982; Phatcharat, Benjakul, & Visessanguan, 2006). If the stability of fish mince can be maintained, it can be used to produce surimi and some other added value products (Shahidi, 2006a)

### **2.3 Production of fish cake**

Fish cake is a 'ready to eat' product in most Asian countries. Each country has a different way to prepare the fish cake. Fish cake can be sliced and used in soups, or it can be fried with some spices to create new flavour for noodle dishes. Vietnamese and Chinese people like the rubbery texture of fish cake while consumers of a Western-style product prefer the medium level of rubbery texture (Snell, 1990).

#### *Collection of raw material*

One or more species of fresh fish is the main material used for fish cake production (Snell, 1990). In Vietnam, pilchard, mackerel, surmullet, horsehead fish, pony fish, tilapia, catfish, snake-head, tench are mainly used to produce fish cake.

#### *Handling*

Handling procedures vary depending on the size of the fish. These may involve cleaning (clean cold water or salted water), heading and gutting, filleting (big fish), separating fish flesh and chopping (Snell, 1990; Tran Thi & Do Minh, 1996).

#### *Mixing*

At this step, ingredients such as starch, spices and functional additives are mixed with the minced fish flesh. Wheat starch, potato starch and corn flour are the most common functional additives used to improve the eating quality of the fish cake. Furthermore, some different functional compounds such as egg white powder, fish protein concentrates, phosphate, modified starch and carrageenan are used with the intention to increase structural stability. Salt, pepper, fresh garlic and onion are added in order to enhance taste. Popular among Asian people is also to use sodium glutamate and sugar, while the European style product does not involve these ingredients. Mixing formulas can vary depending on the properties and composition of the raw fish used (Snell, 1990; Tran Thi & Do Minh, 1996; VMC Group, 2014).

#### *Forming*

The purpose of this step is to use mechanical force to chop fish flesh and break the high-level structure of muscle protein. After mixing the ingredients with fish, fine milling is completed. The parameters that should be controlled are time, temperature and rotation speed of mill. The temperature of the mixture should not exceed 10 °C. The temperature and time strongly affect strength and stability of protein gel. Typically, the temperature of 35 - 40°C and the time of 20 - 30 min is applied in the production of fish cake in Vietnam. However, different parameters should be applied for different raw materials used in the production (Kok & Park, 2007; Kok, 2005; Muoi & Nguyen, 2005; VMC Group, 2014). After gel-forming, the mixture is shaped into balls or cakes of preferred sizes with the use of manual or mechanical tools.

#### *Cooking*

Fish cakes can be cooked in boiling water, steamed or fried in oil. Time of the cooking process should be adjusted to the size of the fish cake. Typically, fish cake is cooked at 90 - 95 °C for 10 - 20 min (VMC Group, 2014).

#### *Packaging and storage*

Vacuum packing is a common method applied for fish cake. The product can be stored frozen or at chilled conditions (0 - 2°C) which depends on the commercial purposes of the product.

## 2.4 Gel formation in fish cake production

Gelation plays an important role to create the quality characteristics of fish cake, which include hardness, stickiness, rubbery, elasticity, sponginess and chewiness (Kok, 2005; Muoi & Nguyen, 2005; Shaviklo, Arason, Thorkelsson, Sveinsdottir, & Martinsdottir, 2010; Snell, 1990). These attributes strongly depend on the quality of myofibrillar protein. Myofibrillar protein contains two major components, myosin and actin. The myosin/actin ratio affects the strength and stability of protein gel (Kok, 2005; Yin, Reed, & Park, 2014). Myosin accounts for 43 - 45% of myofibrillar protein in the muscle fish while actin constitutes approximately one-fifth of all myofibrillar species. The myosin molecule is composed of two large subunits, myosin heavy chain (MHC) and four small subunits, myosin light chain (MLC) (Choi, Cho, & Park, 2000a). The actin is usually presented in a polymerized fibrillar form known as F - actin, and in globular form known as G - actin. Myosin alone can produce excellent gels while actin alone cannot. However, a combination of F - actin and myosin can enhance elasticity of gel. Elasticity will be maximized when the ratio of F - actin and myosin is 0.061 (Sano, F. Noguchi, J. Matsumoto, & Tsuchiya, 1989).

The muscle protein gelation involves two main steps, denaturation and aggregation. When denaturation takes place, the protein unfolds to expose its hydrophobic groups which promote the formation of hydrophobic clusters. Then the combination of hydrophobic areas of proteins leads to aggregation (Smith, 2009). Otherwise, denatured proteins undergo aggregation non-covalently to form a fine elastic network. Myosin heavy chain (MHC) in the muscle of various fish has different cross-linking that affects gel-forming ability of muscle (Benjakul, Visessanguan, Ishizaki, & Tanaka, 2001). There is a long  $\alpha$ -helical and globular region in each MHC. The long  $\alpha$ -helical portions of the MHC wrap around each other to form the rod portion. The globular region links to some locations in the actin molecule for gel-forming. Aggregation is strong when the temperature is over 60 °C, due to thermal denaturation and rheological properties of actomyosin (Kok, 2005). Hence, aggregation depends on fish species and the heating rate. The slower the heating rate, the higher the turbidity of protein gel (Esturk & Park, 2014).

Several factors influence the gelling properties of myofibrillar protein. Different species and freshness of the fish, as well as processing parameters, mainly protein concentration, pH, ionic strength, temperature and time have a strong effect on the gelation (Dong Sun & A Holley, 2011; Tadpitchaysngkoon, Park, & Yongsawatdigul, 2001).

Protein gels generated from various species have different textural properties because of differences in structure, formation, action and appearance of myofibrillar. White muscle usually forms stronger gels than dark muscle (Sun & Holley, 2011). Freshness of the fish also influences on the strength of protein gelation in fish cake. Freshness has influence on the action and appearance of the myofibrillar protein and enzyme systems of the muscle. Enzyme activity influences the gelling properties of mince by hydrolysing myosin or cross-linking myosin. Enzyme activity is highest in fresh fish so protein gels from fresh fish muscle always have the best textural properties. Endogenous proteinases and transglutaminases are the most common enzymes present in fresh fish. They can significantly increase myofibrillar protein gel strength (An, Peters, & Seymour, 1996; Sun & Holley, 2011).

Setting temperature and time of the forming step influences the quality and stability of protein gel. The optimum temperature during the gelation process relates to the habitat temperature of the fish species used to produce the fish cake and may be determined by the heat stability of myosin. Fish myosin from cold water species is more vulnerable to thermal denaturation than

the myosin from warm water species (Kok, 2005; Soottawat, 2003). Lee and Park (1998) reported that the optimum gelling temperatures for pollock is 5 °C and is 25 °C for Pacific whiting. A temperature of 25 °C thus constitutes the optimum condition to improve protein gel quality from tropical fish. Combination of this temperature and formation time can greatly benefit the quality of gel (Soottawat, 2003). The parameters of protein gel formation from Alaska pollock, Atlantic croaker, sardine, threadfin bream, Pacific whiting, or round herring were about 5 – 40 °C for 2 - 16 hours (Tadpitchaysngkoon et al., 2001). The protein gel from Bigeyes Snapper is best at the temperature of 35 °C (Benjakul *et al.*, 2001).

The ingredients added strongly influence the quality of protein gel. Depending on the physico-chemical properties, distribution and volume fraction of the added ingredients, there can be various effects on the textural properties of seafood (Yoon, Lee, & Hufnagel, 1991). Salt, phosphate compounds, protein additives and starch are popularly used in the production of fish cakes to improve textural properties as well as the production of gel. Salt destabilizes native proteins by solubilisation of myofibrillar and causes the protein to unfold. Sodium cations interact with the negative groups (carboxyl) of amino acids while chloride anion may strongly bind with the positive charges of amino acids. This results in alternations in electrostatic and hydrophobic interactions between protein molecules, and dispersion of the proteins which cause changes in the structure of protein (Choi, Cho, & Park, 2000b; Kok, 2005). According to Park and Kok (2005), increasing salt concentration (from 2% to 3%) caused decreases in density of surimi paste, breaking force, deformation and apparent viscosity of surimi gel. Moreover, adding salt is necessary to break ionic linkages and assist better dispersion of the proteins for aggregation and gelation.

Starches have been commonly used in fish cake and surimi production. Starch granules are not soluble in cold water but can swell after water absorption. The swelling and gelatinization ability is directly affected by heating time and temperature. The gelatinization temperature of starch is about 60 – 70 °C. Gelatinized starch granules can help the protein gel matrix firmer and become slightly cohesive. These characteristics of starch granules are very important to control the rheological properties of foods during processing and storage ( Hong, 1997; Nishinari, Zhang, & Ikeda, 2000). Campo & Tovar (2008) reported that “the stiffness and hardness of the surimi gel increased with increasing starch content; the optimum starch content for Alaska pollock sticks was 11%, above which the product becomes hard and brittle” (Campo & Tovar, 2008). Many kinds of starch are used in the production of fish cakes include corn, wheat, potato, tapioca starch, depending on desired textural properties of the product (Akter, Islami, Reza, Shikha, & Kamal, 2013). According to the findings of Prabpree & Pongsawatmanit, (2011), an increase in tapioca content (from 3.5 – 10.5%) resulted in increases in the water holding capacity, shear force, hardness and chewiness of fish sausages and the similar changes were found for thawed sausages.

However, application of starch in the food industry has been improved by using physically and chemically modified starches. Modified starch is obtained from chemical modification which includes substitution and cross-linking. Modified starch has functional properties like phosphate, so these can change the viscosity of hot paste, gel strength, gelatinization temperature, dispersion, and stability at cold temperature. Hence, compared to starch, modified starch is not retrograded during the frozen storage stage therefore fish cake or surimi can avoid water separation and deterioration of product quality (Hong, 1997). Moreover, adding starch into the fish cake or surimi makes the colour of the product brighter (Campo & Tovar, 2008; Prabpree & Pongsawatmanit, 2011).

Phosphates are legally permitted additives to improve eating quality of many foods. They are widely used in fish processing to improve the water holding capacity and the texture of fish products. Depending on the length of the chain, phosphates are classified into several

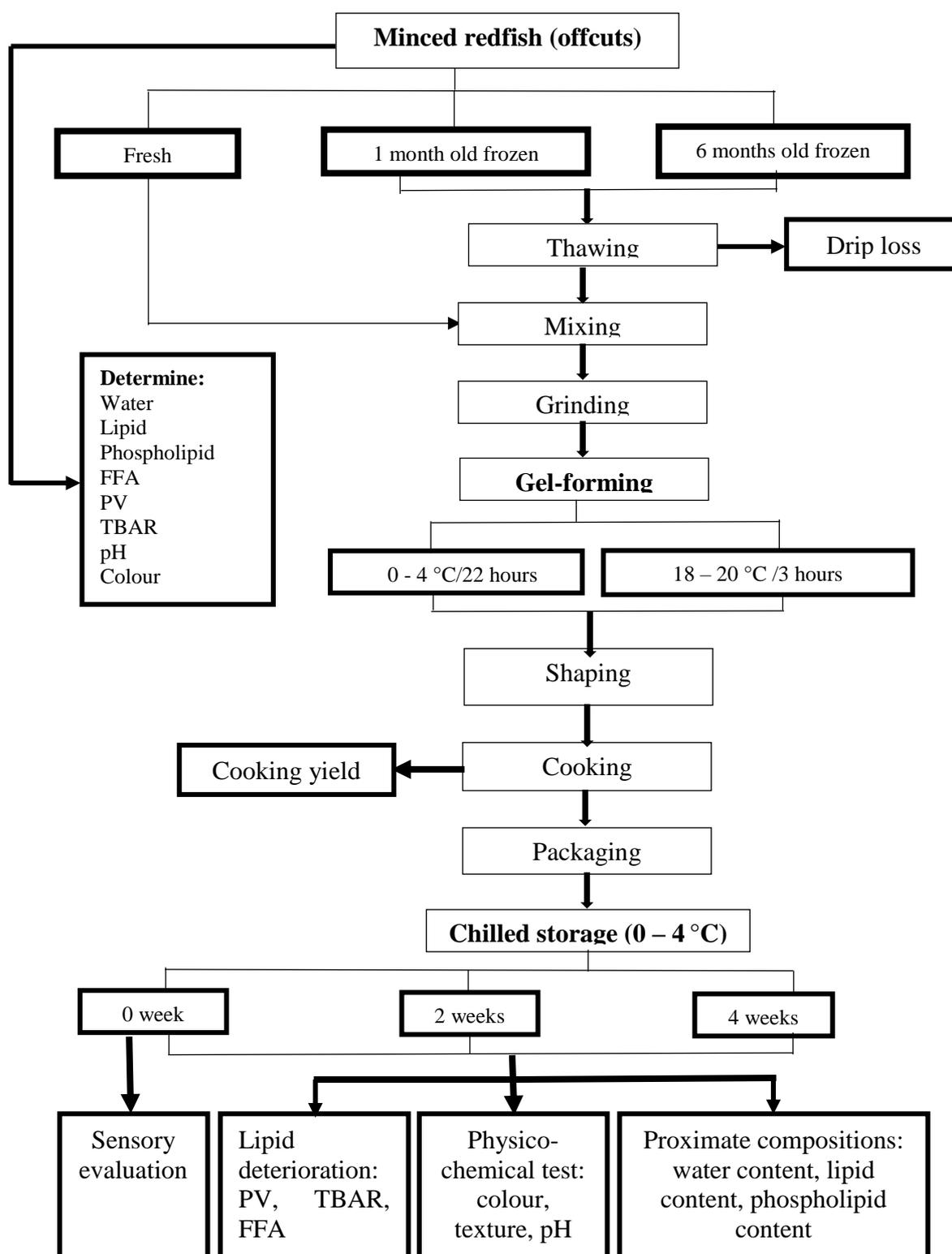
categories, such as simple phosphates, pyrophosphates (2 phosphate groups), tripolyphosphates (3 phosphate groups) and polyphosphates (4 or more phosphate groups). Phosphates used in fish processing are often mixtures of pyrophosphate, tripolyphosphates and polyphosphate compounds (FAO, 2001; SIA, 2012). The mechanism of the action of polyphosphates on proteins is not well understood. However, one hypothesis is that phosphates can affect the interactions between the protein and water, and increase space between muscle fibres which creates more capacity for water holding (Wangtueai, Tongsir, Maneerote, & Supaviriyakorn, 2014). In fish cake production, polyphosphate is mixed with the mince before chopping in order to obtain a binding agent by swelling protein to give the product an improved texture. “The addition of sodium chloride along with phosphates has improved both the interaction with protein and the distribution of flavour of the product” (FAO, 2001). There is a marked increase in the gel strength and water holding capacity of surimi gel when sodium pyrophosphate and  $\text{CaCl}_2$  were added (Julavittayanukul, Benjakul, & Visessanguan, 2006).

The advantages of using protein additives in fish cake production are improvement of gel hardness, elasticity, inhibition of heat-stable proteases, and decrease in degeneration of starch throughout chilled and frozen storage. Dried egg white is a common kind of protein additive used in fish cake production. Dried egg white has better gelling properties than liquid egg white and can increase whiteness and inhibit gel softening (Smith, 2009). These are due to the fact that the ovalbumin in egg white can increase the gel forming ability of the myofibrillar protein. Particularly during heating, ovalbumin can expose its sulfhydryl groups and two disulphide bridges that help to enhance protein-protein interactions (Campo-Deaño & Tovar, 2009). Egg white powder significantly improved texture properties of common carp surimi gel at the level of 3% (Jafarpour, Hajiduon, & Rezale, 2012). The gel strength of Alaska Pollock and Pacific Whiting surimi was the best at the egg albumen content of 1.5% (Campo-Deaño & Tovar, 2009; Jafarpour et al., 2012)

### **3 MATERIALS AND METHODS**

#### **3.1 Experimental design**

The experiment layout is shown in Figure 2. The status of minced redfish offcuts (including fresh, 1 month old frozen, and 6 months old frozen at  $-25^\circ\text{C}$ ), different forming methods (0 – 4  $^\circ\text{C}$  for 22 hours, and room temperature (18 – 20 $^\circ\text{C}$ ) for 3 hours), and chilled storage time (0, 2, 4 weeks) were studied.



**Figure 2: The flowchart of the experiments for producing fish cakes.**

The minced redfish offcuts were transferred from HB Grandi Ltd. (Reykjavik, Iceland) to the lab at Matis Ltd. The fresh material was kept chilled at  $3.5 \pm 0.5$  °C by dried ice in a Styrofoam box. The frozen materials were in 7.5 kg blocks. They were cut into pieces of 1-kilogram weight, then were thawed in the chilled storage at the temperature of 0 - 4 °C for 18 hours in air. Some ingredients were mixed for 2 minutes before grinding. The grinding process was conducted at temperatures below  $12 \pm 2$  °C and for about 15 minutes. The minced mixtures were kept in plastic bags and put in gel-forming conditions (0 - 4°C for 22 hours; room

temperature (18 - 20 °C) for 3 hours). Shaping was done with the use of a tool, which allowed making the fish cakes of the same shape and weight (diameter of 3cm and weigh of  $35 \pm 2$  g). The samples were cooked at 95 °C for 20 minutes (as suggested in Shavikalo (2007)). After cooling for 15 minutes in the chilled storage, the samples were packed in PA bags and kept chilled at 0 - 4 °C. 6 sample groups were prepared and depicted in Table 2. Samples were further analysed after 0, 2 and 4 weeks (Figure 2).

**Table 2: Six experimental groups.**

Forming modes	Raw material		
	Fresh (FR)	1 month old frozen (F1M)	6 months old frozen (F6M)
0 - 4 °C, 22 hours (1)	G11	G21	G31
18 - 20 °C, 3 hours (2)	G12	G22	G32

## 3.2 Materials

### 3.2.1 Golden redfish mince

The study was conducted on golden redfish mince provided HB Grandi Ltd. (Reykjavik, Iceland). General size of the golden redfish processed in Iceland is between 800 - 1200 grams. Three kinds of golden redfish mince were used in the study: fresh, frozen and stored for 1 month and 6 months. The fresh material was caught in January 2016 and kept on ice for 4 days before being filleting and mince was produced. The fresh mince was transferred to the Matis Ltd. Lab and processed to the product the same day. The one-month old frozen mince was produced from redfish that caught in December 2015 and the 6 months old frozen one was caught in July 2015. The offcuts and V-cut were the main parts used to produce the mince. The frozen materials were produced in blocks in a contact plate freezer and were stored at -25 °C. Experiments were carried out at Matis Ltd. – a Food and Biotech R&D institute in Iceland.

### 3.2.2 Added ingredients

All ingredients used for fish cakes production were bought from the local market (Reykjavik, Iceland). Kornax wheat flour was purchased from Lifland Company (Reykjavik, Iceland), modified potato starch from KMC (Brande, Denmark), egg white powder (Actiwhite) from Nordbakels AB - Ó.Johnson & Kaaber (Reykjavik, Iceland), salt from Premier Foods Group Ltd. (Griffiths Way, AL1 2RE, United Kingdom), phosphates from Vaessen - Schoemaker (Deventer, Netherlands) and white pepper powder from the Netherlands producer. Composition of the fish cakes is presented in Table 3.

