EFFECTS OF ADDITIVES ON THE RHEOLOGICAL AND TEXTURAL PROPERTIES OF SURIMI

ROZZAMRI ASHARI

A thesis submitted to

THE UNIVERSITY OF BIRMINGHAM

for the degree of

DOCTOR OF PHILOSOPHY

School of Chemical Engineering

The University of Birmingham

October 2017

TO

my wife Norakasha Rusli

and my daughter Nailiwafa Airisha

THANK YOU

ABSTRACT

Surimi is a concentrated myofibrillar protein added with several additives to stabilize and increase its functionality. It has been used by seafood industries as an intermediate product to produce various seafood analogues. Due to the current increase in health awareness, consumer demands healthier and less sweeter surimi. The objective of this study is to investigate the rheological and textural properties of surimi added with different types of additives which is sugar, salt and sago starch. This study is also done to determine the possibility of reducing the amount of sugar and salt added to surimi. A novelty in this research is using sago starch as an additive to improve the rheological and textural property of surimi. Another concern in the surimi industry is the washing process. The washing step uses 3 washing cycle procedure that produces large amount of water waste. Thus, this study is done to understand the effects of reducing the washing cycle and using nanobubble water on the rheological and textural properties of surimi. Results obtained from this research shows that mannitol yields the best rheological and textural properties when compared to sucrose and sorbitol. Surimi with 4% (*w/*w) salt concentration yields the highest gel strength for 2 months. However, surimi with 1% (*w/w*) salt concentration which possesses a lower gel strength showed frozen stability up to 6 months. Sago starch was found to increase gel strength up to 110% when compared to sample without sago starch. Washing using medium concentrated nanobubble water (11.15 x 10^8 bubbles/ml) was found to display a high gelling strength with only 2 washing cycle when compared to washing using distilled water.

ACKNOWLEDGEMENTS

I would like to thank the God Almighty for His guidance and for the strength I needed the most to complete this research for without it I would not have finished this journey.

I would also like to express my gratitude to my supervisor, Prof. Dr. Mostafa Barigou for his supervision, motivation, guidance, knowledge and support in helping me throughout this challenging journey. Without him, I would not have completed this study.

I would also like to acknowledge my sponsors the Malaysian Ministry of Education and Universiti Putra Malaysia for providing me the financial support and giving me the opportunity to acquire knowledge from a country with different culture and diversity.

To my parents, Ashari Abu Hasan and Zamruddah Zainal Abidin, my wife and daughter, Norakasha Rusli and Nailiwafa Airisha, I greatly thank you all for the inspiration, motivation, sacrifice and moral support throughout this adventure. Thank you for believing in my capabilities. My thanks also go to my family in Malaysia, my friends especially MCIB and all the staff of University of Birmingham especially those from School of Chemical Engineering. Without their kindness and support, this research would not have been an enjoyable ride of learning and discovering new things in life.

THANK YOU

TABLE OF CONTENT

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS AND ABBREVIATIONS

cm	Centimetre
mm	Millimetre
l	Litre
ml	Millilitre
g	Gram
G'	Storage modulus
G''	Loss modulus
G'_o	Energy stored per cycle of sinusoidal shear deformation
G''_o	Energy loss per cycle of sinusoidal shear deformation
rad/s	Radian per second
°	Degree
°C	Degrees Celsius
σ	Oscillation stress
γ̇	Oscillation stain
t	Time
T	Temperature
f	Frequency
Hz	Hertz
F	Force
Pa	Pascal
ω	Frequency (rad/s)
δ	Phase angle
n'	Flow behaviour index for elasticity
n''	Flow behaviour index for viscosity
Na^+	Sodium ion

K^+	Potassium ion
ANOVA	Analysis of variance
BF	Breaking force
C	Control
DF	Breaking deformation
DSC	Differential Scanning Calorimetry
DW	Distilled water
EMC	Expressible moisture content
FTIR	Fourier Transform Infrared
GC/MS	Gas Chromatography Mass Spectrometer
M	Mannitol
MN	Medium concentration nanobubble water
HN	High concentration nanobubble water
HPLC	High Performance Liquid Chromatography
LN	Low concentration nanobubble water
LVR	Linear Viscoelastic Region
SAOS	Small Amplitude Oscillatory Shear
SDS-PAGE	Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis
SU	Sucrose
SO	Sorbitol
SS	Sucrose + Sorbitol
SM	Sucrose + Mannitol
SEM	Scanning Electron Microscope
TEM	Transmission Electron Microscope
TA	Texture analyser
WHC	Water holding capacity
W/W	Weight per weight

CHAPTER 1

Introduction

1.0 INTRODUCTION

1.1 Surimi

Surimi is an intermediate product produced by extracting fish myofibrillar protein and subsequently added with additives. Surimi is usually procured by different seafood analogue manufacturers to produce various kinds of seafood products. Ideally, surimi is made from the meat of white-fleshed fish which is naturally lean and possesses lesser fat. The two most popular fish that are used in the surimi industry are cold water fish Alaska pollock (*Gadus chalcogrammus*) and Pacific whiting (*Merluccius productus*).