**Table 3: Composition of ingredients (%) of the fish cakes made from redfish mince.**

Ingredients*	Composition (%)
Redfish mince	87.1
Wheat flour	6.0
Modified starch	1.5
Egg white powder	3.0
Salt	1.0
Pepper powder	0.3
Tripolyphosphat	0.3
Garlic	0.3
Onion	0.5
Total	100%

\* Recipe modified from Muoi & Nguyen (2005), Olayinka, Tope, Patricia, & Akande, (2009), Shaviklo & Johannsson (2006).

### 3.3 Proximate composition analyses

#### 3.3.1 Water content

Water content was determined according to ISO 6496:1999. About 5 g of sample was placed in the oven to dry for 4h at 103 °C. The samples were removed from the oven and allowed to cool to ambient temperature in a desiccator for about 45 minutes. Results were expressed as g water/100 g sample.

#### 3.3.2 Total lipid content

Total lipid content of the samples were extracted according to the Bligh and Dryer method (Bligh & Dyer, 1959). The lipid content was weighed and expressed as g lipids per 100 g sample.

#### 3.3.3 Phospholipid

The total lipid extracts were used to measure phospholipid content (PL) by the colorimetric method (Stewart, 1980). This method was based on the formation of a complex between phospholipids and ammonium ferrothiocyanate. A standard curve was prepared with phosphatidylcholine in chloroform (5 - 50 µg/ml) by evaluation of absorbance at 488 nm (UV-1800 spectrophotometer. Shimadzu, Kyoto, Japan). The results were expressed as a percentage of the total lipid content.

### 3.4 Physicochemical analyses

#### 3.4.1 Colour measurement

Colour changes of the samples were measured with a Chroma Meter (Model CR-310, Minolta Camera Co. Ltd., Osaka, Japan). The instrument recorded the  $L^*$  value (lightness),  $a^*$  value (redness) and  $b^*$  value (yellowness) on the CIELAB colour scale which was used to calculate the mean and standard deviation. The analyses were performed in 5 times for each sample.

Whiteness was calculated as:  $Whiteness = 100 - ((100 - L^*)^2 + a^2 + b^2)^{1/2}$  (Lertwittayanon, Benjakul, Maqsood, & Encarnacion, 2013)

#### 3.4.2 Cooking yield

After shaping to the same size, pieces were weighed then cooked at 95 °C for 20 minutes. After cooling down for 15 minutes in the chilled conditions, the pieces were weighed again. Cooking yield was calculated as the ratio of the weight of fish cakes after and before cooking.

$$Cooking\ yield\ (\%) = \frac{g\ cooked\ pieces}{g\ pieces\ before\ cooking} \times 100$$

#### 3.4.3 Drip loss during storage

The frozen blocks were weighed, and this weight recorded and then sealed in polyamide bags and kept in running water for 60 minutes for thawing. The thawed mince was drained on a wire mesh for 10 minutes before it was weighed again. The drip loss after frozen storage was determined on weighing of the sample before and after thawing (Joseph & Perigreen, 1983).

#### 3.4.4 Texture measurement

The TA.XT2<sup>®</sup> Texture Analyser (Stable Micro Systems, Haslemere, Surrey, UK) was used to measure texture properties of the samples. The analysis was performed five times for each sample. The Warner-Bratzler shearing blade with a thickness of 3.21 mm, length of 125 mm and width of 70 mm was used to shear and cut through the sample. The speed of the blade was 1.8 mm/s. The results were expressed as the maximum peak force (shear force) required to shear through the sample. Breaking force is the first peak force to be attained at the speed of 2 mm/s.

#### 3.4.5 Ph

The pH of the samples was measured by a digital pH meter (Knick - Portamess 913 pH, Berlin, Germany). The sample of 5 g was mixed with 20 mL of distilled water and the mixture was shaken for 3 minutes before measurements. All samples were measured at room temperature. The results were expressed as a mean value collected from two readings.

### 3.5 Sensory evaluation

#### Generic Descriptive Analysis Method (GDA)

Sensory evaluation of fish cakes was done by ten panellists of Matis Ltd. who had been selected according to the general guidance of International Organization for Standardization (ISO) for the selection, training and monitoring of assessors (ISO 1983). Panellists were trained during the two sessions to evaluate fish cakes from redfish offcuts with the GDA method (Lawless & Heymann, 1999). The panellists observed differences in sensory attributes of the samples and focused on the textural attributes of the fish cakes.

All samples observations were conducted according to the general guidelines of ISO for the design of test rooms (ISO 1988). The samples were cooked for sensory evaluation by putting samples into a steam cook oven (Convotherm OEB/OGB, Eglfing, Bavaria, Germany) at 100 °C for 6 min.

The panellists were trained in recognition of sensory characteristics of the samples and describing the intensity of each attribute for a given sample using a 15 cm unstructured scale (from 0 to 100) (Table 4). The panellists evaluated the cooked samples without prior knowledge about the condition of raw material or storage time, using the list of sensory vocabulary developed during training. A computerized system (FIZZ, Version 2.0, 1994–2000, Biosystèmes, Couternon, France) was used for data recording and for further processing. Average scores of the judges were calculated for each sample assessed and the reported values were the average of the duplicate samples.

**Table 4: Generic descriptive analysis of fish cakes made from redfish mince.**

Attribute	Scale	Definition
<i>ODOUR</i>		
fish cakes	n1    much	traditional fish cakes: white fish, onion, pepper
fish oil	n1    much	odour of fresh fish oil or fresh fish liver (not rancid)
TMA	n1    much	TMA, amine
rancid	n1    much	rancid odour
dried fish	n1    much	processed fish, dried fish, salted fish
<i>FLAVOUR</i>		
fish cakes	n1    much	traditional fish cakes: white fish, onion, pepper
salt	n1    much	salty flavour
fish oil	n1    much	flavour of fresh fish oil or fresh fish liver (not rancid)
TMA	n1    much	TMA, amine
rancid	n1    much	rancid flavour
dried fish	n1    much	processed fish, dried fish, salted fish
<i>TEXTURE</i>		
soft	firm    soft	softness in first bite
juice	dry    juicy	dry - draws liquid from mouth
tender	tough    tender	when chewing a few times
elastic	n1    much	elastic, rubbery
adherence	n1    much	adheres teeth while chewing
grainy	fine    coarse	fine: puree, mash; coarse: coarse grains, sago grains

### 3.6 Lipid deterioration analyses

#### 3.6.1 Lipid hydroperoxide values (PV)

Lipid hydroperoxides (PV) was determined with a modified ferric thiocyanate method (Shantha & Decker, 1994). Lipids were extracted from 5.0 g of the sample and homogenized (Ultra-Turrax T25 basic, IKA Labortechnik, Germany) with 10 mL of ice-cold chloroform: methanol (1:1) solution, containing 500 ppm BHT to prevent further peroxidation during the extraction process. Sodium chloride (0.5 M) was added (5.0 mL) into the mixture and homogenized for additional 10 sec before centrifugation at 5100 rpm for 5 min (TJ-25 Centrifuge, Beckmann Coulter, USA). The chloroform layer was collected (500  $\mu$ L) and completed with 500  $\mu$ L chloroform: methanol solution. A total of 5  $\mu$ L of ammonium thiocyanate (4 M) and ferrous chloride (80 mM) mixture (1:1) was finally added. The samples were incubated at room temperature for 10 min and read at 500 nm (Tecan Sunrise, Austria). A standard curve was prepared using cumene hydroperoxides. The results were expressed as  $\mu$ mol lipid hydroperoxides per kg of sample.

#### 3.6.2 Thiobarbituric acid reactive substances (TBARS)

A modified method of Lemon (1975) was used to analyse TBARS. The sample (5.0 g) was homogenized (Ultra-Turrax T-25 basic, IKA, Germany) with 10.0 mL of trichloroacetic acid (TCA) solution (7.5% TCA, 0.1% propylgallate and 0.1% ethylenediaminetetraacetic acid). The homogenized sample was then centrifuged at 5100 rpm for 20 min (TJ-25 Centrifuge, Beckmann Coulter, USA). Supernatant (0.5 mL) was collected and mixed with the same volume of thiobarbituric acid (0.02 M) and heated in a water bath at 95 °C for 40 min. After cooling down on ice, sample was immediately measured at 530 nm (Tecan Sunrise, Austria). A standard curve was prepared using 1.1.3.3-tetraethoxypropane (TEP). The results were expressed as  $\mu$ mol of malondialdehyde diethyl acetal per kg of sample.

#### 3.6.3 Free fatty acid (FFA)

Free fatty acids (FFA) were determined according to the method of Lowry and Tinsley (1976) with a modification made by Bernardez et al. (2005). The total lipid extract was used for measurement of the FFA. Absorbance was read at 710 nm (UV-1800 spectrophotometer, Shimadzu, Japan). The FFA concentration in the sample was calculated based on a standard curve with use of oleic acid. Results were expressed as mg FFA per 100 g of total lipids.

### 3.7 Statistical analysis

Data was analysed by using Microsoft Office Excel 2013 (Microsoft Inc. Redmond, Wash, USA) and STATISTICA software. Break down & one-way ANOVA, t-test and Tukey's comparison were applied on means for each group. The significance level was set at  $p \leq 0.05$ .

## 4 RESULTS

### 4.1 Attributes of redfish mince materials

The proximate composition and some physico-chemical properties of fresh and frozen redfish mince are presented in Table 5.

Water content was recorded be similar for all of the groups and reached  $79.0 \pm 4.0\%$ . However, results showed a marked difference in lipid content of different raw materials ( $p < 0.05$ ). Fresh

raw mince (caught in January 2016) had the highest lipid content ( $4.0 \pm 0.0\%$ ), 6 months old frozen (caught in July 2015) reached  $3.4 \pm 0.1\%$  and one-month old frozen mince (caught in December 2015) showed the lowest lipid content ( $2.6 \pm 0.1\%$ ).

Table 5 also shows that the phospholipid percent of total lipid in fresh mince was the lowest ( $4.9 \pm 0.3\%$ ), not marked variety with the one in 6 months old frozen mince ( $5.5 \pm 0.1\%$ ) but a little various compare to the one in one-month old frozen mince ( $6.6 \pm 0.2\%$ ). A negative correlation between phospholipid content and total lipid content was indicated ( $r = -0.98$ ,  $p = 0.0001$ ).

The pH of redfish mince was not significant different between three kinds of the material. In general, the values for all groups reached  $6.8 \pm 0.1$ . Table 5 also showed the variety in the colour parameters between materials.

The result of drip loss was different between 1-month old frozen mince and 6 months old frozen mince ( $10.7 \pm 0.3\%$  and  $16.2 \pm 0.9\%$ , respectively). The longer frozen storage time, the higher the drip loss.

**Table 5: Physiochemical properties of various redfish minces.**

Material*	Water content (%)	Lipid content (%)	Phospholipid (% of total lipid)	pH	Drip loss
Fresh raw	79.5	$4.0 \pm 0.0^a$	$4.9 \pm 0.3^a$	$6.7 \pm 0.0^a$	-
Frozen 1 month	79.9	$2.6 \pm 0.1^b$	$6.6 \pm 0.2^b$	$6.8 \pm 0.1^a$	$10.7 \pm 0.3^a$
Frozen 6 months	79.6	$3.4 \pm 0.1^c$	$5.5 \pm 0.1^a$	$6.8 \pm 0.1^a$	$16.2 \pm 0.9^b$

Values represent mean  $\pm$  SD; measurements were performed as a single analysis for water, duplicates for lipid content, phospholipid content and pH, quintuplicate for drip loss. <sup>a-c</sup> Different letters in the same column indicate a significant difference between raw materials ( $p < 0.05$ ).

Some indicators of lipid deterioration in fresh and frozen redfish mince are shown in Table 6.

A negligible high value of  $L^*$  belonged to frozen redfish mince stored for one month ( $58.4 \pm 0.7$ ) and six months ( $57.9 \pm 0.2$ ) in comparison to fresh material ( $54.4 \pm 0.5$ ) ( $p < 0.05$ ). Additionally, redness ( $a^*$  values) and yellowness ( $b^*$  values) were recorded to be significantly affected by different raw material condition. Highest  $a^*$  values were reached for fresh mince ( $7.5 \pm 0.3$ ), followed by 6-month-old frozen mince ( $4.6 \pm 0.1$ ) and the lowest redness was recorded for one-month old mince ( $3.2 \pm 0.2$ ). Furthermore, analysis of the yellowness showed the highest values for 6 months old frozen mince ( $8.5 \pm 0.3$ ), followed by one-month old frozen mince ( $6.9 \pm 0.2$ ) and fresh raw material ( $5.8 \pm 0.3$ ).

Compared to the fresh mince, PV of the one-month old frozen mince ( $6.1 \pm 3.6 \mu\text{mol/kg}$ ) was not significantly different while PV of the 6 months old frozen was twice as high ( $16.5 \pm 2.2 \mu\text{mol/kg}$ ) ( $p < 0.05$ ).

TBARS and FFA of fresh mince were observed at the lowest values ( $5.78 \pm 1.3 \mu\text{mol MDA/kg}$ ,  $14.3 \pm 0.1 \text{ mg/100g lipid}$ , respectively) in comparison to the ones of frozen minces. However, there was no significant difference in TBARS and FFA between one month old ( $8.4 \pm 1.1 \mu\text{mol MDA/kg}$ ,  $29.4 \pm 0.8 \text{ mg/100g lipid}$ , respectively) and 6 months old frozen minces ( $9.9 \pm 0.4 \mu\text{mol MDA/kg}$ ,  $30.6 \pm 0.0 \text{ mg/100g lipid}$ , respectively).

**Table 6: Lipid deterioration of redfish mince.**

Material*	PV ( $\mu\text{mol/kg}$ )	TBARS ( $\mu\text{mol/kg}$ )	FFA ( $\text{mg/100g}$ lipid)	Colour		
				L* value	a* value	b* value
Fresh raw	7.3 $\pm$ 1.5a	5.78 $\pm$ 1.3a	14.3 $\pm$ 0.1a	54.4 $\pm$ 0.5 <sup>a</sup>	7.5 $\pm$ 0.3 <sup>a</sup>	5.8 $\pm$ 0.3 <sup>a</sup>
Frozen 1 month	6.1 $\pm$ 3.6a	8.4 $\pm$ 1.1b	29.4 $\pm$ 0.8b	58.4 $\pm$ 0.7 <sup>b</sup>	3.2 $\pm$ 0.2 <sup>b</sup>	6.9 $\pm$ 0.2 <sup>b</sup>
Frozen 6 months	16.5 $\pm$ 2.2b	9.9 $\pm$ 0.4b	30.6 $\pm$ 0.0b	57.9 $\pm$ 0.2 <sup>b</sup>	4.6 $\pm$ 0.1 <sup>c</sup>	8.5 $\pm$ 0.3 <sup>c</sup>

Values represent mean  $\pm$  stdv; measurements were performed as duplicates for FFA, triplicate for PV and TBARS, quintuplicate for colour. <sup>a-c</sup> Different letters in the same column indicate a significant difference between raw materials ( $p < 0.05$ ).

## 4.2 Proximate composition of fish cakes

### 4.2.1 Changes in water content

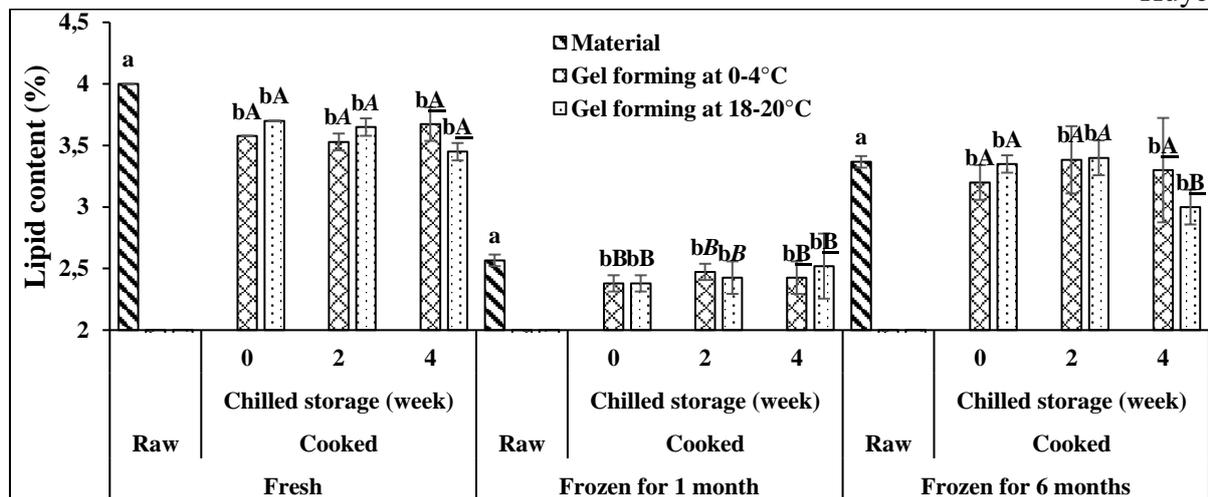
Water contents of the raw material and fish cakes groups are listed in Table 7. Water content of fish cakes decreased by around 10% in comparison to the one of raw material. Variety of time and temperature in gel-forming step did not affect water content of fish cakes. There was no marked changes in water content during chilled storage time.

**Table 7: The water content of fish cakes made from redfish minces during chilled storage time.**

Raw material	Fresh		1-month frozen storage		6 months frozen storage	
	0 - 4°C	18 - 20°C	0 - 4°C	18 - 20°C	0 - 4°C	18 - 20°C
Forming method						
Week 0	70.7	69.9	71.6	71.5	70.4	70.0
Week 2	70.8	69.8	71.9	71.8	70.5	70.2
Week 4	70.9	69.9	71.6	71.5	70.4	70.1

### 4.2.2 Changes in total lipid content

Lipid content of raw materials and the fish cakes groups is illustrated in Figure 3. Fish cakes groups from one-month frozen mince showed lowest lipid content in comparison to other fish cakes groups. Compared to the raw material, lipid content of the fish cakes groups slightly decreased. For example, a highest lipid content was reached for fresh mince ( $4.0 \pm 0.0\%$ ), followed by fish cakes made from this material and formed at  $0 - 4^\circ\text{C}$  ( $3.6 \pm 0.0\%$ ). Fish cakes from the same raw material did not show a marked difference in lipid content. Two different forming conditions used in this study did not affect lipid content of fish cakes. Fish cakes made from 6 months frozen mince showed a lipid content of  $3.2 \pm 0.1\%$  for forming at  $0 - 4^\circ\text{C}$  and a similar content of  $3.3 \pm 0.1\%$  for forming at  $18 - 20^\circ\text{C}$ . Stable lipid content was observed during chilled storage time. Lipid content was correlated negatively with water content ( $r = -0.83$ ,  $p = -0.00002$ ).

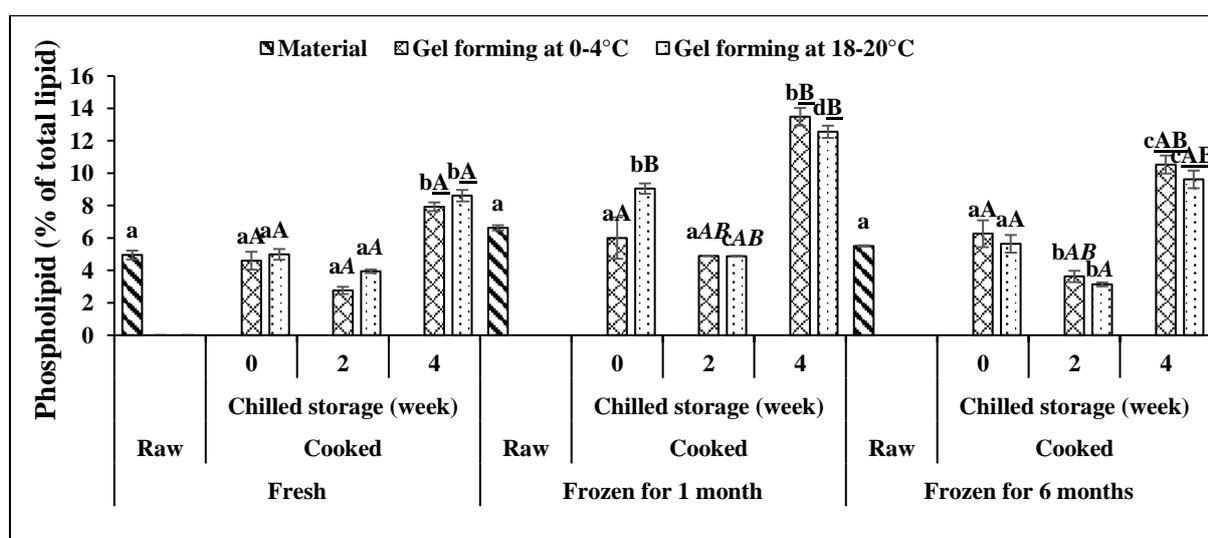


**Figure 3: The lipid content of fish cakes made from Redfish minces during chilled storage time.** Bars represent Means+SD, duplicated; letters for significant difference ( $p < 0.05$ ):  $a-d$  letters for material and product during storage time,  $A-D$  letters for groups after cooking (0 week),  $A-D$  letters for groups after 2 weeks,  $A-D$  letters for groups after 4 weeks.

#### 4.2.3 Changes in phospholipid content

Phospholipid is a complex lipid which links with protein in cell membrane. Figure 4 illustrates changes of phospholipid from initial raw material and fish cakes as a product in 4 weeks under chilled storage. There was no significant difference in phospholipid content between raw material and product. Particularly, only fish cakes from one month frozen mince and formed at 18–20 °C had a significant increase in phospholipid compare to the raw material.

During two first weeks under chilled storage, phospholipid of fish cakes from fresh mince and from one month frozen mince (forming at 0–4 °C) were stable while other fish cakes groups showed a marked decrease in content ( $p < 0.0001$ ). There were considerable increase in phospholipid of all of the fish cakes groups after the second week ( $p = 0.0001$ ). Faster increase was observed in fish cakes from frozen mince ( $p = 0.0001$ ). A positive correlation between phospholipid of fish cakes and storage time ( $r = 0.57$ ,  $p = 0.0003$ ) was indicated.



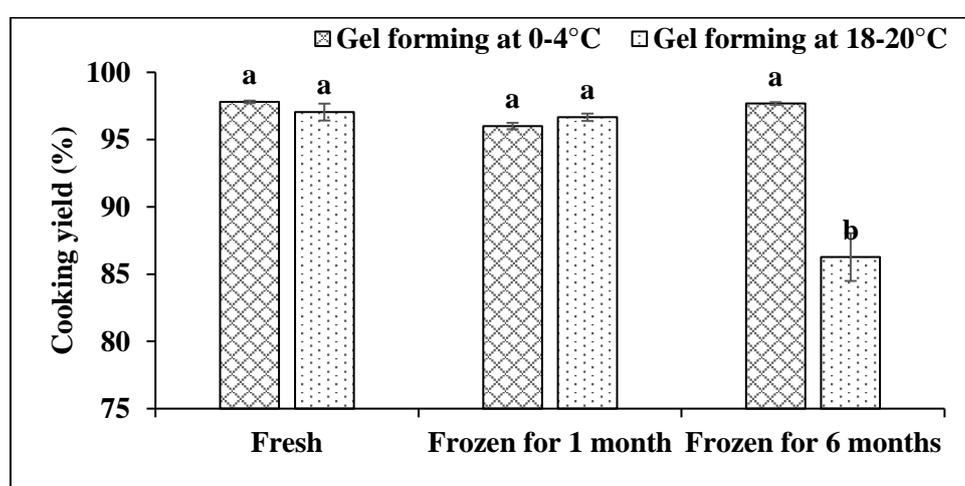
**Figure 4: The phospholipid content of fish cakes made from redfish minces during chilled storage time.** Bars represent Means+SD, duplicated; letters for significant difference ( $p < 0.05$ ):  $a-d$  letters for material

and product during storage time, <sup>A-D</sup> letters for groups after cooking (0 week), <sup>A-D</sup> letters for groups after 2 weeks, <sup>A-D</sup> letters for groups after 4 weeks.

### 4.3 Sensory evaluation and physicochemical properties of fish cakes from redfish mince

#### 4.3.1 Cooking yields of fish cakes

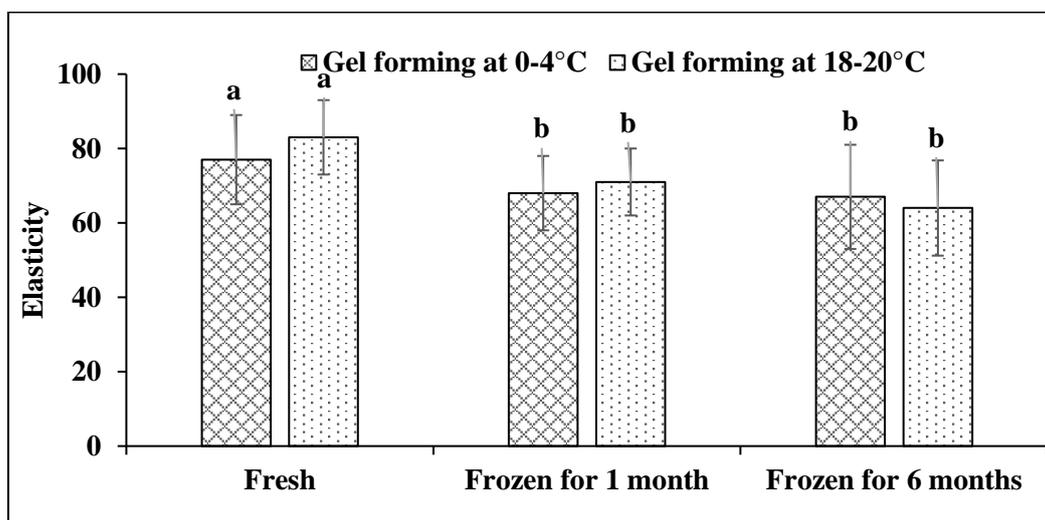
Cooking yields of fish cakes made from different raw material and with various forming conditions are presented in Figure 5. In general, cooking yield of fish cakes prepared in this study was high, about 96 – 97%, except for fish cakes made from 6 months old frozen mince and formed at room temperature ( $86 \pm 1.7\%$ ). There was no significant difference in cooking yield between fish cakes made from fresh mince and one month old frozen. Forming conditions did not affect cooking yield of fish cakes from fresh mince and one month old frozen. However, forming at 0 – 4 °C for 22 hours could be more suitable with 6 months old frozen mince to maintain a high cooking yield.



**Figure 5: The cooking yield of fish cakes made from redfish minces.** Bars represent SD, replication is 5 times, and letters on the top of bars indicate significant difference ( $p < 0.05$ ) between fish cakes groups made from different materials and formed with various forming conditions.

#### 4.3.2 Sensory evaluation of fish cakes

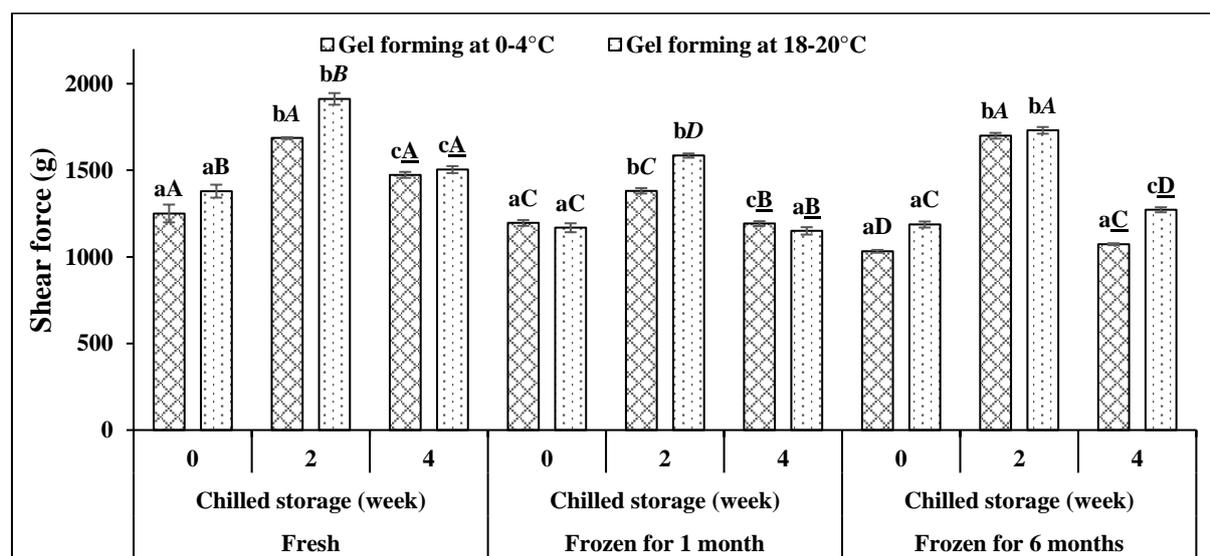
Sensory scores of different fish cakes groups were evaluated by the GDA method. The attributes of odour, flavour and texture were evaluated by using a 15 cm unstructured scale (from 0 to 100). Overall, no significant difference in odour and flavour of all fish cakes groups was observed. Odour of fish cakes could be recognized well with the score fluctuating from 37 to 47. Fish cakes have hardly any odour of fish oil, TMA, rancid and dried fish. Similar scores for flavour of fish cakes, fish oil, TMA and rancid were recorded. Flavour of salt was very slight with the scores fluctuating from 35 to 43. Flavour of dried fish was rather small (9 - 16). Textural attributes had considerable difference in elasticity of fish cakes made from fresh mince compare to the one made from frozen mince ( $p = 0.002$ ). The results are shown in Figure 6. All of the elasticity scores of fish cakes groups were larger than 64 therefore this sensory attribute could insure eating quality of these fish cakes. Elasticity of fish cakes did not differ between two forming conditions.



**Figure 6:** The elasticity score of fish cakes made from redfish minces. Bars represent SD, duplicated, letters on the top of bars indicate significant difference ( $p < 0.05$ ) between fish cakes groups made from different materials and formed with various forming conditions.

#### 4.3.3 Changes in textural properties

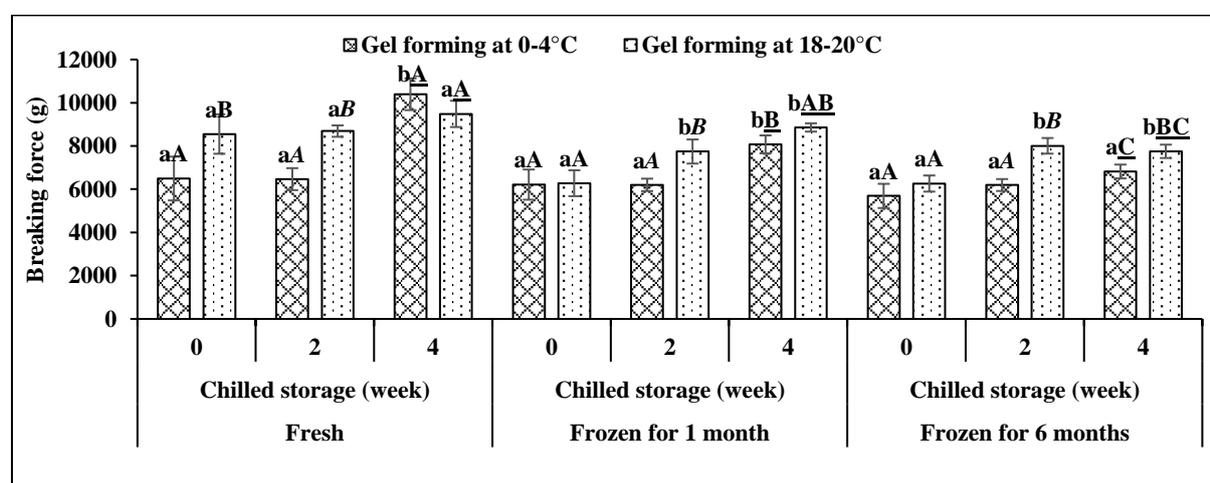
Textural properties were evaluated as shear force, hardness and breaking force by analyses of texture analyser. Shear force relates to tenderness of fish cakes which can be called as elasticity of sensory evaluation. There was a positive correlation between shear force and elasticity ( $r = 0.8$ ,  $p = 0.04$ ). Shear force of fish cakes after cooking and during chilled storage is showed in Figure 7. Shear force of fish cakes from fresh mince was higher then from frozen mince ( $p = 0.0001$ ). There was no significant differences in shear force of fish cakes from 1 month and 6 months old frozen mince after cooking. Forming at 18 – 20 °C raised shear force of fish cakes from fresh mince and 6 months frozen ( $p = 0.0001$ ), not from one month frozen mince.



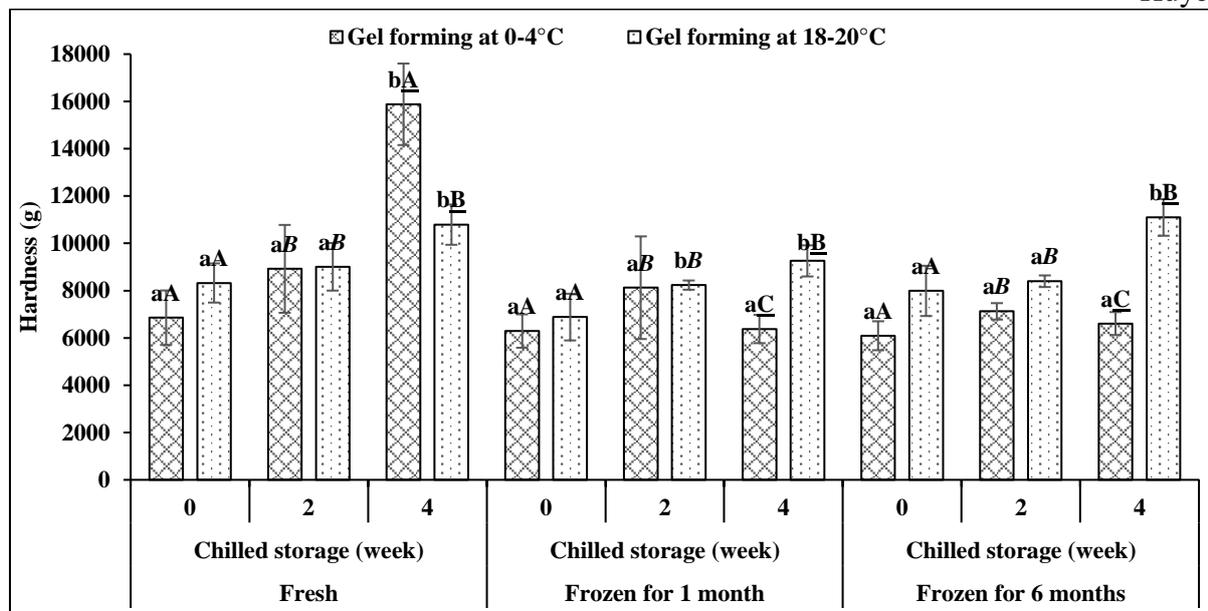
**Figure 7:** The shear force of fish cakes made from redfish minces during chilled storage time. Bars represent SD, quintuplicated, letters for significant difference ( $p < 0.05$ ): a – d letters for groups during storage time, A – D letters for groups after cooking (0 week), A – D letters for groups after 2 weeks, A – D letters for groups after 4 weeks.

Fish cakes are based on protein gel formation. Breaking force and hardness are main parameters to estimate gel strength. The results are showed in Figures 8 and 9. In general, there was no significant difference in breaking force and hardness of fish cakes after cooking made from various raw material. For fish cakes from fresh mince, forming at 18 – 20 °C could improve breaking force more than that of 0 – 4 °C ( $p = 0.0001$ ). However breaking force of fish cakes from both one month frozen mince and 6 months frozen mince showed independence on forming condition after cooking. Additionally, there was increase ( $p = 0.0001$ ) in breaking force of fish cakes with forming at 18 – 20 °C after second week under chilled storage. Breaking force of fish cakes from fresh mince showed a marked high value at fourth week of storage in comparison to those from frozen mince ( $p = 0.0001$ ). A similar observation in hardness of fish cakes from fresh mince was recorded. However, forming at 0 – 4 °C seemed to bring a stability in hardness of fish cakes from frozen mince during 4 weeks storage. Hardness of the others of the fish cakes groups increased after second week. A positive correlation between hardness and storage time ( $r = 0.49$ ,  $p = 0.000001$ ) was observed.

Breaking force increased with storage time ( $p = 0.0001$ ). After first 2 weeks of chilled storage, breaking force was relatively stable then increased significantly ( $p = 0.0001$ ) at fourth week (a exception for fish cakes from 6 months frozen and formed at 18 - 20 °C). The relationship between breaking force and storage time is a positive correlation ( $r = 0.58$ ,  $p = 0.000001$ ).



**Figure 8: The breaking force of fish cakes made from Redfish minces during chilled storage time. Bars represent SD, quintuplicated; letters for significant difference are same as in Figure 7**



**Figure 9: The hardness of fish cakes made from redfish minces during chilled storage time. Bars represent SD, quintuplicated; letters for significant difference are same as in Figure 7.**

#### 4.3.4 Changes in colour

There was a significant change in colour of fish cakes compared to raw material. The result of colour parameters is shown in Table 8. After cooking, there were a marked increase in  $L^*$  value ( $p < 0.001$ ), a significant decrease in  $a^*$  value ( $p < 0.001$ ) and a considerable increase in  $b^*$  value ( $p < 0.001$ ) compared to the ones of raw materials. The lightness of fish cakes from the same raw material is not significantly different. However, there were marked differences in the lightness, redness and yellowness of fish cakes from frozen mince in comparison to the ones from fresh mince ( $p < 0.0002$ ). In general, the lightness of fish cakes made from fresh mince was recorded to be lower than those made from frozen mince. The highest redness was reached for fish cakes from fresh mince, followed by fish cakes from six months frozen mince, lowest  $a^*$  value was recorded for fish cakes from one-month frozen mince. An exception for fish cakes from six months frozen, no marked variety in  $b^*$  values between rest fish cakes groups was indicated.