Figure 1.1 Surimi blocks made from different types of fish

In mid 1980s, the high demand of surimi caused certain decrease in fish population which forced the United States of America to introduce a fishing quota (Guenneugues and Morrissey, 2005). This consequently opened up a new direction for surimi manufacturers. Extensive researches were carried out in order to increase the quality and yield of surimi due to the decrease in fish

harvesting. Surimi's quality then started to be graded from high to low as characterised by its amount of myofibrillar protein, additives and the types of fish used.

1.2 The benefits of surimi

Ever since its inception, surimi has been considered as one of the most popular seafood intermediate products. Seafood product manufacturers often favour surimi over fresh commodities to produce various seafood analogues. By using surimi, consumers who are allergic to or dislike consuming fresh seafood have the chance to consume seafood in another way. Most processed seafood products which are produced by surimi tend to possess similar (or almost similar) texture and taste as the fresh seafood commodities.

Figure 1.2 Various seafood products produced by surimi

Being processed from concentrated myofibrillar protein, surimi is known as a major source for protein (Colmenero, 2014) which is essential to humans since it serves as building blocks for the body tissue and also a source for energy (Lemon, 2000). The essential fatty acids which are unique and only present in fish meats such as omega-3 (ω-3) and omega-6 (ω-6) can also be found in surimi (Tahergorabi *et al*., 2013). Thus, consuming surimi can provide the essential nutrients required by our body. This in turn increases the marketability of surimi as it offers nutrients in the dietary intake.

Another major benefit of surimi is its *halal* status (majority seafoods are accepted as *halal* by the Muslims which approximately make up ≈25% of the global population). Compared to meats (chicken, beef, mutton, *etc*.), seafoods do not require any *halal* slaughtering procedure and are processed thoroughly through washing to prevent contamination from blood, and cutting into small pieces for packaging purposes. As a product of seafoods, surimi is therefore accepted as *halal* (provided that the hygiene and sanitation procedures at processing plants are adhered) and saves the manufacturers from applying for additional *halal* certification and authentication procedures (Feriyanto *et al*., 2016).

As surimi is already processed, it can be stored for much longer as compared to fresh seafoods. Surimi quality can be maintained by frozen storage up to six months compared to fresh seafoods. This is highly convenient for deep sea fishing (where fishermen spend longer time at sea) as surimi offers them another option in preserving seafoods and maintaining its quality. Some deep

sea fishing ships equip themselves with surimi processing equipment to produce fresh surimi and maintain quality of their harvest.

1.3 Surimi quality

Surimi is just an intermediate product for seafood manufacturers to produce a variety of seafood based products. Thus, different type of seafood products requires different range of surimi quality. However, the main characteristic to determine surimi quality is its gel strength and whiteness. Table 1.1 shows a comparison of two surimi quality.

Table 1.1: Comparison of Primary Grade and Recovery Grade Surimi (Park and Lin, 2005)

ATTRIBUTES	PRIMARY GRADE	RECOVERY GRADE
Moisture (%)	75.6	78.9
Gel Strength (g/cm)	575	231
Breaking Deformation (cm)	1.51	1.50
Colour L* Value	81.5	80.9
Colour a* Value	4.5	4.4

Surimi manufacturers have graded surimi according to their gel strength. High grade surimi are labelled as SA (600-700g/cm), FA (500-600g/cm) and AA (400-500g/cm). Low grade surimi is labelled as KA 300-399 (g/cm), KB and RA. Whiteness are usually measured using colorimeter which consists of L* Value for difference in lightness and darkness and a* Value for difference in red and green.

1.4 Rheology and gel strength

The most important characteristics in determining the quality of surimi is the gel strength. However, estimating the gel strength of surimi based on the rheological properties of the surimi paste is a challenge (Kim, Park and Yoon, 2005). Kim, Park and Yoon (2005) states that shear stress is related to force whereas shear strain is related to deformation. Many researchers develop various models and predict gel characteristics nowadays using rheological tests. Dynamic rheological tests have been used as one of the method to determine the characteristics of surimi paste and also surimi gel. Thus, a new area of research is ventured to determine the texture of surimi by understanding the rheological properties of surimi paste.

G' and G'' value are currently being used to characterise food gel properties (Kim, Park and Yoon, 2005). Campo-Deaño et. al, (2009B) and Dileep et. al, (2005) states that higher G' value associated with higher rigidity and denser structure thus producing a higher gel strength. Thus, relating the rheology and texture of samples.