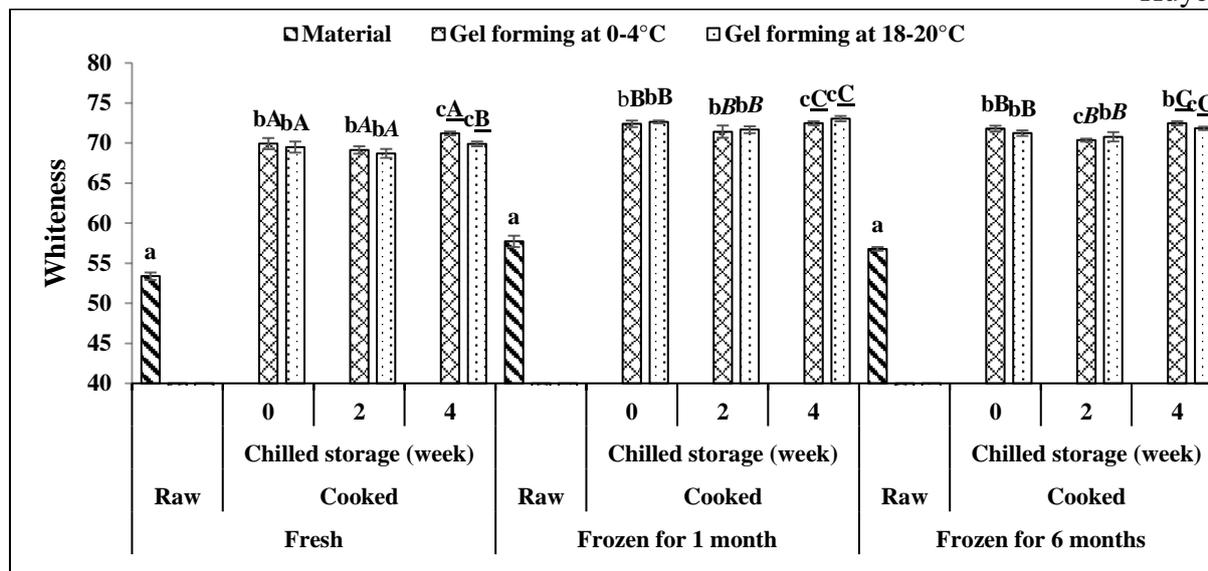
There was no significant difference in lightness of fish cakes after chilled storage up to for weeks, although a slight increase in  $L^*$  value can be observed ( $p < 0.05$ ). After initial 2 weeks under chilled storage,  $L^*$  value of G11, G32 were not significantly different, however a significant variety after week 4 occurred.  $L^*$  value of other groups increased significantly from week 0 to week 2 then dropped. Yellowness of all of fish cakes groups was very stable during storage time. The redness of fish cakes decreased with increasing the storage time. This decrease was faster in first two weeks. There was a negative correlation between  $a^*$  value of fish cakes groups and storage time ( $r = -0.42$ ,  $p = 0.00003$ ) and a negative correlation between  $b^*$  value of fish cakes from 6 months frozen mince and storage time ( $r = -0.37$ ,  $p = 0.0003$ ). Furthermore, water content and  $b^*$  value showed a negative correlation ( $r = -0.8$ ,  $p = 0.00007$ ). Lipid content had positive correlation with  $a^*$  value ( $r = 0.81$ ,  $p = 0.00005$ ) and with  $b^*$  value ( $r = 0.67$ ,  $p = 0.002$ ).  $L^*$  value also correlated negatively with shear force ( $r = -0.76$ ,  $p = 0.0002$ ) and with lipid content ( $r = -0.71$ ,  $p = 0.0009$ ).

**Table 8: Changes in colour of material and fish cakes made from redfish minces during chilled storage time.**

	L* value			a* value			b* value		
	Week 0	Week 2	Week 4	Week 0	Week 2	Week 4	Week 0	Week 2	Week 4
G11	72.7±0.9 <sup>aA</sup>	71.9±0.5 <sup>aA</sup>	74.1±0.1 <sup>bA</sup>	0.7±0.2 <sup>aA</sup>	-0.1±0.0 <sup>bA</sup>	-0.3±0.1 <sup>bA</sup>	12.6±0.4 <sup>aA</sup>	12.7±0.3 <sup>aA</sup>	12.5±0.4 <sup>aA</sup>
G12	72.4±0.8 <sup>aA</sup>	71.5±0.6 <sup>abA</sup>	72.8±0.3 <sup>aB</sup>	0.7±0.2 <sup>aA</sup>	-0.0±0.0 <sup>bA</sup>	-0.1±0.1 <sup>bB</sup>	12.9±0.2 <sup>aA</sup>	12.9±0.2 <sup>aA</sup>	12.9±0.3 <sup>aA</sup>
G21	75.3±0.5 <sup>aB</sup>	74.1±0.8 <sup>bB</sup>	75.3±0.2 <sup>aC</sup>	-0.7±0.1 <sup>aB</sup>	-1.1±0.1 <sup>bB</sup>	-1.1±0.0 <sup>bC</sup>	12.3±0.2 <sup>aA</sup>	12.0±0.3 <sup>aA</sup>	12.0±0.5 <sup>aA</sup>
G22	75.7±0.3 <sup>aB</sup>	74.4±0.5 <sup>bB</sup>	75.8±0.3 <sup>aC</sup>	-0.8±0.1 <sup>aB</sup>	-1.2±0.1 <sup>bB</sup>	-1.2±0.1 <sup>bC</sup>	12.5±0.4 <sup>aA</sup>	12.1±0.3 <sup>aA</sup>	11.8±0.4 <sup>aB</sup>
G31	75.2±0.4 <sup>aB</sup>	73.2±0.3 <sup>bC</sup>	74.5±0.4 <sup>aC</sup>	-0.1±0.1 <sup>aC</sup>	-0.4±0.0 <sup>bC</sup>	-0.5±0.1 <sup>cC</sup>	13.4±0.3 <sup>aA</sup> B	12.7±0.2 <sup>aA</sup>	12.5±0.3 <sup>bA</sup>
G32	74.6±0.5 <sup>aB</sup>	73.9±0.6 <sup>aC</sup>	74.8±0.2 <sup>aC</sup>	-0.3±0.1 <sup>aC</sup>	-0.7±0.1 <sup>bD</sup>	-0.7±0.1 <sup>bA</sup>	13.5±0.3 <sup>aA</sup>	13.1±0.1 <sup>aA</sup> B	12.6±0.3 <sup>bA</sup>
FM		54.4±0.5			7.5±0.3			5.8±0.3	
F1M		58.4±0.7			3.2±0.2			6.9±0.2	
F6M		57.9±0.2			4.6±0.1			8.5±0.3	

FM: fresh mince, F1M: frozen 1 month, F6M: frozen 6 months, G11: fresh mince, forming at 0 – 4 °C, G12: fresh mince, forming at 18 – 20 °C, G21: frozen 1 month, forming at 0 – 4 °C, G22: frozen 1 month, forming at 18 – 20 °C, G31: frozen 6 months, forming at 0 – 4 °C, G32: frozen 6 months, forming at 18 – 20 °C (Table 2). Values represent Mean ± SD, replication is 5 times. Treatment means with different superscripts the same row for each parameter of each group (<sup>a-c</sup>), and the same column for each parameter between 6 groups (<sup>A-C</sup>) differ significantly ( $p < 0.05$ ).

Figure 10 shows changes in whiteness of raw material and fish cake groups. Compared to whiteness of raw materials, all the fish cakes groups had a marked increase in whiteness due to increase of L\* and decline of a\* after cooking. A significant difference in whiteness was recorded for fish cakes from fresh mince, not for fish cakes from frozen mince ( $p = 0.0001$ ). At the same forming condition and at week 0, whiteness of fish cakes from fresh mince ( $69.9 \pm 0.69$ ) was lower than those made from frozen mince ( $72.6 \pm 0.17$ ). Whiteness of fish cakes made from one month frozen mince did not differ with the ones made from 6 months frozen mince. There was no considerable difference in whiteness between fish cakes from frozen mince in the various forming conditions while the forming temperature of 18 – 20 °C made a slight decrease in whiteness of fish cakes from fresh mince at week 4. Increasing the storage time from 0 to 2 weeks did not change whiteness of all fish cakes groups but after that whiteness went up significantly at fourth week ( $p = 0.0001$ ). There was positive correlation between L\* value with whiteness of fish cakes ( $r = 0.98$ ,  $p = 0.0$ ) while a\* value was correlated negatively with whiteness ( $r = -0.72$ ,  $p = 0.0007$ ).



**Figure 10: Changes in whiteness of material and fish cakes made from redfish minces during chilled storage time. Bars represent SD, quintuplicated, letters on the top of bars indicate significant difference ( $p < 0.05$ ): a – d letters for material and product during storage time, A – D letters for groups after cooking (0 week), A – D letters for groups after 2 weeks, A – D letters for groups after 4 weeks.**

#### 4.3.5 Changes in pH

Changes in pH of raw materials and the fish cakes groups are illustrated in Figure 11. There was a remarkable increase in pH of fish cakes in comparison to the raw material ( $p < 0.05$ ). Fresh mince showed pH of 6.6 but two different fish cakes groups from the same raw material had pH of 7.2. Furthermore, pH did not differ between the fish cakes at week 0, 2 and 4. Fish cakes made from the same raw material had similar pH values. However, a marked decrease was evident in pH of fish cakes after 4 weeks under chilled storage ( $p = 0.001$ ). A tendency to decrease in pH was faster in the two initial weeks of storage and slower in the following two weeks. Negative correlation between decreasing pH of fish cakes and storage time was indicated ( $r = -0.82$ ,  $p < 0.0001$ ).

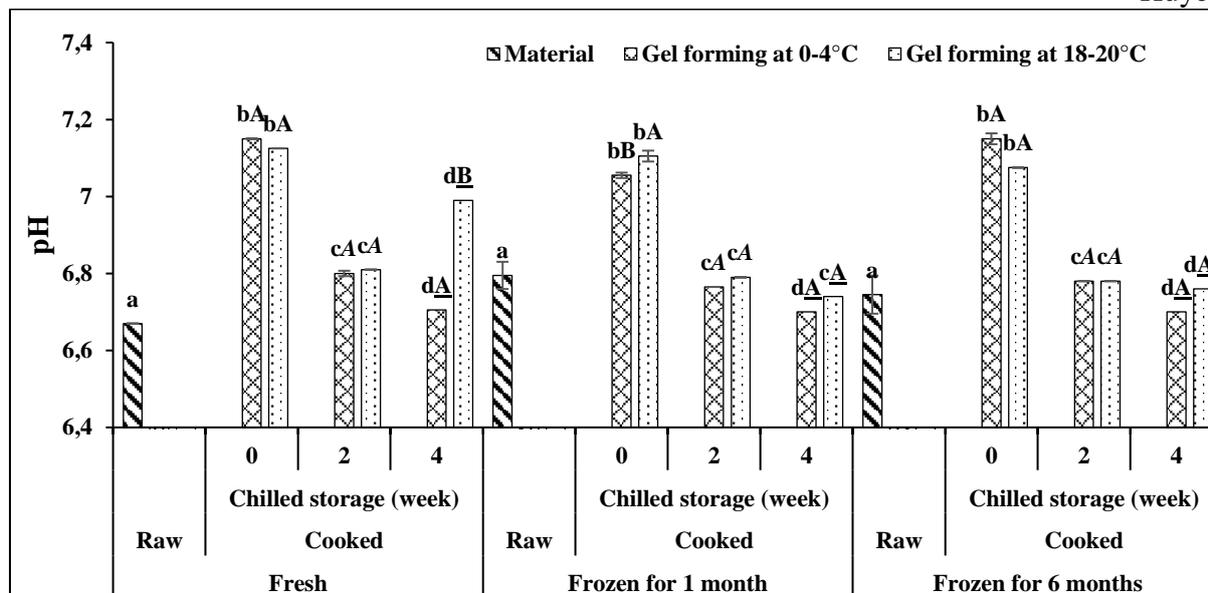


Figure 11: The pH of fish cakes made from redfish minces during chilled storage time. Bars represent SD, duplicated, letters on the top of bars indicate significant difference ( $p < 0.05$ ): a – d letters for material and product during storage time, A – D letters for groups after cooking (0 week), A – D letters for groups after 2 weeks, A – D letters for groups after 4 weeks.

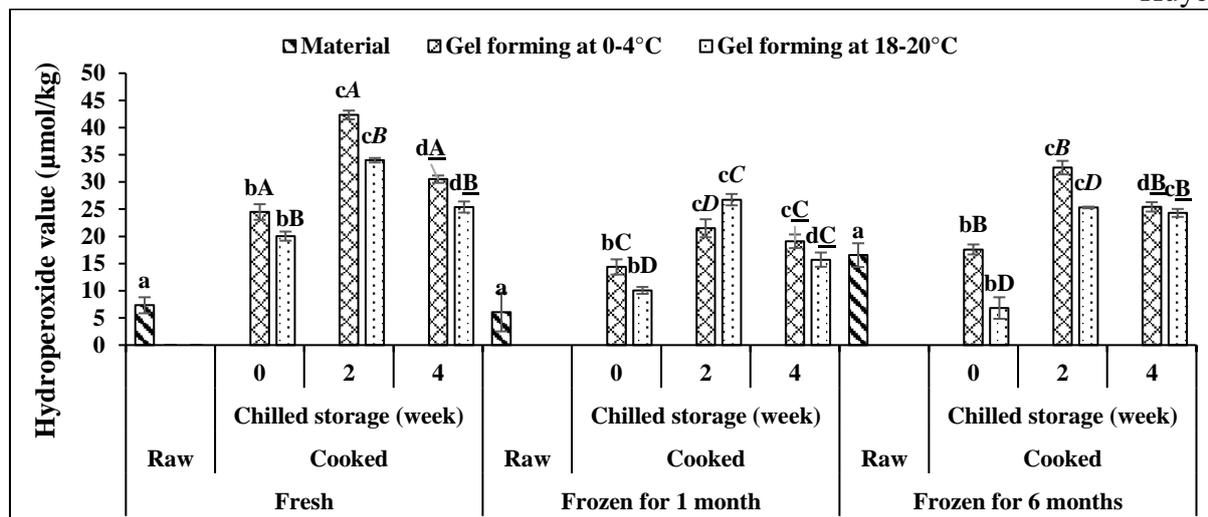
#### 4.4 Lipid deterioration in fish cakes made from various redfish mince and in different forming conditions during chilling storage time

##### 4.4.1 Changes in PV

Hydroperoxide is primary lipid oxidation product. Changes in PV of raw material and fish cakes are showed in Figure 12. For fish cakes made from fresh mince and frozen 1-month mince, PV increased significantly after cooking. PV for fish cakes made from 6 months frozen mince and formed at 0 – 4 °C had no marked change. PV showed a remarkable decline for fish cakes made from 6 months frozen mince and formed at 18 – 20 °C for 3 hours after cooking. Significant increase in PV ( $p < 0.0001$ ) of other fish cakes groups after cooking was attributed to high level of lipid oxidation during cooking.

Forming condition affected PV of fish cakes. For fish cakes made from the same raw material, forming at 0 – 4 °C for 22 hours caused higher PV development compared to those formed at 18 – 20 °C for 3 hours ( $p < 0.001$ ). PV increased very fast in initial 2 weeks ( $p < 0.0001$ ) then went down significantly at week 4 ( $p < 0.05$ ). However, there was still a positive correlation between PV of fish cakes and chilled storage time ( $r = 0.37$ ,  $p = 0.005$ ).

A positive correlation between PV and shear force was recorded ( $r = 0.74$ ,  $p = 0.0004$ ) while PV was correlated negatively with lightness of fish cakes ( $r = -0.68$ ,  $p = 0.002$ ).



**Figure 12:** The hydroperoxide values of fish cakes made from Redfish minces during chilled storage time. Bars represent SD, triplicated; letters for significant difference ( $p < 0.05$ ): a – d letters for material and product during storage time, A – D letters for groups after cooking (0 week), A – D letters for groups after 2 weeks, A – D letters for groups after 4 weeks.

#### 4.4.2 Changes in TBARS

TBARS is one of the most widely used test to assess the degree of lipid oxidation. TBARS level of fish cakes made from different raw material and formed with various conditions are showed in Figure 13. Comparing fish cakes with raw material after cooking, TBARS of fish cakes from fresh mince increased significantly and only slightly for fish cakes made from frozen minces. Particularly, TBARS of fresh mince was just  $5.2 \pm 0.2$   $\mu\text{mol MDA/kg}$  while TBARS of fish cakes made from fresh mince went up to  $13.6 \pm 0.4$   $\mu\text{mol MDA/kg}$  (for forming at  $0 - 4$   $^{\circ}\text{C}$  for 22 hours). Additionally, TBARS reached  $8.2 \pm 0.8$   $\mu\text{mol MDA/kg}$  for one-month frozen mince, then increased slightly up to  $9.9 \pm 0.3$   $\mu\text{mol MDA/kg}$  for fish cakes made from one month frozen mince. There was no significant difference in TBARS of fish cakes formed with various conditions after cooking. For fish cakes made from one month frozen mince, TBARS did not differ with various forming conditions during storage time. With the storage time, TBARS of fish cakes from frozen mince did not change significantly while those made from fresh mince increased ( $p < 0.0001$ ) during first 2 weeks of storage (from  $13.6 \pm 0.4$   $\mu\text{mol MDA/kg}$  to  $21.7 \pm 2.2$   $\mu\text{mol MDA/kg}$  for fish cake formed at  $0 - 4$   $^{\circ}\text{C}$ , from  $13.9 \pm 0.6$   $\mu\text{mol MDA/kg}$  to  $20.7 \pm 2.1$   $\mu\text{mol MDA/kg}$  for fish cake formed at  $18 - 20$   $^{\circ}\text{C}$ ) then values were rather stable until fourth week. TBARS was correlated negatively with lightness of fish cakes ( $r = -0.64$ ,  $p = 0.005$ ).

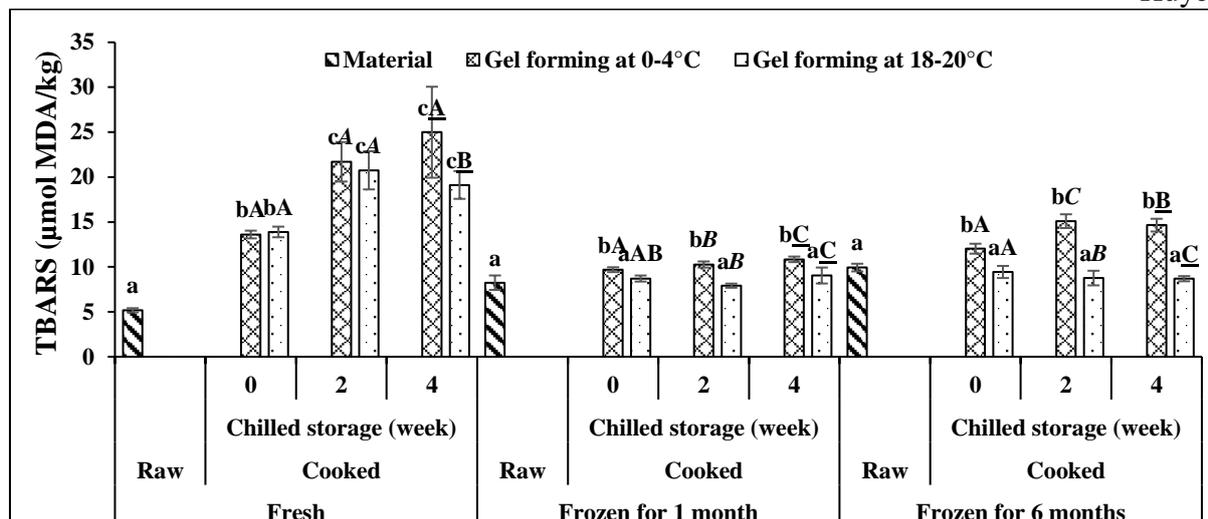


Figure 13: The TBARS values of fish cakes made from Redfish minces during chilled storage time. Bars represent SD, triplicated; letters for significant difference ( $p < 0.05$ ): a – d letters for material and product during storage time, A – D letters for groups after cooking (0 week), A – D letters for groups after 2 weeks, A – D letters for groups after 4 weeks.