LVR range for maximum stress (σ_{max}), maximum strain (γ_{max}), and maximum phase angle (δ_{max}) can also use as an indicator to predict the quality of surimi (Campo-Deaño et al., 2009B). Campo-Deaño and Tovar (2009A) measured the maximum stress and maximum strain by plotting a stress *versus* strain curve from which a linear graph would be obtained. The maximum point before the graph starts to decrease will be taken as maximum stress (σ_{max}), maximum strain (γ_{max}), and maximum phase angle (δ_{max}) before the sample deformed.

Solo-de-Zaldívar *et al*., (2014*A*) stated that higher values of σ_{max} and γ_{max} indicated that the protein structure of the fish paste has higher flexibility and denser network.

Other than that, frequency sweep has also been used to predict or characterise the quality of surimi. Sample is categorised as less stable if a material is frequency-dependent (Binsi *et al.,* 2009; Dileep *et al.,* 2005). In addition, the more frequency-dependent the sample is, the more it displays a fluid-like behaviour rather than elastic behaviour (Campo-Deaño *et al.,* 2009B). Campo and Tovar (2008) uses a power law model based on the frequency sweep where G'_o and G''_o represents the resistance to elastic and viscous deformation. Thus, relating gel structure with the viscoelastic moduli. The difference between G'_o and G''_o can be considered or measured as gel strength (Campo and Tovar, 2008).

Temperature sweep test are usually used to determine the gelation temperature of surimi gel. However, the G' and G'' value can be considered as the density of myofibrillar gel network formed during gelation. Higher gelation temperature also indicates higher amount of energy to dissociate chemical interactions and bonds within the protein network (Cando et al., 2006). This is also an indicator of rigidity and gel strength of the surimi.

Rheological tests have successfully used to determine the quality parameters of surimi. However, researchers can never relate the experimental data of rheology with actual data from texture. Furthermore, no relationship has been

reported between the surimi paste and surimi gel characteristics. By predicting the texture quality of surimi using rheological analysis of surimi paste; manufacturers and food researchers can save time and cost determining the product quality.

1.5 Challenges in surimi processing

Due to the popular demand of surimi, surimi manufacturers have been increasing the manufacturing capacity which inadvertently affects the environment. Fish population was deemed to decrease due to the increased surimi productivity especially in Alaskan pollock and Pacific whiting (Guenneugues and Morissey, 2005). Another effect of uncontrolled surimi processing is the increase amount of water waste. Surimi requires a large amount of water (1:3 mince/water) during processing to prevent microbial growth, increase quality and better shelf life which in turn causes deteriorating effect to the environment. It is reported that approximately 5.7L of water is needed to process a kilogram of fish to produce surimi (Morrissey, Lin and Ismond, 2005). Therefore, an alternative to increase surimi productivity whilst sustaining the environment is urgently warranted.

Surimi which is made of concentrated fish protein has a high tendency of contamination and protein denaturation. Food manufacturers add several additives to preserve and improve the product quality. One of the most popular additives is sugar. However, high amount of sugar will impart a sweet taste which is not altogether favourable to certain consumers. Most consumers prefer the original fresh seafood taste in their seafood products. Another

popular additive incorporated during surimi processing is salt. Salt is known to increase the quality of surimi especially its texture. However, increased intake of salt causes several health complications such as cardiovascular diseases (Hyseni *et al*., 2017). Furthermore, the increase in health awareness has changed the perspective of consumers on daily dietary intake. Educated and health-conscious consumers prefer a healthy diet with less sugar and salt in their foods. As a result, surimi products nowadays are getting less and less popular due to the incorporation of these additives.

Surimi quality has always been determined by its rheological and textural properties especially gel strength. Therefore, in the present work, in order to determine the gel strength of the surimi, fish paste was subjected to heat treatment which changes it into gel consistency. The present work was carried out to test the possibility of predicting the gel strength of surimi in its paste form by understanding the rheological properties of the surimi paste and conducting several rheological tests. Positive results obtained from this research might benefit the surimi manufacturers and also food engineers to further save time and cost by gathering information on gel strength without having to process the surimi paste to gel state. The vast application of rheology has allowed researchers to determine various food properties and producing numerous prediction models which could be utilised by the surimi industry to further optimise surimi and surimi-products processing.

1.6 Research objectives

The present work was done to determine the effects of various additives and washing effects on the quality of surimi. Nanobubble water was used to determine its ability to increase surimi quality and decrease water waste during surimi processing. Rheological testing was selected as a tool to determine the quality of surimi. Rheology properties were studied to determine the correlation between surimi paste form and its gel quality. The sample was analysed in paste form to predict the gel quality. Other than that, rheological tests were also conducted to understand the gelation mechanism of the surimi. Ultimately, the present work was done in the search for alternative way to improve surimi quality and produce a healthier surimi while preserving the environment. Specific objectives are listed below:

1) To understand the effects of different additives on the rheological properties and texture of surimi fish paste and fish gel. Sugar, salt and sago starch was selected as additives in order to improve the rheological and gelling properties of surimi fish paste and gel,
2) To determine the effects of using mannitol as an alternative cryoprotectant and comparing the rheological and textural properties of surimi added with mannitol with commercial sugar,
3) To study the effects of using nanobubbles to improve the quality of surimi and reduce the amount of washing cycle used during processing, and
4) To investigate the feasibility of using rheological to crudely predict the texture quality of surimi fish gel.

CHAPTER 2

Literature Review

2.0 LITERATURE REVIEW

2.1 Surimi

Over the recent decades, inexpensive and abundant fish which is unpopular has been transformed into high quality food products by processing it into surimi (Yang, 1998). Surimi is widely used in the food industry as a raw material for various food analogues to replace expensive seafood such as scallops, shrimps, lobster and crabs (Campo and Tovar, 2008). Other than that, surimi-based products are widely known and in demand due to its desired textural properties and health benefits to the consumers (Benjakul *et al.,* 2005).

It was reported that surimi produced around the world has increased up to from 500,000 metric tonnes in the 1990s to 750,000 metric tonnes by 2010 currently due to its popularity (Guennegues and Lanelli, 2013; Guennegues and Morrisey, 2005). Furthermore, at a surimi forum held in Astoria, Oregon (2017), Guennegues reported that the current surimi production for 2017 is about 800,000 metric tonnes. Surimi is also popular due to its unique gelling properties which consist of concentrated myofibrillar protein. This causes surimi to be used extensively as a food base for a variety of seafood products (Benjakul *et al.,* 2003). With current growing market and increasing numbers of health-conscious consumers, research on fortification of surimi has also been created by the industry to increase the quality of surimi (Solo-de-Zaldívar *et al.,* 2014B; Tahergorabi and Jaczynski, 2012B; Pietrowski *et al.,* 2011).

Surimi is a thermo-irreversible concentrated fish protein which forms a gelling network upon heating. The most essential physical characteristics to determine the quality of the surimi is its ability to form gel (Dileep *et al.,* 2011; Park and Lin, 2005). The gel strength of surimi has been the main attribute in categorising the quality and price of the surimi (An, Peters and Seymour, 1996). Other than its gel strength, surimi is also well known for its whiteness which is considered as another key indicator for surimi quality (Tahergorabi and Jaczynski, 2012B; Tabilo-Munizaga and Barbosa-Cánovas, 2005B). Surimi is mainly processed from Alaska pollock. It has been reported that more than 50% of world surimi production is made from this type of fish (Geunnegues and Morrissey, 2005). Due to its desired quality, surimi manufacturers highly prefer white-fleshed fish with low fat contents (lean meat) for surimi production

2.1.1 Surimi processing

There are several steps in surimi processing. Figure 2.1 shows the surimi processing from raw fish to frozen storage. Raw fish is first cleaned, cut, gutted, deboned and finally minced to transform the fish meat into a fish paste. Cleaning and gutting is done to remove internal organs which have high amounts of proteolytic enzymes that will disrupt the protein gel network formation during gelation (Pippatsattayanuwong, 1996). In addition, mincing is done thoroughly in order to obtain a homogenous blend of fish paste. The fish paste particle size should be taken into consideration; smaller size will be washed away during the washing process thus reducing the recovery of surimi (Park and Lin, 2005).

The washing step during surimi processing is important as it determines the gelling quality of the surimi. Washing removes lipid, blood, enzymes and sarcoplasmic protein which disrupts the formation of gelling network and also increases the myofibrillar concentration of the surimi for better gelling characteristics (Campo-Deaño *et al*., 2010; Pippatsattayanuwong, 1996). Washing is usually repeated three times and salt is added during the final wash. The sample is filtered after each washing step and finally undergoes filtration using screw press with pore size of 0.5 – 1.5 mm (Park and Lin, 2005). The surimi is then added with cryoprotectants and some chelating agents to increase shelf life and retain its mechanical properties during frozen storage.