#### 4.4.3 Change in FFA

Changes in FFA content of mince and the fish cakes during chilled storage are showed in Figures 14. There was a marked decrease in FFA content of fish cakes after cooking ( $p < 0.05$ ). Figure 14 shows that FFA content of fish cakes corresponded to this value of initial raw material. There was no significant difference in FFA between fish cakes produced at the forming temperature of 0 – 4 °C and at 18 – 20 °C. During storage time, FFA content fluctuated, an exception, a slight decline in FFA level of fish cakes made from frozen one month old mince and formed at 18 – 20 °C, no significant difference in FFA during storage time was observed in other fish cakes groups.

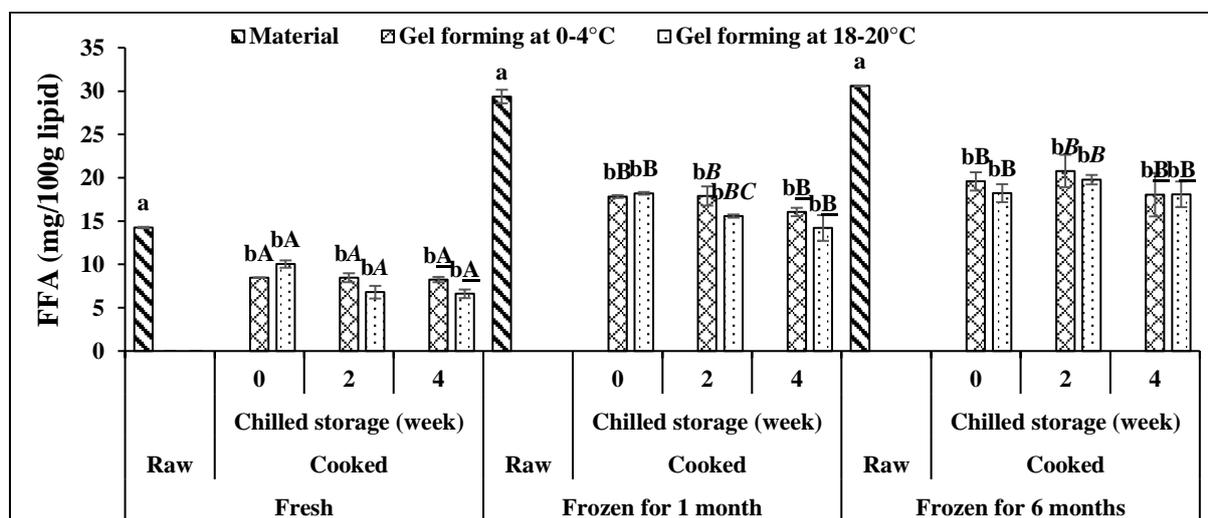


Figure 14: The FFA content of fish cakes made from redfish minces during chilled storage time. Bars represent SD, duplicated; letters for significant difference ( $p < 0.05$ ): a – d letters for material and product during storage time, A – D letters for groups after cooking (0 week), A – D letters for groups after 2 weeks, A – D letters for groups after 4 weeks.

## 5 DISCUSSION

### 5.1 Effect of different raw material on quality of fish cakes

Attributes of raw material strongly affected quality of the products. A different characteristic of the three kinds of redfish mince used in this study was related to changes in drip loss, lipid content, colour and properties of lipid deterioration. Drip loss of six-month-old frozen mince was higher than that of one-month old frozen mince. Typically, long time of frozen storage can increase drip loss because of protein denaturation, which may result in decrease of water holding capacity thus loss of liquid (water, protein, lipid) during thawing (Burgaard, 2010). A similar observation was reported by Joseph & Perigreen (1983), where drip loss during thawing of frozen threadfin bream mince increased from 5% to 13% during 35 weeks.

Significant difference in lipid content of raw material may be due to the time of catch and variability of the offcuts from redfish. Overall, the lipid content is often impacted by migration and spawning time, while the lowest values occurs during the post-spawning period (FAO, 2002; Waters, 1982). According to Yhoemke (2009), redfish spawn from August to November and that may be a reason of decrease in the lipid content of the mince from redfish caught in December 2015. Furthermore, main compositions of redfish mince in this study were from offcuts and V-cut that may had more lipids than fillets. Various processing dates may vary in composition of redfish mince and lipid oxidation during frozen storage often attribute to lipid content (Takahashi & Zama, 1986).

Frozen mince had the higher lightness ( $L^*$  value) than fresh mince.  $L^*$  value is related to dehydration and having a slight contribution of lipid oxidation process which can cause the tendency to develop yellow colour during storage. Result recorded in the present study are in agreement to changes in the colour of mackerel muscle during 6 months of frozen storage (Paola & Isabel, 2015). Lipid oxidation can be observed as increase in PV and TBARS with extended frozen storage time (Joseph & Perigreen, 1983; Karami et al., 2013; Sankar & Raghunath, 1995b). Furthermore, lipid hydrolysis by enzyme systems also happens during frozen storage, which can lead to marked increase in FFA of six months frozen mince and decrease in phospholipid content (Nazemroaya, Sahari, & Rezaei, 2011; Sankar & Raghunath, 1995a; Takahashi & Zama, 1986; Van der Spoel et al., 2015).

Cooking process impacted on various attributes of material to cause the difference in physico-chemical properties of the fish cakes. During cooking, chemical and physical reactions occur, which are often associated with an increase in digestibility, due to protein denaturation and lipid hydrolysis by heat (Sun, 2012). Ktari & Trabelsi (2015) revealed that after steaming and cooking by microware, the Zebra blenny muscle underwent a loss in weight corresponding to 17.3% and 55.12% of initial weight, respectively. Steaming made a decrease in lipid content of Zebra blenny fillets while cooking by microware caused increase slightly in lipid content. In this study, decrease in lipid content also is due to addition of some ingredients and effect of heat treatment. Cooking resulted liquid loss that included lipid of fish cake. Lipid deterioration, thermolysis and lipid oxidation occurring throughout the heat treatment cause decline in lipid content (Tenyang et al., 2013). Domiszewski et al., (2011) observed that cooking striped catfish fillets caused an average 10% loss of fat. 8% of loss fat was revealed for zebra blenny fillets by (Ktari & Trabelsi, 2015).

A significant decrease in FFA after cooking is known as result of heat treatment (Sun, 2012). FFA formation may be also due to derivation from phospholipids and triacylglycerides. However, Nazemroaya et al., (2011) found that in frozen stored skipjack tuna, the majority of FFA was formed from triacylglycerides in the dark muscle, but, in the light tissue, phospholipids hydrolysis was a main progress to form FFA. In this study, no marked negative

correlation between FFA and phospholipid was observed; therefore FFA can be formed from triacylglycerides. Tenyang et al (2013) revealed that FFA in catfish dropped significantly after boiling and frying while this content increased if catfish was smoked (Tenyang et al., 2013). This decrease can be due to the interaction between free fatty acids and proteins (Joseph & Perigreen, 1983).

Decreasing in FFA during cooking was also revealed for Zebra blenny fillets (Ktari & Trabelsi, 2015), catfish (Tenyang et al., 2013). This decline could be a result of evaporation of volatile FFA during heating and action of various enzymes which can cause degradation of FFA (O'keefe, 2008). Additionally, there was no significant difference in phospholipid content between raw material and product. However, it did not demonstrate that phospholipid content was constant during processing. After cooking, a decline in phospholipid may be due to their hydrolysis and oxidation reactions while an increase in phospholipid can be also attributed to decreasing lipid content. Therefore, balance of two processes may result in stability of phospholipids.

Changes in PV and TBARS of fish cakes made from fresh mince showed remarkable varieties compared to fish cakes made from one-month frozen mince and 6 months frozen mince. Increased frozen storage time can strongly affect lipid oxidation development.

Physical properties of the fish cakes corresponded with respective raw material. Fish cakes produced from fresh mince, are more elastic than others. This is due to activity of endogenous proteinases and transglutaminases in fresh mince which increase myofibrillar protein gel strength (An et al., 1996). The lowest cooking yield of fish cakes made from 6-month frozen mince relates to increased protein denaturation during frozen storage, which led to decrease in water holding capacity throughout heat treatment.

Colour parameters of fish cakes corresponded to the raw material. Frozen mince was lighter than fresh mince. Leaching out of muscle pigment known as exudates as well as the leaching of white connective tissue containing collagen located between the segments of muscles during cooking could attribute to increase in lightness (Martin Xavier, Ravishankar, Bindu, & Srinivasa Gopal, 2013). Addition of wheat flour and modified potato starch could attribute to these changes as well (Prabpre & Pongsawatmanit, 2011). Decline of redness may be caused by the heat treatment thus induced denaturation of myoglobin and oxidation of carotenoid pigments. Heating may cause a brown discolouration in white flesh fish therefore increase in yellowness can be recorded (Martin Xavier et al., 2013). Similar observations were presented by Kok (2005) and Xavier et al., (2013). Changes in redness and yellowness is similar to the report of Naourez et al. (2015) although they revealed that lightness of Zebra Blenny fillets slightly decreased after steam cooking process from 80.6 down to 78.9 (Ktari & Trabelsi, 2015).

## **5.2 Effect of various forming condition on quality of fish cakes**

Two forming conditions were used in present study: (1) at 0 – 4 °C for 22 hours and (2) at 18 - 20 °C for 3 hours. Different forming conditions affected mainly textural properties and hydroperoxide value of fish cakes after cooking as well as during chilled storage. The high values of breaking force and shear force relate to strength of protein gel, tenderness and cohesive of food. For fish cakes made from fresh mince, forming at 18 - 20 °C brought a marked increase in shear force in comparison to forming at 0 - 4 °C.

Forming is a process to stabilize protein gel that affects texture characteristics of fish cakes as well as surimi. There are four main types of bonds formed in gel to support the network structure: salt linkages, hydrogen bonds, hydrophobic interaction and covalent disulphide bonds. In this study, three main bonds to build up gel network of fish cakes may be suggested such as salt linkages, hydrogen bonds and covalent disulphide. Forming at 0 - 4 °C can enhance

hydrogen bonds while covalent disulphide bonds can be supported by forming at 18 - 20 °C (Choi et al., 2000a). Intermolecular salt linkage formed among charged amino acids of the protein chain in the presence of salt is attributed to gel strength (Kok & Park, 2007). Forming at 18 – 20 °C for 22 hours brought stronger protein gel to fish cakes made from fresh mince, thus a high action of transglutaminase may be a reason of this difference.

Free water in fish flesh converts to bound water in the progress of gel formation. Decrease in cooking yield of fish cakes made from 6 months old frozen and formed at 18 – 20 °C demonstrated that water holding capacity of these fish cakes was lower than others. This can be explained by transglutaminase activity in 6 months old frozen mince which may be weak while the forming temperature of 18 – 20 °C was not suitable for formation of hydrogen bonds.

Forming at 0 - 4 °C for 22 hours resulted in significant increase of PV while forming at 18 - 20 °C for 3 hours caused remarkable decline in PV of fish cakes made from frozen for 6 months mince. Lower temperature and longer time of forming condition caused greater development of lipid oxidation. However, depending on the rate of PV and its break-down, change of PV could increase or decrease. Hydroperoxide known as a primary product of oxidation can be broken down to secondary product then carbonyl compounds such as aldehydes (Sankar & Raghunath, 1995b).

In general, forming conditions (temperature and time) can be adjusted to obtain texture of fish cakes responding to demands of consumers.

### **5.3 Quality changes of fish cakes during chilled storage time**

Preservation of fish cakes in this study was performed in chilled condition (0 – 4 °C). Lipid and water contents were quite stable during the 4 weeks of chilled storage. Breaking force and hardness increased with increasing of storage time while pH decreased during storage time. This result is in opposition to observations of Kok (2005) for surimi from threadfin bream, that decreasing in pH caused a decline in breaking force. This may be due to that decreasing pH was not enough to cause protein denaturation while cool temperature may be a good condition to stabilize protein gel after cooking.

Whiteness of fish cakes was not affected by storage time as suggested by Gates (2010), colour of cooked food is rather stable during chilled and frozen storage. Texture properties (breaking force, hardness and shear force) showed similar changes in stability. Slight increase of texture properties was observed until second week then went up significantly in the next 2 weeks. Protein gel product often need more time for stability after cooking. These changes in texture of fish cakes are similar to the result of Kok (2005), revealed for surimi from threadfin bream, and result of Yousefi & Moosavi-Nasab (2014) for texture of sausage from talang queenfish mince and surimi.

PV increased after 4 weeks of chilled storage in which a fast increase from week 0 to week 2 then it decreased. PV at fourth week was still higher than that at week 0. Perez-Alonso et al (2003) indicated a similar trend for PV from Atlantic pomfret (Ray's bream) during chilled storage. However, with observations about non-significant different changes of FFA and TBARS values as well as changes in phospholipid, it is necessary to suggest analyses of fatty acid profile that can obtain clarity about interactions in processing and storing of fish cakes since lipid hydrolysis and oxidation are complex progresses.

Correlations between lipid deterioration products (lipid content, TBARS and PV) with colour parameters shows interrelated effects of lipid quality and sensory attribute of fish cakes made from different raw material and formed at various conditions (Belitz, Grosch, & Schieberle, 2009).

## 6 CONCLUSIONS

There are many factors that affect the quality of the fish cake. Focus of this study was on two main factors: condition of raw material and gel-forming conditions. Results showed that there was no significant difference in sensory quality between fish cakes made from fresh mince and frozen mince, an exception for being the difference in elasticity. There is a slight difference in colour, but the whiteness of all fish cakes groups was over 70 which could be able to ensure eating quality. Textural properties of fish cakes made from fresh mince did not differ with those from frozen mince. After cooking, hardness and breaking force were similar for all the groups with the same forming condition. Texture of fish cake became stable during chilled storage. Lipid and phospholipid content as well as FFA level of fish cakes were corresponding to the raw material which was used to produce them. Cooking caused some significant varieties in quality of lipid compounds such as increases in PV and TBAR and decrease in FFA.

Forming at 0 – 4 °C and 18 – 20 °C seemed to affect the tenderness and cohesive (shear force) of the fish cakes and caused few differences in textural properties during storage time. Different forming conditions did not cause differences in colour, pH, water content, lipid content, FFA, PL, and TBARS, however forming at 18 -20 °C for 3 hours attributed to lowering PV of fish cakes formed at 0 – 4 °C for 22 hours.

Through storage time, although texture of fish cake did not change much, decreasing quality of lipid compounds was evaluated by increase in TBARS and PV. Most of changes in lipid deterioration were recorded, although they did not allow suggesting shelf life of fish cakes.

In general, one month old and 6 months old frozen redfish mince can be recommended for fish cakes production with acceptable quality similar to fresh redfish mince. Changes in lipid quality of fish cakes should be studied more deeply to improve their shelf life. However, a notice concerned that insuring quality of the frozen mince at -25 °C and avoiding fluctuation of storage temperature may play an important role.

Some recommendations for next studies:

- Further studies should analyse fatty acid profile in redfish mince as well as in fish cakes in order to monitor changes in lipid quality during processing and storing.
- It is necessary to experiment with more different forming conditions to reveal the best model of forming
- The next studies should compare effects of cooking methods on quality and quality changes of fish cakes during storage
- Experiments on different addition of ingredients are relatively important to satisfy demand of consumers
- Research on determining shelf life of fish cakes from redfish mince.

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## APPENDICES

## Appendix 1: Sensory evaluation of fish cake groups after cooking

Table 9: The result of sensory evaluation of 6 fish cake groups.

attribute	G11	G12	G21	G22	G31	G32	p-value
<i>ODOUR</i>							
fish cake	47	41	39	42	44	37	0.177
fish oil	4	6	3	7	4	7	0.261
TMA	1	1	1	0	1	0	0.356
rancid	1	1	1	1	0	0	0.173
dried fish	7	9	9	7	6	6	0.210
<i>FLAVOUR</i>							
fish cake	43	39	35	35	39	37	0.070
salt	20	18	16	16	22	20	0.171
fish oil	6	5	4	7	4	4	0.155
TMA	2	3	1	0	1	0	0.254
rancid	0	1	1	1	1	0	0.795
dried fish	16	18 <sup>a</sup>	12	10	16 <sup>a</sup>	9 <sup>b</sup>	0.006
<i>TEXTURE</i>							
soft	31	30	37	30	38	38	0.018
juice	26	23	28	27	27	30	0.192
tender	58	56	64	63	61	63	0.114
elastic	76	83 <sup>a</sup>	68 <sup>b</sup>	71 <sup>b</sup>	67 <sup>b</sup>	64 <sup>b</sup>	0.002
adherence	3	4	4	4	2	4	0.787
grainy	65	63	60	65	60	57	0.293

**Appendix 2: T-test, Tukey HSD test for quality indicators of fish cake**

df: degree of freedom; t-value: statistic from T-test; Std.Dev: standard deviation; Valid N: Number of samples; M: mean; Material: FM: fresh mince, F1M: frozen 1 month, F6M: frozen 6 months. Forming 1: 0 - 4°C/ 22 hours; forming 2: 18 - 20°C; Time '0': week 0; time '2': week 2; time '4': week 4.