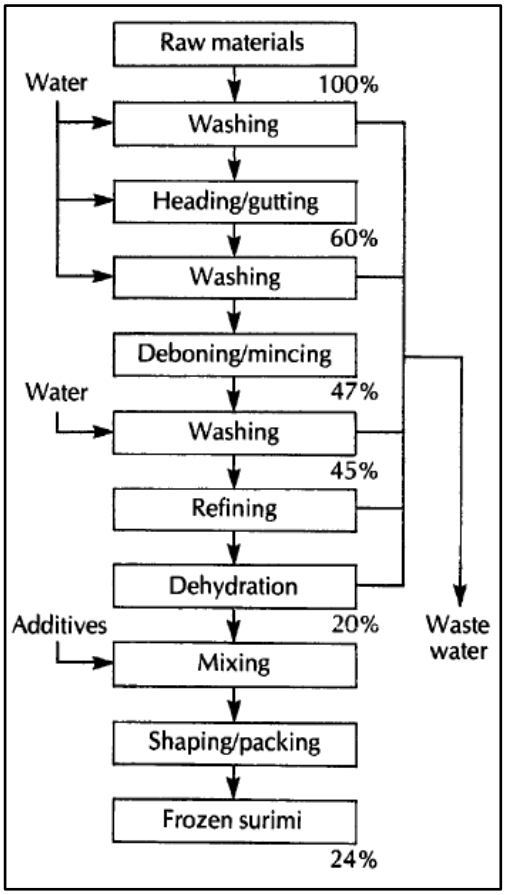


Figure 2.1: Flow diagram of surimi processing
(Park and Lin, 2005)

2.1.2 Surimi gelation

Gelation is the most important quality of the surimi (Campo-Deaño *et al*., 2009B; Esturk, 2003; Sultanbawa and Li-Chan, 1998). Myofibrillar protein represents 66 –77% of the fish composition, and plays a major role in surimi gelation (Yang, 1998). Gelation of myofibrillar protein is induced by heat which forms three-dimensional protein network (Mosavi-Nasab, 2003; Esturk, 2003). The mechanism of myofibrillar gelation involves multiple molecular interaction and bonds such as hydrogen bond, covalent bond, ionic bond, and hydrophobic bond (Lanier, Carvajal and Yongsawatdigul, 2005; Esturk, 2003; Niwa, 1992).

Gelation of myofibrillar protein to form protein networks are a combination of several type of bonds. Among the contributing bonds is the hydrogen bond. Hydrogen bond is important as it binds water within the system to produce hydrogel (Lanier, Carvajal and Yongsawatdigul, 2005; Niwa, 1992; Lanier, 1986). Water molecules are attracted to the exposed amino acid on the protein surface due to its polarity (Esturk, 2003). Ionic bonds are responsible for gel networks stability. The interaction between positively charged and negatively charged amino acid gives structure to the protein gel network (Lanier, Carvajal and Yongsawatdigul, 2006;). These two bonds are affected by temperature thus they are regarded as temperature-dependent bonds. Hydrogen and ionic bonds are stable at low temperature and become less stable as temperature increases (Damodoran, 1997). Therefore, the gelation texture of myofibrillar protein is temperature-dependent. However, covalent bonds which react due to the interaction of sulphide bonds present in cysteine are not temperature-

dependent. This makes covalent bonds as one of the main binders of protein at higher temperature. Another bond which contributes to the quality of gelation is hydrophobic bond. Hydrophobic bond occurs when protein denaturation unfolds and exposes hydrophobic sites of the protein network (Lanier, Carvajal and Yongsawatdigul, 2005; Esturk, 2003).

At low temperature, gels are stabilised by the hydrogen and ionic bonds. However, as temperature increases, covalent bonds and hydrophobic bonds become stronger and affect the gelation network of fish gel. Protein denaturation occurs at higher temperature which unfolds and exposes multiple amino acids. Exposed amino acid causes protein-protein interaction which increases the covalent and hydrophobic bonds (Moosavi-Nasab, 2003). Presence of multiple bonds contributes to the uniqueness of surimi gel. Only fish protein gelation (myofibrillar protein) consists of two gelation points. One gelation point will produce reversible gel with increase and decrease of temperature, and the other will establish a non-reversible gel (Carvajal, Lanier and Macdonald, 2005; Yoon, Gunasekaran and Park, 2004).

Lanier (1986) explained that among the early quality attributes of gelation are strength and elasticity. Furthermore, the gel structure created must be able to enclose water, fat, and other constituents to be considered as a quality gel. However, some constituents that are present in the fish inhibit myofibrillar gelation such as sarcoplasmic protein (Lanier, Carvajal and Yongsawatdigul, 2005; Yang, 1998). The heat-stable proteases which are present within the sarcoplasmic protein tend to degrade the textural properties at temperature

range of 50 to 70°C. Sarcoplasmic proteins possess low water holding capacity and do not gel with myosin cross-linking during gelation which causes a poor gelation of surimi (Benjakul *et al.,* 2010).

2.2 Effects of additives

The expansion of surimi industry over the years has forced manufacturers to research for various methods to sustain and produce higher quality surimi. Hence, additives were one of the options developed by the researchers. Additives are added to improve surimi's mechanical and functional properties (Ramírez *et al.,* 2011). Cryoprotectants, salt, starch and other additives have been added to improve the characteristics of the final products. Each of these additives has their own unique characteristics which improves the quality of surimi.