Differences significant (p< 0.05) were marked red colour

**Table 10: Tukey HSD test for cooking yield of 6 fish cake groups.**

			Tukey HSD test; Variable: Cooking yield (Spreadsheet29) Marked differences are significant at p < .05000					
MATERIAL FORMING			{1}	{2}	{3}	{4}	{5}	{6}
			M=97.803	M=97.044	M=96.005	M=96.658	M=97.683	M=86.266
FR	1	{1}		0.650632	<b>0.015318</b>	0.230080	0.999880	<b>0.000138</b>
FR	2	{2}	0.650632		0.324011	0.968542	0.789321	<b>0.000138</b>
F1M	1	{3}	<b>0.015318</b>	0.324011		0.774753	<b>0.026596</b>	<b>0.000138</b>
F1M	2	{4}	0.230080	0.968542	0.774753		0.337581	<b>0.000138</b>
F6M	1	{5}	0.999880	0.789321	<b>0.026596</b>	0.337581		<b>0.000138</b>
F6M	2	{6}	<b>0.000138</b>	<b>0.000138</b>	<b>0.000138</b>	<b>0.000138</b>	<b>0.000138</b>	

**Table 11: Tukey HSD test for L\* value of 6 fish cake groups.**

		Tukey HSD test; Variable: L- value (Spreadsheet29) Marked differences are significant at p < .05000																	
MATERIAL FORMING TIME		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)
		M=72.698	M=71.874	M=74.080	M=72.378	M=71.484	M=72.798	M=75.328	M=74.110	M=75.250	M=75.662	M=74.402	M=75.794	M=75.188	M=73.216	M=75.472	M=74.638	M=73.912	M=74.826
FR	1 0 (1)		0.476165	<b>0.005482</b>	0.999905	<b>0.028735</b>	1.000000	<b>0.000177</b>	<b>0.004014</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000294</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.973515</b>	<b>0.000177</b>	<b>0.000182</b>	<b>0.028735</b>	<b>0.000177</b>
FR	1 2 (2)	0.476165		<b>0.000177</b>	0.979576	0.998787	0.278210	<b>0.000177</b>	<b>0.000246</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000175</b>	<b>0.000177</b>						
FR	1 4 (3)	<b>0.005482</b>	<b>0.000177</b>		<b>0.000305</b>	<b>0.000177</b>	<b>0.015033</b>	<b>0.020875</b>	1.000000	<b>0.042870</b>	<b>0.000725</b>	<b>0.999898</b>	<b>0.000280</b>	<b>0.073155</b>	<b>0.390888</b>	<b>0.004936</b>	<b>0.948711</b>	1.000000	<b>0.650953</b>
FR	2 0 (4)	0.999905	0.979576	<b>0.000305</b>		0.331906	0.997046	<b>0.000177</b>	<b>0.000255</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000175</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.445673</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.001141</b>	<b>0.000177</b>
FR	2 2 (5)	<b>0.028735</b>	<b>0.998787</b>	<b>0.000177</b>	<b>0.000177</b>		<b>0.010946</b>	<b>0.000177</b>	<b>0.000255</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000177</b>						
FR	2 4 (6)	1.000000	0.278210	<b>0.015033</b>	<b>0.997046</b>	<b>0.010946</b>		<b>0.000177</b>	<b>0.011168</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000599</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.997222</b>	<b>0.000177</b>	<b>0.000190</b>	<b>0.069592</b>	<b>0.000175</b>
F1M	1 0 (7)	<b>0.000177</b>	<b>0.000177</b>	<b>0.020875</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000177</b>		<b>0.027692</b>	<b>0.000956</b>	0.274825	0.999831	0.274825	0.990720	1.000000	<b>0.000175</b>	1.000000	0.767861	<b>0.003851</b>
F1M	1 2 (8)	<b>0.004014</b>	<b>0.000177</b>	1.000000	<b>0.000255</b>	<b>0.000177</b>	<b>0.011168</b>	<b>0.027692</b>		<b>0.005765</b>	<b>0.000956</b>	<b>0.999970</b>	<b>0.000338</b>	<b>0.093395</b>	<b>0.331906</b>	<b>0.006725</b>	<b>0.968411</b>	1.000000	<b>0.715532</b>
F1M	1 4 (9)	<b>0.000177</b>	<b>0.000177</b>	<b>0.042870</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000177</b>	1.000000	<b>0.055765</b>		<b>0.997645</b>	<b>0.424317</b>	<b>0.958781</b>	1.000000	<b>0.000175</b>	1.000000	<b>0.838225</b>	<b>0.008600</b>	<b>0.996711</b>
F1M	2 0 (10)	<b>0.000177</b>	<b>0.000177</b>	<b>0.000725</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.999831</b>	<b>0.000956</b>	<b>0.997645</b>		<b>0.018612</b>	1.000000	<b>0.988924</b>	<b>0.000177</b>	1.000000	<b>0.141562</b>	<b>0.002241</b>	<b>0.449988</b>
F1M	2 2 (11)	<b>0.000294</b>	<b>0.000177</b>	0.999898	<b>0.000175</b>	<b>0.000177</b>	<b>0.000599</b>	0.274825	0.999970	0.424317	<b>0.018612</b>		<b>0.004936</b>	<b>0.561202</b>	<b>0.037135</b>	<b>0.089522</b>	0.999999	0.984490	<b>0.996711</b>
F1M	2 4 (12)	<b>0.000177</b>	<b>0.000177</b>	<b>0.000280</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.990720</b>	<b>0.000338</b>	<b>0.958781</b>	1.000000	<b>0.004936</b>		<b>0.901255</b>	<b>0.000177</b>	<b>0.999898</b>	<b>0.048511</b>	<b>0.000188</b>	<b>0.209907</b>
F6M	1 0 (13)	<b>0.000177</b>	<b>0.000177</b>	<b>0.073155</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000177</b>	1.000000	<b>0.093395</b>	1.000000	<b>0.988924</b>	<b>0.561202</b>	<b>0.901255</b>		<b>0.000180</b>	<b>0.999898</b>	<b>0.954655</b>	<b>0.015937</b>	<b>0.999521</b>
F6M	1 2 (14)	<b>0.973515</b>	<b>0.008246</b>	<b>0.390888</b>	<b>0.445673</b>	<b>0.000255</b>	<b>0.997222</b>	<b>0.000177</b>	<b>0.331906</b>	<b>0.000175</b>	<b>0.000177</b>	<b>0.037135</b>	<b>0.000177</b>	<b>0.000180</b>		<b>0.000177</b>	<b>0.003611</b>	<b>0.756155</b>	<b>0.000570</b>
F6M	1 4 (15)	<b>0.000177</b>	<b>0.000177</b>	<b>0.004936</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000177</b>	1.000000	<b>0.006725</b>	1.000000	1.000000	<b>0.095225</b>	<b>0.999898</b>	<b>0.999982</b>	<b>0.000177</b>		<b>0.454320</b>	<b>0.000888</b>	<b>0.845405</b>
F6M	2 0 (16)	<b>0.000182</b>	<b>0.000177</b>	<b>0.948711</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000198</b>	<b>0.767861</b>	<b>0.968411</b>	<b>0.893825</b>	<b>0.141562</b>	<b>0.000177</b>	<b>0.954655</b>	<b>0.003611</b>	<b>0.454320</b>		<b>0.694420</b>	<b>1.000000</b>	<b>0.295477</b>
F6M	2 2 (17)	<b>0.028735</b>	<b>0.000175</b>	1.000000	<b>0.001141</b>	<b>0.000177</b>	<b>0.069592</b>	<b>0.003851</b>	1.000000	<b>0.008600</b>	<b>0.000241</b>	<b>0.984490</b>	<b>0.000180</b>	<b>0.015937</b>	<b>0.756155</b>	<b>0.000888</b>	<b>0.694420</b>		<b>0.295477</b>
F6M	2 4 (18)	<b>0.000177</b>	<b>0.000177</b>	<b>0.650953</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000175</b>	<b>0.980344</b>	<b>0.715532</b>	<b>0.996711</b>	<b>0.449988</b>	<b>0.996711</b>	<b>0.209907</b>	<b>0.999521</b>	<b>0.000570</b>	<b>0.845405</b>	1.000000	<b>0.295477</b>	

**Table 12: Tukey HSD test for a\* value of 6 fish cake groups.**

		Tukey HSD test; Variable: a value (FULL DATA) Marked differences are significant at p < .05000																	
MATERIAL FORMING TIME		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)
		M=67000	M=1160	M=3440	M=67800	M=0200	M=0840	M=6500	M=1.108	M=1.124	M=7700	M=1.246	M=1.216	M=1340	M=3960	M=5360	M=2780	M=6940	M=6760
FR	1 0 (1)		<b>0.000177</b>	<b>0.000177</b>	1.000000	<b>0.000177</b>													
FR	1 2 (2)	<b>0.000177</b>		<b>0.134165</b>	<b>0.000177</b>	0.995691	1.000000	<b>0.000177</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000177</b>	1.000000	<b>0.017500</b>	<b>0.000186</b>	<b>0.680086</b>	<b>0.000177</b>	<b>0.000177</b>
FR	1 4 (3)	<b>0.000177</b>	<b>0.134165</b>		<b>0.000177</b>	<b>0.002296</b>	<b>0.040672</b>	<b>0.005375</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000183</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.236624</b>	<b>0.999999</b>	<b>0.382036</b>	<b>0.999963</b>	<b>0.000719</b>	<b>0.001581</b>
FR	2 0 (4)	1.000000	<b>0.000177</b>	<b>0.000177</b>		<b>0.000177</b>													
FR	2 2 (5)	<b>0.000177</b>	<b>0.995691</b>	<b>0.002296</b>	<b>0.000177</b>		<b>0.999976</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.974646</b>	<b>0.000307</b>	<b>0.000177</b>	<b>0.044089</b>	<b>0.000177</b>	<b>0.000177</b>
FR	2 4 (6)	<b>0.000177</b>	1.000000	<b>0.040672</b>	<b>0.000177</b>	<b>0.999976</b>		<b>0.000177</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.999999</b>	<b>0.004053</b>	<b>0.000178</b>	<b>0.363946</b>	<b>0.000177</b>	<b>0.000177</b>
F1M	1 0 (7)	<b>0.000177</b>	<b>0.000177</b>	<b>0.005375</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000177</b>		<b>0.000178</b>	<b>0.000178</b>	<b>0.959623</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.051685</b>	<b>0.974646</b>	<b>0.000339</b>	1.000000	1.000000
F1M	1 2 (8)	<b>0.000177</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000178</b>		1.000000	<b>0.001203</b>	<b>0.877791</b>	<b>0.984979</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000189</b>	<b>0.000181</b>	<b>0.000181</b>
F1M	1 4 (9)	<b>0.000177</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000178</b>	1.000000		<b>0.000614</b>	<b>0.953391</b>	<b>0.997365</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000182</b>	<b>0.000179</b>	<b>0.000179</b>
F1M	2 0 (10)	<b>0.000177</b>	<b>0.000177</b>	<b>0.000183</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.959623</b>	<b>0.001203</b>	<b>0.000614</b>		<b>0.000178</b>	<b>0.000179</b>	<b>0.000177</b>	<b>0.000322</b>	<b>0.109088</b>	<b>0.000178</b>	<b>0.999754</b>	<b>0.996610</b>
F1M	2 2 (11)	<b>0.000177</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.877791</b>	<b>0.953391</b>	<b>0.000178</b>		1.000000		<b>0.000177</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000177</b>
F1M	2 4 (12)	<b>0.000177</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.000177</b>	<b>0.984979</b>	<b>0.997365</b>	<b>0.000179</b>	1.000000		<b>0.000177</b>						
F6M	1 0 (13)	<b>0.000177</b>	1.000000	<b>0.236624</b>	<b>0.000177&lt;/</b>														

**Table 13: Tukey HSD test for b\* value of 6 fish cake groups.**

		Tukey HSD test; Variable: b value (Spreadsheet29)																	
		Marked differences are significant at p < .05000																	
		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)
		M=12.566	M=12.694	M=12.546	M=12.914	M=12.880	M=12.902	M=12.352	M=12.042	M=11.968	M=12.476	M=12.072	M=11.794	M=13.378	M=12.684	M=12.466	M=13.562	M=13.134	M=12.614
FR	1 0 (1)		1.00000	1.00000	0.957761	0.983780	0.969100	0.999821	0.481831	0.255943	1.00000	0.587415	0.029770	0.016425	1.00000	1.00000	0.000877	0.338715	1.00000
FR	1 2 (2)	1.00000		0.99999	0.99974	0.999974	0.999974	0.999871	0.963761	0.143574	0.056743	0.999772	0.200175	0.004062	0.097778	1.00000	0.999593	0.006815	0.768356
FR	1 4 (3)	1.00000	0.99999		0.932524	0.970744	0.948663	0.999953	0.552000	0.309814	1.00000	0.657462	0.039612	0.012061	1.00000	1.00000	0.000660	0.282035	1.00000
FR	2 0 (4)	0.957761	0.99974	0.932524		1.00000	1.00000	0.356871	0.006390	0.001913	0.774370	0.10320	0.000236	0.691494	0.999545	0.743642	0.150317	0.99974	0.989794
FR	2 2 (5)	0.983780	0.99974	0.970744	1.00000		1.00000	0.468057	0.010988	0.003343	0.864727	0.017453	0.000236	0.573251	0.999945	0.840651	0.100232	0.996433	0.997293
FR	2 4 (6)	0.969100	0.99987	0.948663	1.00000	1.00000		0.468057	0.007744	0.007744	0.808933	0.012462	0.000253	0.650556	0.999772	0.780312	0.130741	0.999492	0.993378
F1M	1 0 (7)	0.999821	0.963761	0.99995	0.356871	0.468057	0.394612		0.985724	1.00000	0.786190	1.00000	0.995132	0.369245	0.000578	0.972325	1.00000	0.000186	0.025733
F1M	1 2 (8)	0.481831	0.143574	0.552000	0.006390	0.010988	0.007744	0.985724		1.00000	0.786190	1.00000	0.998832	0.000178	0.160888	0.814424	0.000177	0.000283	0.326903
F1M	1 4 (9)	0.255943	0.056743	0.309814	0.001913	0.003343	0.002326	0.985724	1.00000		0.537852	1.00000	0.999990	0.000178	0.064845	0.573251	0.000177	0.000200	0.153778
F1M	2 0 (10)	1.00000	0.999772	1.00000	0.774370	0.864727	0.808933	1.00000	0.786190	0.537852		0.864727	0.100232	0.003932	0.999877	1.00000	0.000296	0.133844	1.00000
F1M	2 2 (11)	0.587415	0.200175	0.657462	0.010320	0.017453	0.012462	0.995132	1.00000	0.864727	0.537852		0.995503	0.000178	0.222263	0.886548	0.000177	0.000377	0.420850
F1M	2 4 (12)	0.029770	0.004062	0.039612	0.000236	0.000236	0.000236	0.369245	0.998832	0.999990	0.100232	0.995503		0.000177	0.004771	0.113283	0.000177	0.000178	0.014536
F6M	1 0 (13)	0.016425	0.097778	0.012061	0.000236	0.000236	0.000236	0.369245	0.998832	0.999990	0.100232	0.995503	0.000177		0.004771	0.003343	0.000177	0.999045	0.033405
F6M	1 2 (14)	1.00000	1.00000	1.00000	0.999545	0.999945	0.999772	0.723225	0.160888	0.064845	0.999877	0.222263	0.004771	0.086241		0.999772	0.005805	0.737311	1.00000
F6M	1 4 (15)	1.00000	0.99999	1.00000	0.743642	0.840651	0.780312	1.00000	0.814424	0.573251	1.00000	0.865454	0.113283	0.003343	0.999772		0.000274	0.118886	0.999999
F6M	2 0 (16)	0.000877	0.006815	0.000660	0.150317	0.100232	0.130741	0.000186	0.000178	0.000236	0.000177	0.000177	0.000177	0.000177	0.999977	0.005805	0.000274	0.803363	0.001853
F6M	2 2 (17)	0.338715	0.768356	0.282035	0.99974	0.998433	0.999492	0.257333	0.000236	0.000236	0.133844	0.000178	0.000178	0.000178	0.999045	0.737311	0.118886	0.803363	0.495723
F6M	2 4 (18)	1.00000	1.00000	1.00000	0.989794	0.997293	0.993378	0.997733	0.326903	0.153778	1.00000	0.420850	0.014536	0.033405	1.00000	0.99999	0.001853	0.495723	

**Table 14: Tukey HSD test for whiteness of 6 fish cake groups.**

		Tukey HSD test; Variable: Whiteness (Spreadsheet29)																	
		Marked differences are significant at p < .05000																	
		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)
		M=69.931	M=69.141	M=71.199	M=69.497	M=68.709	M=69.892	M=72.399	M=71.424	M=72.482	M=72.636	M=71.668	M=73.044	M=71.808	M=70.360	M=72.478	M=71.235	M=70.784	M=71.833
FR	1 0 (1)		0.317891	0.002756	0.982980	0.004825	1.00000	0.000177	0.000293	0.000177	0.000177	0.000180	0.000177	0.000177	0.984402	0.000177	0.001804	0.202143	0.000178
FR	1 2 (2)	0.317891		0.000177	0.997972	0.983637	0.403146	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.004945	0.000177	0.000177	0.000185	0.000177
FR	1 4 (3)	0.002756	0.000177		0.000182	0.000177	0.001765	0.000216	0.999996	0.002315	0.000447	0.964395	0.000178	0.757207	0.225132	0.002441	1.00000	0.998896	0.700558
FR	2 0 (4)	0.982980	0.997972	0.000182		0.321527	0.993426	0.000177	0.000178	0.000177	0.000177	0.000177	0.000177	0.187080	0.000177	0.000180	0.000177	0.000222	0.000177
FR	2 2 (5)	0.004825	0.097778	0.000177	0.321527		0.007546	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177
FR	2 4 (6)	1.00000	0.403146	0.001765	0.993426	0.007546		0.000177	0.000243	0.000177	0.000177	0.000177	0.000178	0.965144	0.000177	0.001162	0.149815	0.000178	
F1M	1 0 (7)	0.000177	0.000177	0.006210	0.000177	0.000177	0.000177		0.069954	0.069954	1.00000	0.999991	0.452857	0.674683	0.797165	0.000177	1.00000	0.009433	0.000195
F1M	1 2 (8)	0.000293	0.000177	0.99999	0.000178	0.000177	0.000243	0.069954		0.030405	0.030405	0.99998	0.005393	0.000194	0.995122	0.028271	0.031811	1.00000	0.684595
F1M	1 4 (9)	0.000177	0.000177	0.002315	0.000177	0.000177	0.000177	1.00000	0.030405		1.00000	0.269327	0.853755	0.599811	0.000177	1.00000	0.003668	0.000182	0.661400
F1M	2 0 (10)	0.000177	0.000177	0.000447	0.000177	0.000177	0.000177	0.999991	0.005393	1.00000		0.075000	0.990937	0.244598	0.000177	1.00000	0.000624	0.000178	0.290333
F1M	2 2 (11)	0.000180	0.000177	0.964395	0.000177	0.000177	0.000178	0.99998	0.005393	0.269327	0.075000		0.000805	1.00000	0.001734	0.277588	0.983144	0.157691	1.00000
F1M	2 4 (12)	0.000177	0.000177	0.000178	0.000177	0.000177	0.000177	0.674683	0.000194	0.853755	0.990937	0.000805		0.004117	0.000177	0.846100	0.000178	0.000177	0.005485
F6M	1 0 (13)	0.000178	0.000177	0.757207	0.000177	0.000177	0.000178	0.797165	0.995122	0.599811	0.244598	1.00000	0.004117		0.000400	0.610888	0.831607	0.043025	1.00000
F6M	1 2 (14)	0.984402	0.000494	0.225132	0.187080	0.000182	0.965144	0.000177	0.028271	0.000177	0.000177	0.001734	0.000177	0.000400		0.000177	0.170364	0.986494	0.000342
F6M	1 4 (15)	0.000177	0.000177	0.002441	0.000177	0.000177	0.000177	1.00000	0.031811	1.00000	1.00000	0.277588	0.846100	0.610888	0.000177		0.003752	0.000182	0.672153
F6M	2 0 (16)	0.001804	0.000177	1.00000	0.000180	0.000177	0.001162	0.009433	1.00000	0.003668	0.000624	0.983144	0.000178	0.831607	0.170364	0.000375		0.974892	0.782738
F6M	2 2 (17)	0.202143	0.000185	0.988896	0.002227	0.000177	0.149815	0.000195	0.684595	0.000182	0.000178	0.157691	0.000177	0.043025	0.986494	0.000182	0.974892		0.335332
F6M	2 4 (18)	0.000178	0.000177	0.700558	0.000177	0.000177	0.000178	0.844264	0.990583	0.661400	0.290333	1.00000	0.005485	1.00000	0.000342	0.672153	0.782738	0.033533	