2.2.1 Effects of cryoprotectants

Cryoprotectants are used in the surimi industry to retain the functional properties of myofibrillar protein which in turn prevents protein denaturation during frozen storage (Carvajal, Lanier and MacDonald, 2005; MacDonald and Lanier, 1994; Park, Lanier, and Green, 1988). Cryoprotectants added have the ability to stabilise actomyosin, prevent moisture loss, increase surface tension and also prevent loss of protein solubility (Parvathy and George, 2014; Somjit *et al.,* 2005; Sultanbawa and Li-Chan, 1998; Park, Lanier, and Green, 1988). Protein denaturation during frozen storage cause aggregation of protein, changes in texture and decrease in water holding capacity. These characteristics reduce the quality of surimi (Campo-Deaño *et al.,* 2009;

Moosavi-Nasab, 2003). Furthermore, prolonged storage under freezing temperature will cause undesirable changes in colour (Esturk, 2003). Cryoprotectant reduces the rate of protein denaturation and aggregation during frozen storage thus retaining the quality of the surimi for a longer period (Zhou *et al*., 2006; Mosavi-Nasab, 2003). There are various types and forms of cryoprotectants which come in different compounds such as low molecular weight sugars, amino acids, carboxylic acids and polyphosphates (Ramírez *et al*., 2011; Zhou *et al*., 2006; Auh *et al*., 1999;).

Cryoprotectants maintain the functional properties of the surimi during freezing by inhibiting the hydrophobic interaction of the proteins. Hydrophobic interactions will cause textural changes and reduce the water holding capacity of water for gelling (Campo-Deaño *et al.,* 2009). Most commonly used cryoprotectants in the industry are sucrose and sorbitol (Carvajal, Lanier and MacDonald, 2005; Auh *et al.,* 1999). Majority surimi uses a blend of sucrose and sorbitol (1:1) at 8% concentration (w/w) (Zhou *et al.,* 2006). However, the drawback of these sugars from health-conscious consumers' perceptive is that they introduce unnecessary sweetness and calorie to the surimi final product (Nopianti *et al.,* 2012; Zhou *et al*., 2006; Sultanbawa and Li-Chan, 1998). On the other hand, Auh *et al.* (1999) also reported that by adding other types of highly concentrated branched oligosaccharides to surimi fish paste, the hardness of the surimi gel prepared increased compared to addition of sucrose and sorbitol, and the gel displayed a much denser microstructure and lower water holding capacity which resulted in the gel being more compact and rigid.

So far, cryoprotectants have been reported to successfully increase the shelf life of the surimi during frozen storage up to 10°C while preserving its quality (Campo-Deaño *et al.,* 2010). Sodium tripolyphosphate ($Na_5P_3O_{10}$) and tetrasodium pyrophosphate ($Na_4P_2O_7$) have also been added in surimi as a chelating agent to inhibit the metal ions in surimi and prevent ionic bonds occurring during frozen storage (Park, 2006). In addition, the effectiveness of cryoprotectants has been reported to be correlated with the storage temperature. The myofibrillar protein quality of a common carp (European carp; *Cyprinus carpio*) could be retained significantly at -3°C compared to -1°C with addition of cryoprotectants (Liu *et al*., 2014). However, there are still discrete reports on the functionality of cryoprotectants to increase the gelling ability of fish protein.

2.2.1.1Sorbitol and Mannitol

Sorbitol and mannitol have been used commercially by various industries for more than a century. Sorbitol and mannitol are isomers with the same amount of carbon (Figure 2.2; $C_6H_{14}O_6$).

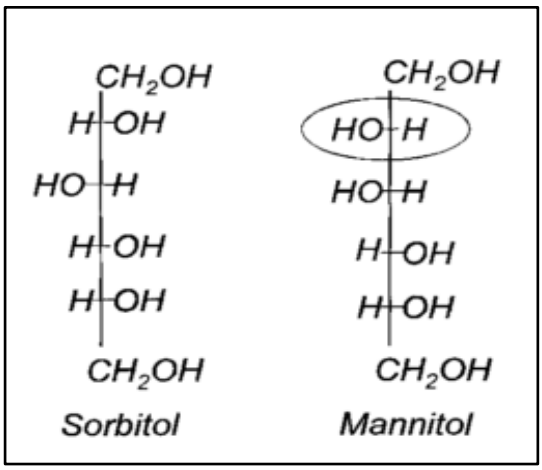


Figure 2.2: The chemical structures of sorbitol and mannitol (adapted from Nabors, 2001)

The difference between these two sugars is the degree of solubility in water. Mannitol is reported to have lower solubility in water compared to sorbitol. In other words, sorbitol is hygroscopic whereas mannitol is non-hygroscopic (Nabors, 2001). Another difference between these two isomers is their sweetness. Sorbitol was reported to be sweeter and has higher calorie than mannitol (Nabors, 2001). Table 2.1 summarises the characteristics of these two sugar polyols (alcohols).

Table 2.1: Characteristics of sorbitol and mannitol

Characteristics	Sorbitol	Mannitol
Sweetness	60% of sucrose	50% sucrose
Taste	Sweet / cool	Sweet / cool
Odour	None	None
Moisture sensitivity	Hygroscopic	Non-hygroscopic
Solubility	235 g / 100 g of water	23 g / 100 g of water
Caloric value	2.6 cal/g	1.6 cal/g
Melting point	100˚C	164˚C
Chemical stability	Does not darken or decompose at elevated temperatures or in the presence of amines	Does not undergo Maillard reaction

The usage of sorbitol in surimi industry has been well known because of its hygroscopic property which allows for moisture equilibrium during frozen storage thus increasing the shelf life of surimi. The use of mannitol on the other hand, has been reported to have poor cryoprotectant ability (Yoon and Lee, 1990). To our knowledge, no further research agreeing on this is available at present. Therefore, the effects of mannitol on surimi fish paste

should be further investigated as mannitol itself has lower calorie and sweetness as compared to sorbitol.

2.2.2 Effects of salt

Salt is added in the manufacturing of surimi to enhance gelling by extracting the myofibrillar protein and forming viscous protein paste which is elastic when heated (Greiff *et al*., 2015; Tahergorabi and Jaczynski, 2012A). Gelation of surimi improves when salt is added because naturally myofibrillar protein is a salt soluble protein (Damodaran, 1997). Salt added causes protein to unfold exposing the negative-charged amino acid that will bind with water thus increasing water binding capacity of the protein. This consequently increases protein solubility in water (Esturk, 2003). Higher percentage of protein solubility will result in stronger and higher quality surimi gel. Tahergorabi and Jaczynski (2012A) reported that as concentration of salt increases, the endothermic transition and myofibrillar gelation point increase to a higher temperature which in turn indicates that addition of salt requires more energy to unfold protein. Usually, 2 – 3% of table salt (sodium chloride; NaCl) is added to the surimi, and this is considered as the suitable amount to provide the best gelling texture on surimi (Amiza and Ain, 2012).

Myofibrillar protein solubilisation is important as it effects a lot of surimi functional properties especially gelation (Lanier, Carvajal and Yongsawatdigul, 2005; Esturk 2003; Niwa, 1992). Minced fish which is washed with water and salt will form a continuous matrix which allows for some additives to be entrapped within its network. Some additives trapped in this network are

beneficial as they help improve the gelling strength of surimi, but some are detrimental (Ramírez *et al*., 2011). Furthermore, washing with salt solutions minimises myofibrillar loss during washing (Pippatsattayanuwong, 1996). Núñex-Flores *et al.* (2018) reported that without any addition of salt, surimi gel becomes more aggregated, less elastic and less protein network formed which compromises the protein structure stability resulting in poor gel quality.

Nevertheless, at higher concentration of salt, the gel strength of myofibrillar protein was found to decrease due to the salting out effect (Esturk, 2003). Increased amount of salt present within the gelation system causes more undesired protein-protein network rather than the desired protein-solvent interaction (Carvajal, Lanier and MacDonald, 2005). Furthermore, salt present causes competition for water causing less protein-solvent interaction which decreases the protein solubility.

Another concern for the high amount of salt is its effect on consumers' health. High consumption of sodium could lead to health deterioration and incur health risk such as high blood pressure and stroke (Greiff *et al*., 2015). In order to introduce healthier surimi products, Tahergorabi and Jaczynski (2012A) substituted the Na^+ ions with K^+ ions which possess anti-hypertensive properties as opposed to sodium which induces hypertension. It was found that at equal concentration, potassium chloride (KCl) displayed similar characteristics with surimi added with sodium chloride. However, the researchers did not reduce the amount of additive used to maintain the surimi quality. Greiff *et al*. (2015) replaced sodium with magnesium (Mg) to determine

if it was feasible. Magnesium used at equal molarity to sodium was found to decrease the water holding capacity of surimi gel thus making it pointless to replace sodium. Greiff *et al*. (2015) also reported that sodium displayed the highest surimi gel breaking force when compared to potassium or magnesium.