**Table 15: Tukey HSD test for Braking force of 6 fish cake groups.**

		Tukey HSD test; Variable: Braking force (Spreadsheet29)																	
		Marked differences are significant at p < .05000																	
		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)
		M=6493.4	M=6466.9	M=10392	M=8553.5	M=8887.0	M=9484.4	M=6213.4	M=6321.6	M=8073.5	M=6278.1	M=7743.9	M=8857.1	M=5690.1	M=6613.7	M=6819.0	M=6261.8	M=8167.2	M=7752.2
FR	1 0 (1)		1.00000	0.000177	0.000198	0.000176	0.000177	0.99998	1.00000	0.002596	1.00000	0.050976	0.000176	0.666465	1.00000	0.999961	1.00000	0.001075	0.047687
FR	1 2 (2)	1.00000		0.000177	0.000184	0.000176	0.000177	0.99999	1.00000	0.002015	1.00000	0.041101	0.000176	0.718533	1.00000	0.999886	1.00000	0.000854	0.038386
FR	1 4 (3)	0.000177	0.000177		0.000322	0.005357	0.452684	0.000177	0.000177	0.990176	0.000177	0.000177	0.004023	0.000177	0.000177	0.000177	0.000177	0.000177	0.000178
FR	2 0 (4)	0.000198	0.000184	0.000322		0.999946	0.406625	0.000178	0.000178	0.994805	0.000178	0.653712	0.999986	0.000177	0.000216	0.000644	0.000178	0.999622	0.670403
FR	2 2 (5)	0.000178	0.000178	0.005357	0.999946		0.955010	0.000177	0.000177	0.645623	0.000177	0.114882	1.00000	0.000177	0.000178	0.000180	0.000177	0.000177	0.218663
FR	2 4 (6)	0.000177	0.000177	0.452684	0.406625	0.955010		0.000177	0.000177	0.012861	0.000177	0.012861	0.000614	0.932404	0.000177	0.000177	0.000177	0.000177	0.002935
F1M	1 0 (7)	0.999996	0.999996	0.000177	0.000178	0.000177	0.000177		1.00000	0.000284	1.00000	0.004188	0.000177	0.987020	0.999400	0.949407	1.00000	0.000211	0.030871
F1M																			

**Table 17: Tukey HSD test for shear force of 6 fish cake groups.**

		Tukey HSD test; Variable: Shear force (Spreadsheet29)																	
		Marked differences are significant at p < .05000																	
		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)
MATERIAL FORMING TIME		M=1250.8	M=1686.2	M=1473.6	M=1379.5	M=1912.4	M=1504.2	M=1196.2	M=1420.1	M=1192.3	M=1168.2	M=1584.9	M=1150.2	M=1032.2	M=1699.4	M=1073.2	M=1186.9	M=1730.8	M=1272.0
FR	1 0 (1)		0.000177	0.000177	0.000177	0.000177	0.000177	0.034517	0.000177	0.015351	0.000207	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.991046
FR	1 2 (2)			0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.201156
FR	1 4 (3)				0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177
FR	2 0 (4)					0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177
FR	2 2 (5)						0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177
FR	2 4 (6)							0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177
F1M	1 0 (7)								0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177
F1M	1 2 (8)									0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177
F1M	1 4 (9)										0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177
F1M	2 0 (10)											0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177
F1M	2 2 (11)												0.000177	0.000177	0.000177	0.000177	0.000177	0.000177	0.000177
F1M	2 4 (12)													0.000177	0.000177	0.000177	0.000177	0.000177	0.000177
F6M	1 0 (13)														0.000177	0.000177	0.000177	0.000177	0.000177
F6M	1 2 (14)															0.000177	0.000177	0.000177	0.000177
F6M	1 4 (15)																0.000177	0.000177	0.000177
F6M	2 0 (16)																	0.000177	0.000177
F6M	2 2 (17)																		0.000177
F6M	2 4 (18)																		

**Table 18: Tukey HSD test for pH of 6 fish cake groups.**

		Tukey HSD test; Variable: pH (FULL DATA)																	
		Marked differences are significant at p < .05000																	
		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)
Material Forming Time		M=7.1500	M=6.8000	M=6.7050	M=7.1250	M=6.8100	M=6.9300	M=7.0550	M=6.7650	M=6.7000	M=7.1050	M=6.7900	M=6.7400	M=7.1500	M=6.7800	M=6.7000	M=7.0750	M=6.7800	M=6.7600
FR	1 0 (1)		0.000181	0.000181	0.992871	0.000181	0.000181	0.006407	0.000181	0.000181	0.612484	0.000181	0.000181	1.000000	0.000181	0.000181	0.051324	0.000181	0.000181
FR	1 2 (2)			0.006407	0.000181	1.000000	0.000181	0.000181	0.890110	0.003818	0.000181	1.000000	0.211959	0.000181	0.999373	0.003818	0.000181	0.999373	0.766869
FR	1 4 (3)				0.000181	0.002305	0.000181	0.000181	0.211959	1.000000	0.000181	0.016289	0.890110	0.000181	0.051324	1.000000	0.000181	0.051324	0.316525
FR	2 0 (4)					0.000259	0.000181	0.000181	0.000181	0.000181	0.999373	0.000181	0.000181	0.992871	0.000181	0.000181	0.455429	0.000181	0.000181
FR	2 2 (5)						0.000181	0.000181	0.000181	0.000181	0.000181	0.999373	0.000181	0.000181	0.963647	0.001427	0.000181	0.963647	0.455429
FR	2 4 (6)							0.000181	0.000181	0.000181	0.000181	0.000181	0.000181	0.000181	0.000181	0.000181	0.000181	0.000181	0.000181
F1M	1 0 (7)								0.000181	0.000181	0.000181	0.000181	0.000181	0.000181	0.000181	0.000181	0.000181	0.000181	0.000181
F1M	1 2 (8)									0.000181	0.000181	0.000181	0.000181	0.000181	0.000181	0.000181	0.000181	0.000181	0.000181
F1M	1 4 (9)										0.000181	0.000181	0.000181	0.000181	0.000181	0.000181	0.000181	0.000181	0.000181
F1M	2 0 (10)											0.000181	0.000181	0.000181	0.000181	0.000181	0.000181	0.000181	0.000181
F1M	2 2 (11)												0.000181	0.000181	0.000181	0.000181	0.000181	0.000181	0.000181
F1M	2 4 (12)													0.000181	0.000181	0.000181	0.000181	0.000181	0.000181
F6M	1 0 (13)														0.000181	0.000181	0.000181	0.000181	0.000181
F6M	1 2 (14)															0.000181	0.000181	0.000181	0.000181
F6M	1 4 (15)																0.000181	0.000181	0.000181
F6M	2 0 (16)																	0.000181	0.000181
F6M	2 2 (17)																		0.000181
F6M	2 4 (18)																		

**Table 19: Tukey HSD test for lipid content of 6 fish cake groups.**

		Tukey HSD test; Variable: Lipid content (FULL DATA)																	
		Marked differences are significant at p < .05000																	
		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)
Material Forming Time		M=3.5770	M=3.5286	M=3.6736	M=3.6993	M=3.6493	M=3.4493	M=2.3796	M=2.4729	M=2.4263	M=2.3796	M=2.4263	M=2.5196	M=3.1994	M=3.3836	M=3.2993	M=3.3493	M=3.3993	M=2.9994
FR	1 0 (1)		1.000000	1.000000	0.999993	1.000000	0.999987	0.000235	0.000359	0.000277	0.000235	0.000277	0.000513	0.654349	0.997746	0.938758	0.982424	0.999137	0.114396
FR	1 2 (2)			0.999930	0.999462	0.999994	1.000000	0.000279	0.000519	0.000362	0.000279	0.000362	0.000784	0.818911	0.999930	0.987426	0.999036	0.999985	0.190520
FR	1 4 (3)				1.000000	1.000000	0.989760	0.000194	0.000232	0.000205	0.000194	0.000205	0.000273	0.321603	0.916686	0.666466	0.833460	0.943908	0.037783
FR	2 0 (4)					1.000000	0.999993	0.000190	0.000214	0.000198	0.000190	0.000214	0.000248	0.254063	0.857394	0.000248	0.000248	0.752714	0.895914
FR	2 2 (5)						0.999994	0.000199	0.000215	0.000199	0.000199	0.000215	0.000306	0.395740	0.955984	0.000306	0.000306	0.895914	0.973071
FR	2 4 (6)							0.000454	0.001081	0.000689	0.000454	0.000689	0.001195	0.973071	1.000000	0.999891	1.000000	1.000000	0.395740
F1M	1 0 (7)								1.000000	1.000000	1.000000	1.000000	0.999956	0.006510	0.000822	0.002007	0.001161	0.000710	0.071316
F1M	1 2 (8)									1.000000	1.000000	1.000000	1.000000	0.002198	0.002223	0.006007	0.003306	0.001863	0.195887
F1M	1 4 (9)										1.000000	1.000000	1.000000	0.011474	0.001328	0.003438	0.001933	0.001122	0.120054
F1M	2 0 (10)											1.000000	1.000000	0.000182	0.000822	0.002007	0.001161	0.000710	0.071316
F1M	2 2 (11)												1.000000	0.011474	0.001328	0.003438	0.001933	0.001122	0.120054
F1M	2 4 (12)														0.000381	0.001328	0.003438	0.001933	0.001122
F6M	1 0 (13)															0.000182	0.000822	0.002007	0.001161
F6M	1 2 (14)																0.000182	0.000822	0.002007
F6M	1 4 (15)																	0.000182	0.000822
F6M	2 0 (16)																		0.000182
F6M	2 2 (17)																		
F6M	2 4 (18)																		

**Table 20: Tukey HSD test for FFA content of 6 fish cake groups.**

		Tukey HSD test; Variable: FFA (FULL DATA)																	
		Marked differences are significant at p < .05000																	
		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)
Material Forming Time		M=8.4651	M=8.4608	M=8.2481	M=10.037	M=6.7903	M=6.6049	M=17.831	M=17.890	M=16.046	M=18.202	M=15.572	M=14.201	M=19.574	M=20.782	M=18.047	M=18.224	M=19.777	M=18.088
FR	1 0 (1)		1.000000	1.000000	0.978094	0.962984	0.919355	0.000182	0.000182	0.000250	0.000182	0.000362	0.002821	0.000181	0.000181	0.000182	0.000182	0.000181	0.000182
FR	1 2 (2)			1.000000	0.977574	0.963735	0.920627	0.000182	0.000182	0.000250	0.000182	0.000362	0.002795	0.000181	0.000181	0.000182	0.000182	0.000181	0.000182
FR	1 4 (3)				0.988918	0.968264	0.920627	0.000182	0.000182	0.000250	0.000182	0.000362	0.002795	0.000181	0.000181	0.000182	0.000182	0.000181	0.000182
FR	2 0 (4)					0.244699	0.												

**Table 21: Tukey HSD test for phospholipid content of 6 fish cake groups.**

		Tukey HSD test: Variable: Phospholipid (FULL DATA)																	
		Marked differences are significant at p < .05000																	
		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)
		M=4.5998	M=2.7661	M=7.9327	M=4.9896	M=3.9515	M=8.6120	M=5.9955	M=4.8984	M=13.480	M=9.0498	M=4.8817	M=12.553	M=6.2650	M=3.6264	M=10.533	M=5.6401	M=3.1391	M=9.6154
FR	1 0 (1)																		
FR	1 2 (2)	0.102736																	
FR	1 4 (3)	0.000462	0.000181																
FR	2 0 (4)	0.999991	0.023981	0.001556															
FR	2 2 (5)	0.995217	0.646607	0.000197	0.809117														
FR	2 4 (6)	0.000195	0.000181	0.992363	0.000266	0.000182													
F1M	1 0 (7)	0.405281	0.000624	0.070746	0.839507	0.047586	0.005220												
F1M	1 2 (8)	1.000000	0.034032	0.001136	1.000000	0.888293	0.000240	0.747761											
F1M	1 4 (9)	0.000181	0.000181	0.000181	0.000181	0.000181	0.000181	0.000181	0.000181										
F1M	2 0 (10)	0.000183	0.000181	0.725606	0.000193	0.000181	0.999955	0.001060	0.000189	0.000183									
F1M	2 2 (11)	1.000000	0.036293	0.001071	1.000000	0.900380	0.000236	0.729256	1.000000	0.000181	0.000188								
F1M	2 4 (12)	0.000181	0.000181	0.000182	0.000181	0.000181	0.000201	0.000181	0.000181	0.902816	0.000319	0.000181							
F6M	1 0 (13)	0.182655	0.000321	0.181206	0.540156	0.016933	0.014863	1.000000	0.436292	0.000181	0.002757	0.418280	0.000181						
F6M	1 2 (14)	0.867507	0.942004	0.000185	0.439982	0.999999	0.000181	0.013641	0.544130	0.000181	0.000181	0.563847	0.000181	0.0004794					
F6M	1 4 (15)	0.000181	0.000181	0.005552	0.000181	0.000181	0.075017	0.000182	0.000181	0.001536	0.319123	0.000181	0.052015	0.000185	0.000181				
F6M	2 0 (16)	0.806954	0.001989	0.018359	0.995049	0.169181	0.001408	0.999998	0.982655	0.000181	0.000387	0.978982	0.000181	0.986735	0.053294	0.000181			
F6M	2 2 (17)	0.340248	0.999995	0.000182	0.096805	0.962604	0.000181	0.002120	0.132172	0.000181	0.000181	0.141068	0.000181	0.000844	0.999820	0.000181	0.008163		
F6M	2 4 (18)	0.000181	0.000181	0.172482	0.000182	0.000181	0.841784	0.000267	0.000182	0.000209	0.998910	0.000182	0.001583	0.000443	0.000181	0.908738	0.000198	0.000181	

**Table 22: Tukey HSD test for TBARS value of 6 fish cake groups.**

		Tukey HSD test: Variable: TBAR (FULL DATA)																	
		Marked differences are significant at p < .05000																	
		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)
		M=13.604	M=21.699	M=24.988	M=13.889	M=20.747	M=19.122	M=9.6930	M=10.267	M=10.843	M=8.7038	M=7.9129	M=9.0408	M=12.026	M=15.091	M=14.648	M=9.4357	M=8.7567	M=8.6884
FR	1 0 (1)																		
FR	1 2 (2)	0.000180	0.000180	0.000167	1.000000	0.000335	0.008460	0.191846	0.425681	0.729082	0.031318	0.005793	0.060919	0.977432	0.998704	0.999987	0.125223	0.034855	0.030349
FR	1 4 (3)	0.000167	0.450057		0.000195	0.999997	0.814335	0.000167	0.000167	0.000167	0.000167	0.000167	0.000167	0.000167	0.000168	0.000813	0.000379	0.000167	0.000167
FR	2 0 (4)	0.000167	0.000195	0.000167	0.110249	0.003936	0.000167	0.000167	0.000167	0.000167	0.000167	0.000167	0.000167	0.000167	0.000168	0.000168	0.000167	0.000167	0.000167
FR	2 2 (5)	1.000000	0.000195	0.000167	0.000517	0.119248	0.015645	0.119248	0.294762	0.578878	0.017305	0.003083	0.034759	0.985281	1.000000	0.074915	0.019346	0.016750	0.000167
FR	2 4 (6)	0.000335	0.999997	0.110249	0.000517	0.996433	0.000167	0.000168	0.000168	0.000168	0.000167	0.000167	0.000167	0.000169	0.006271	0.002352	0.000167	0.000167	0.000167
F1M	1 0 (7)	0.008460	0.814335	0.003936	0.015645	0.996433	0.000168	0.000168	0.000168	0.000168	0.000168	0.000168	0.000168	0.000356	0.158009	0.072118	0.000168	0.000168	0.000168
F1M	1 2 (8)	0.191846	0.000167	0.000167	0.119248	0.000167	0.000168	1.000000	1.000000	0.999951	0.999994	0.906164	1.000000	0.902594	0.010965	0.027997	1.000000	0.999997	0.999993
F1M	1 4 (9)	0.425681	0.000167	0.000167	0.294762	0.000168	0.000169	1.000000	1.000000	0.997690	0.896388	0.999885	0.991658	0.036485	0.085685	1.000000	0.085685	1.000000	0.997413
F1M	2 0 (10)	0.729082	0.000167	0.000167	0.578878	0.000168	0.000175	0.999951	1.000000	0.949694	0.641417	0.989369	0.999928	0.108865	0.226052	0.999333	0.959093	0.946688	0.000167
F1M	2 2 (11)	0.031318	0.000167	0.000167	0.017305	0.000168	0.000168	0.999994	0.997690	0.949694	1.000000	1.000000	0.432937	0.001270	0.003309	0.000167	0.000167	0.000167	0.000167
F1M	2 4 (12)	0.005793	0.000167	0.000167	0.003083	0.000167	0.000167	0.990614	0.896388	0.641417	1.000000	0.999963	0.137548	0.000321	0.000638	0.988293	0.999999	1.000000	0.000167
F6M	1 0 (13)	0.060919	0.000167	0.000167	0.034759	0.000167	0.000168	1.000000	0.999885	0.989369	1.000000	0.999963	0.611677	0.026171	0.006965	1.000000	0.405994	1.000000	1.000000
F6M	1 2 (14)	0.977432	0.000168	0.000167	0.985281	0.000169	0.000356	0.902594	0.991658	0.999928	0.432937	0.137548	0.611677	0.568771	0.006265	0.000167	0.000167	0.000167	0.000167
F6M	1 4 (15)	0.998704	0.000813	0.000168	0.999911	0.006271	0.158009	0.010965	0.036485	0.108865	0.001270	0.000321	0.002617	0.568771	0.006965	1.000000	0.006265	0.000167	0.000167
F6M	2 0 (16)	0.999987	0.000379	0.000168	1.000000	0.002352	0.072118	0.027997	0.085685	0.226052	0.003309	0.000638	0.006965	0.794889	1.000000	0.000167	0.000167	0.000167	0.000167
F6M	2 2 (17)	0.125223	0.000167	0.000167	0.074915	0.000167	0.000168	1.000000	1.000000	0.999333	1.000000	0.998293	1.000000	0.808457	0.006265	0.016353	1.000000	1.000000	1.000000
F6M	2 4 (18)	0.034855	0.000167	0.000167	0.019346	0.000167	0.000168	0.999997	0.998448	0.959093	1.000000	0.999999	1.000000	0.459944	0.001417	0.003718	1.000000	1.000000	1.000000

**Table 23: Tukey HSD test for PV value of 6 fish cake groups.**

		Tukey HSD test: Variable: PV (FULL DATA)																	
		Marked differences are significant at p < .05000																	
		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)
		M=24.472	M=42.331	M=30.513	M=20.040	M=33.995	M=25.388	M=14.370	M=21.486	M=19.089	M=10.055	M=26.743	M=15.714	M=17.589	M=32.638	M=25.417	M=6.8177	M=25.311	M=24.308
FR	1 0 (1)																		
FR	1 2 (2)	0.000167	0.000175	0.002501	0.000167	0.999845	0.000167	0.143204	0.000167	0.000263	0.000167	0.544409	0.000167	0.000168	0.000167	0.999768	0.000167	0.999952	1.000000
FR	1 4 (3)	0.000175	0.000167	0.000167	0.000167	0.000167	0.000167	0.000167	0.000167	0.000167	0.000167	0.000167	0.000167	0.000167	0.000167	0.651255	0.000420	0.000167	0.000167
FR	2 0 (4)	0.002501	0.000167	0.000167	0.000167	0.000167	0.000167	0.000198	0.973926	0.999748	0.000167	0.000168	0.003453	0.416202	0.000167	0.000264	0.000167	0.000306	0.004109
FR	2 2 (5)	0.000167	0.000167	0.040697	0.000167	0.000167	0.000167	0.000167	0.000167	0.000167	0.000167	0.000168	0.000167	0.000167	0.985643	0.000167	0.000167	0.000167	0.000167
FR	2 4 (6)	0.999845	0.000167	0.000397	0.000274	0.000167	0.000167	0.000167	0.012327	0.000170	0.000167	0.985830	0.000167	0.000167	0.000168	1.000000	0.000167	1.000000	0.998795
F1M	1 0 (7)	0.000167	0.000167	0.000167	0.000198	0.000167	0.000167	0.000168	0.000168	0.001089	0.003562	0.986944	0.081406	0.000167	0.000167	0.000167	0.000167	0.000167	0.000167
F1M	1 2 (8)	0.143204	0.000167	0.000167	0.973926	0.000167	0.012327	0.000168	0.000168	0.452915	0.000167	0.000313	0.000188	0.012520	0.000167	0.011323	0.000167	0.015470	0.206057
F1M	1 4 (9)	0.000263	0.000167	0.000167	0.999748	0.000167	0.000170	0.001089	0.452915	0.000167	0.000167	0.000168	0.054349	0.964218	0.000167	0.000167	0.000167	0.000167	0.000333
F1M	2 0 (10)	0.000167	0.000167	0.000167	0.000167	0.000167	0.000167	0.003562	0.000167	0.000167	0.000167	0.000167	0.000200	0.000167	0.000200	0.000167	0.077822	0.000167	0.000167
F1M	2 2 (11)	0.544409	0.000167	0.018154	0.000168	0.000168	0.985830	0.000167	0.000313	0.000168	0.000167	0.000167	0.000167	0.000167	0.000168	0.988518	0.000167	0.976110	0.426928
F1M	2 4 (12)	0.000167	0.000167	0.000167	0.003453	0.000167	0.000167	0.986944	0.000168	0.054349	0.000200	0.000167	0.000167	0.817805	0.000167	0.000167	0.000167	0.000167	0.000167
F6M	1 0 (13)	0.000168	0.000167	0.000167	0.416202	0.000167	0.000167	0.08140											

**Table 25: T-test for lipid content, pH, FFA and phospholipid content between fish cake from fresh mince and from frozen 1-month mince.**

T-tests; Grouping: Material (FULL DATA)											
Group 1: FR											
Group 2: F1M											
Variable	Mean FR	Mean F1M	t-value	df	p	Valid N FR	Valid N F1M	Std.Dev. FR	Std.Dev. F1M	F-ratio Variances	p Variances
Lipid content	3.596173	2.43405	25.5739	22	0.000000	12	12	0.106709	0.115725	1.176119	0.79267
pH	6.930000	6.85917	1.0103	22	0.323348	12	12	0.176892	0.166431	1.129667	0.84336
FFA	8.101110	16.62345	-14.3143	22	0.000000	12	12	1.254174	1.637282	1.704243	0.39016
Phospholipid	5.475288	8.47646	-2.4161	22	0.024436	12	12	2.210675	3.691727	2.788749	0.10331

**Table 26: T-test for PV and TBARS values between fish cake from fresh mince and from frozen 1 month mince.**

T-tests; Grouping: material (FULL DATA)											
Group 1: FR											
Group 2: F1M											
Variable	Mean FR	Mean F1M	t-value	df	p	Valid N FR	Valid N F1M	Std.Dev. FR	Std.Dev. F1M	F-ratio Variances	p Variances
TBAR	19.00830	9.41009	8.395936	34	0.000000	18	18	4.729114	1.076882	19.28517	0.000000
PV	29.45649	17.90941	5.222869	34	0.000009	18	18	7.525949	5.598495	1.80709	0.232638

**Table 27: T-test for L\*, a\*, b\* values, whiteness, breaking force, hardness and shear force between fish cake from fresh mince and from frozen 6 months mince.**

T-tests; Grouping: MATERIAL (FULL DATA)											
Group 1: FR											
Group 2: F6M											
Variable	Mean FR	Mean F6M	t-value	df	p	Valid N FR	Valid N F6M	Std.Dev. FR	Std.Dev. F6M	F-ratio Variances	p Variances
L- value	72.552	74.542	-8.23870	58	0.000000	30	30	0.998	0.868	1.321286	0.457758
a value	0.131	-0.452	6.66577	58	0.000000	30	30	0.422	0.226	3.497505	0.001184
b value	12.750	12.973	-2.06588	58	0.043317	30	30	0.326	0.492	2.279663	0.030007
Whiteness	69.728	71.416	-7.60737	58	0.000000	30	30	0.927	0.787	1.387554	0.382968
Breaking force	8379.497	6884.000	4.39220	58	0.000048	30	30	1624.927	915.208	3.152301	0.002816
Hardness	9628.627	7894.267	3.17798	58	0.002375	30	30	2412.254	1765.250	1.867385	0.098118
Shear force	1534.473	1332.410	3.06509	58	0.003301	30	30	219.596	286.631	1.703713	0.157328

**Table 28: T-test for lipid content, pH, FFA and phospholipid content between fish cake from fresh mince and from frozen 6 months mince.**

T-tests; Grouping: Material (FULL DATA)											
Group 1: FR											
Group 2: F6M											
Variable	Mean FR	Mean F6M	t-value	df	p	Valid N FR	Valid N F6M	Std.Dev. FR	Std.Dev. F6M	F-ratio Variances	p Variances
Lipid content	3.596173	3.27173	4.5375	22	0.000162	12	12	0.106709	0.223532	4.388054	0.021327
pH	6.930000	6.87417	0.7643	22	0.452831	12	12	0.176892	0.180980	1.046751	0.940957
FFA	8.101110	19.08189	-18.8954	22	0.000000	12	12	1.254174	1.574694	1.576439	0.462462
Phospholipid	5.475288	6.46987	-0.9389	22	0.357971	12	12	2.210675	2.928831	1.755249	0.364789

**Table 29: T-test for PV and TBARS values between fish cake from fresh mince and from frozen 6 months mince.**

T-tests; Grouping: material (FULL DATA)											
Group 1: FR											
Group 2: F6M											
Variable	Mean FR	Mean F6M	t-value	df	p	Valid N FR	Valid N F6M	Std.Dev. FR	Std.Dev. F6M	F-ratio Variances	p Variances
TBAR	19.00830	11.44113	5.840591	34	0.000001	18	18	4.729114	2.801915	2.848719	0.0373
PV	29.45649	22.01354	2.807010	34	0.008219	18	18	7.525949	8.361452	1.234357	0.6691

**Table 30: T-test for lipid content, pH, FFA and phospholipid content between fish cake from frozen 1-month mince and from frozen 6 months mince.**

T-tests; Grouping: Material (FULL DATA)										
Group 1: F1M										
Group 2: F6M										
Variable	Mean F1M	Mean F6M	t-value	df	p	Valid N F1M	Valid N F6M	Std.Dev. F1M	Std.Dev. F6M	p
Lipid content	2.43405	3.27173	-11.5282	22	0.000000	12	12	0.115725	0.2	
pH	6.85917	6.87417	-0.2113	22	0.834571	12	12	0.166431	0.1	
FFA	16.62345	19.08189	-3.7490	22	0.001110	12	12	1.637282	1.4	
Phospholipid	8.47646	6.46987	1.4750	22	0.154369	12	12	3.691727	2.9	

**Table 31: T-test for PV and TBARS values between fish cake from frozen 1-month mince and from frozen 6 months mince.**

T-tests; Grouping: material (FULL DATA)											
Group 1: F1M											
Group 2: F6M											
Variable	Mean F1M	Mean F6M	t-value	df	p	Valid N F1M	Valid N F6M	Std.Dev. F1M	Std.Dev. F6M	F-ratio Variances	p Variances
TBAR	9.41009	11.44113	-2.87066	34	0.007000	18	18	1.076882	2.801915	6.769768	0.000271
PV	17.90941	22.01354	-1.73039	34	0.092629	18	18	5.598495	8.361452	2.230595	0.107614

**Table 32: T-test for L\*, a\*, b\* values, whiteness, breaking force, hardness and shear force between fish cake formed at 0 - 4°C/22 hours and 18 - 20°C/3 hours.**

T-tests; Grouping: FORMING (FULL DATA)											
Group 1: 1											
Group 2: 2											
Variable	Mean 1	Mean 2	t-value	df	p	Valid N 1	Valid N 2	Std.Dev. 1	Std.Dev. 2	F-ratio Variances	p Variances
L- value	74.135	73.988	0.49491	88	0.621897	45	45	1.328	1.484	1.248854	0.464097
a value	-0.415	-0.478	0.52933	88	0.597907	45	45	0.530	0.599	1.277835	0.419368
b value	12.522	12.705	-1.60658	88	0.111729	45	45	0.494	0.586	1.405894	0.262372
Whiteness	71.247	71.033	0.76073	88	0.448854	45	45	1.220	1.438	1.387855	0.280808
Breaking force	7009.280	7998.342	-3.55451	88	0.000612	45	45	1455.325	1168.847	1.550259	0.149825
Hardness	7807.496	8892.262	-2.50310	88	0.014157	45	45	2510.343	1466.156	2.931609	0.000526
Shear force	1336.020	1432.127	-1.83752	88	0.069508	45	45	236.706	258.978	1.197041	0.553364

**Table 33: T-test for lipid content, pH, FFA and phospholipid content between fish cake formed at 0 - 4°C/22 hours and 18 - 20°C/3 hours.**

Variable	T-tests; Grouping: Forming (FULL DATA)										
	Mean 1	Mean 2	t-value	df	p	Valid N 1	Valid N 2	Std.Dev. 1	Std.Dev. 2	F-ratio Variances	p Variances
Lipid content	3.10448	3.09682	0.043591	34	0.965485	18	18	0.531175	0.523286	1.030379	0.951523
pH	6.86722	6.90833	-0.709492	34	0.482856	18	18	0.188736	0.157527	1.435489	0.463903
FFA	15.03805	14.16626	0.518748	34	0.607297	18	18	5.070160	5.013058	1.022911	0.963296
Phospholipid	6.67745	6.93696	-0.241435	34	0.810668	18	18	3.381340	3.059695	1.221297	0.684899

**Table 34: T-test for PV and TBARS values between fish cake formed at 0 - 4°C/22 hours and 18 - 20°C/3 hours.**

Variable	T-tests; Grouping: forming (FULL DATA)										
	Mean 1	Mean 2	t-value	df	p	Valid N 1	Valid N 2	Std.Dev. 1	Std.Dev. 2	F-ratio Variances	p Variances
TBAR	14.76225	11.81076	2.138795	52	0.037169	27	27	5.311238	4.817466	1.215498	0.62243
PV	25.32286	20.93010	1.924561	52	0.059764	27	27	8.405026	8.367636	1.008957	0.98203

### Appendix 3: Linear regression for quality parameters of fish cake with chilled storage time

**Table 35: Linear regression for L\* value of 6 fish cake groups with chilled storage time.**

Regression Summary for Dependent Variable: L- value (FULL DATA)						
R= .11362618 R <sup>2</sup> = .01291091 Adjusted R <sup>2</sup> = .00169399						
F(1,88)=1.1510 p<.28627 Std.Error of estimate: 1.4007						
N=90	b*	Std.Err. of b*	b	Std.Err. of b	t(88)	p-value
Intercept			73.86767	0.233445	316.4243	0.000000
TIME	0.113626	0.105910	0.09700	0.090413	1.0729	0.286269

**Table 36: Linear regression for a\* value of 6 fish cake groups with chilled storage time.**

Regression Summary for Dependent Variable: a value (FULL DATA)						
R= .42468439 R <sup>2</sup> = .18035683 Adjusted R <sup>2</sup> = .17104271						
F(1,88)=19.364 p<.00003 Std.Error of estimate: .51283						
N=90	b*	Std.Err. of b*	b	Std.Err. of b	t(88)	p-value
Intercept			-0.155556	0.085471	-1.81998	0.072162
TIME	-0.424684	0.096510	-0.145667	0.033103	-4.40043	0.000030

**Table 37: Linear regression for b\* value of 6 fish cake groups with chilled storage time.**

Regression Summary for Dependent Variable: b value (FULL DATA)						
R= .37018340 R <sup>2</sup> = .13703575 Adjusted R <sup>2</sup> = .12722934						
F(1,88)=13.974 p<.00033 Std.Error of estimate: .51078						
N=90	b*	Std.Err. of b*	b	Std.Err. of b	t(88)	p-value
Intercept			12.86006	0.085129	151.0648	0.000000
TIME	-0.370183	0.099027	-0.12325	0.032970	-3.7382	0.000329

**Table 38: Linear regression for whiteness of 6 fish cake groups with chilled storage time.**

Regression Summary for Dependent Variable: Whiteness (FULL DATA)						
R= .17596294 R <sup>2</sup> = .03096296 Adjusted R <sup>2</sup> = .01995117						
F(1,88)=2.8118 p<.09712 Std.Error of estimate: 1.3170						
N=90	b*	Std.Err. of b*	b	Std.Err. of b	t(88)	p-value
Intercept			70.85486	0.219506	322.7927	0.000000
TIME	0.175963	0.104937	0.14256	0.085014	1.6768	0.097122

**Table 39: Linear regression for breaking force of 6 fish cake groups with chilled storage time.**

Regression Summary for Dependent Variable: Breaking force (FULL DATA)						
R= .57954141 R <sup>2</sup> = .33586824 Adjusted R <sup>2</sup> = .32832129						
F(1,88)=44.504 p<.00000 Std.Error of estimate: 1150.3						
N=90	b*	Std.Err. of b*	b	Std.Err. of b	t(88)	p-value
Intercept			6513.168	191.7092	33.97420	0.000000
TIME	0.579541	0.086873	495.322	74.2487	6.67112	0.000000

**Table 40: Linear regression for hardness of 6 fish cake groups with chilled storage time.**

Regression Summary for Dependent Variable: Hardness (FULL DATA)						
R= .49070749 R <sup>2</sup> = .24079384 Adjusted R <sup>2</sup> = .23216650						
F(1,88)=27.911 p<.00000 Std.Error of estimate: 1853.8						
N=90	b*	Std.Err. of b*	b	Std.Err. of b	t(88)	p-value
Intercept			7085.511	308.9682	22.93281	0.000000
TIME	0.490707	0.092883	632.184	119.6629	5.28304	0.000001

**Table 41: Linear regression for shear force of 6 fish cake groups with chilled storage time.**

Regression Summary for Dependent Variable: Shear force (FULL DATA)						
R= .12295696 R <sup>2</sup> = .01511841 Adjusted R <sup>2</sup> = .00392658						
F(1,88)=1.3508 p<.24827 Std.Error of estimate: 250.89						
N=90	b*	Std.Err. of b*	b	Std.Err. of b	t(88)	p-value
Intercept			1346.428	41.81472	32.19986	0.000000
TIME	0.122957	0.105791	18.823	16.19477	1.16226	0.248273

**Table 42: Linear regression for lipid content of 6 fish cake groups with chilled storage time.**

Regression Summary for Dependent Variable: Lipid content (FULL DATA)						
R= .02876048 R <sup>2</sup> = .00082717 Adjusted R <sup>2</sup> = -----						
F(1,34)=.02815 p<.86776 Std.Eror of estimate: .52704						
N=36	b*	Std.Err. of b*	b	Std.Err. of b	t(34)	p-value
Intercept			3.118700	0.138888	22.45483	0.000000
Time	-0.028760	0.171428	-0.009025	0.053791	-0.16777	0.867758

**Table 43: Linear regression for pH value of 6 fish cake groups with chilled storage time.**

Regression Summary for Dependent Variable: pH (FULL DATA)						
R= .82562140 R <sup>2</sup> = .68165070 Adjusted R <sup>2</sup> = .67228748						
F(1,34)=72.801 p<.00000 Std.Eror of estimate: .09880						
N=36	b*	Std.Err. of b*	b	Std.Err. of b	t(34)	p-value
Intercept			7.059861	0.026037	271.1449	0.000000
Time	-0.825621	0.096764	-0.086042	0.010084	-8.5323	0.000000

**Table 44: Linear regression for FFA value of 6 fish cake groups with chilled storage time.**

Regression Summary for Dependent Variable: FFA (FULL DATA)						
R= .15351060 R <sup>2</sup> = .02356550 Adjusted R <sup>2</sup> = -----						
F(1,34)=.82056 p<.37139 Std.Eror of estimate: 5.0016						
N=36	b*	Std.Err. of b*	b	Std.Err. of b	t(34)	p-value
Intercept			15.52698	1.318039	11.78036	0.000000
Time	-0.153511	0.169466	-0.46241	0.510474	-0.90585	0.371390

**Table 45: Linear regression for phospholipid value of 6 fish cake groups with chilled storage time.**

Regression Summary for Dependent Variable: Phospholipid (FULL DATA)						
R= .56811068 R <sup>2</sup> = .32274975 Adjusted R <sup>2</sup> = .30283062						
F(1,34)=16.203 p<.00030 Std.Error of estimate: 2.6559						
N=36	b*	Std.Err. of b*	b	Std.Err. of b	t(34)	p-value
Intercept			4.624952	0.699893	6.608082	0.000000
Time	0.568111	0.141135	1.091127	0.271067	4.025296	0.000301

**Table 46: Linear regression for TBARS value of 6 fish cake groups with chilled storage time.**

Regression Summary for Dependent Variable: TBAR (FULL DATA)						
R= .26192711 R <sup>2</sup> = .06860581 Adjusted R <sup>2</sup> = .05069438						
F(1,52)=3.8303 p<.05571 Std.Error of estimate: 5.1040						
N=54	b*	Std.Err. of b*	b	Std.Err. of b	t(52)	p-value
Intercept			11.62164	1.098216	10.58229	0.000000
time	0.261927	0.133834	0.83243	0.425337	1.95711	0.055713

**Table 47: Linear regression for PV value of 6 fish cake groups with chilled storage time.**

Regression Summary for Dependent Variable: PV (FULL DATA)						
R= .37612652 R <sup>2</sup> = .14147116 Adjusted R <sup>2</sup> = .12496099						
F(1,52)=8.5687 p<.00506 Std.Error of estimate: 8.0425						
N=54	b*	Std.Err. of b*	b	Std.Err. of b	t(52)	p-value
Intercept			19.20276	1.730473	11.09682	0.000000
time	0.376127	0.128492	1.96186	0.670209	2.92724	0.005063

## Appendix 4: Some pictures of material and products



Figure 15: Six fish cake groups.



Figure 16: Preparing samples for textural analyses.