2.2.3 Effects of starch

Another commonly used additive to improve surimi gelling is starch. Its unique ability to absorb water and swell helps improve the gel structure of surimi (Ramírez *et al*., 2011). Starch is a type of carbohydrate in a form of polysaccharide which can be found in various plant parts such as seeds, roots and tubers (Yang, 1998). Starch consists of amylose (linear) and amylopectin (branched). Its unique characteristic which is the ability to gelatinise is the reason for it being widely used in food processing industry to further improve and increase food product quality especially in thermal processing (Yang, 1998). When heat is applied to starch granules, swelling occurs which causes the granules to expand several times larger than its original size, and this process is irreversible (Ahmad *et al.,* 1999). However, to control swelling to suit its functional properties, temperature and time of swelling is considered and crucial.

Starch acts as filler which strengthens the gel structure of surimi (Ramírez *et al*., 2011; Campo-Deaño and Tovar, 2008; Park, 2005; Yang, 1998). It also promotes better gelling, firmness and higher elasticity (Elgadir *et al*., 2012, Park, 2005). Due to starch uniqueness, surimi manufacturers can produce surimi with lesser amount of fish protein whilst maintaining the gel strength of

surimi with addition of starch (Teng, Chin and Yusof, 2011; Campo-Deaño and Tovar, 2008). However, the perfect combination of type of starch and its gelatinisation temperature is crucial in order to produce high quality surimi (Park, 2005).

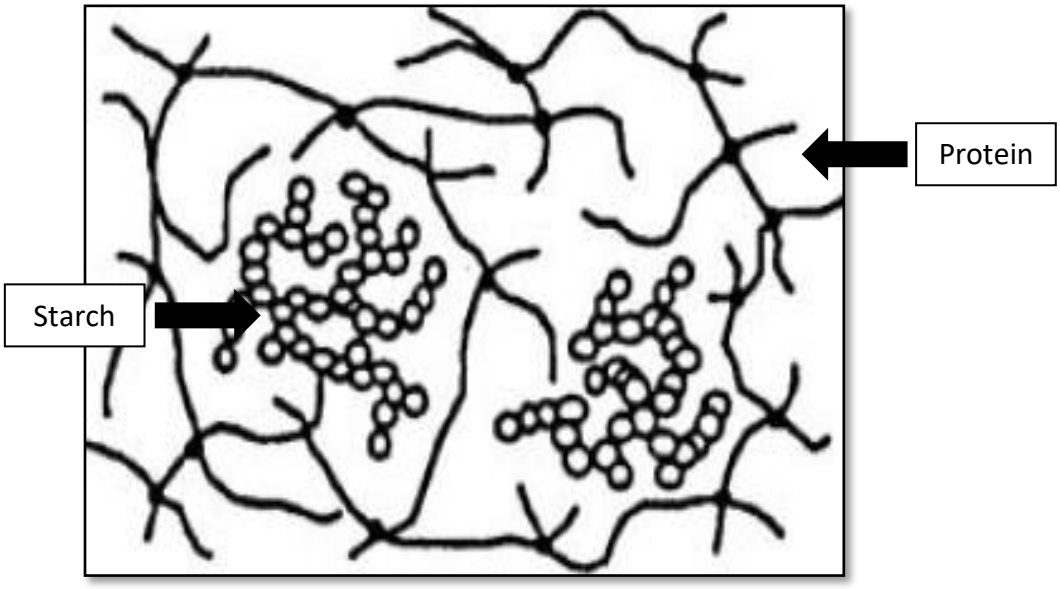


Figure 2.3: Effect of starch on the protein-protein interaction (adapted from Park, 2005)

Campo-Deaño and Tovar (2008) found that starch increased the hardness and stiffness of surimi thus making it less viscoelastic and more brittle. Park (2006) also reported that although potato starch increased the gel strength of surimi due to its large granule, the surimi gel did not possess long shelf life as it underwent retrogradation (*i.e.*, amylose and amylopectin chains in gelatinized starch realign themselves as the heated starch cools) during frozen storage thus releasing water and causing syneresis (*i.e.*, expulsion of liquid from gel). This causes surimi to be more susceptible to protein denaturation during frozen storage. The optimum amount of starch for Alaska pollock surimi was

reported to be at 11% (w/w) and for Pacific whiting 11-15% (w/w). Addition of starch above this range could deteriorate the gel matrix due to the competition of water for gelation (Campo-Deaño and Tovar, 2008; Park, 2005).

2.3 Effects of washing

Washing is one of the important steps in surimi processing as it dictates the quality of the surimi (Ablamowicz, 2007). Washing removes blood, lipids, water soluble proteins and unwanted components which contribute to fish spoilage thus concentrating the myofibrillar protein content (Karthikeyan, Dileep and Shamasundar, 2006). Washing also removes myoglobin and haemoglobin which cause the presence of red hue in the otherwise white-coloured surimi (Park and Lin, 2005; Esturk, 2003). Red hue decreases the quality of surimi due to the decrease in whiteness index. By removing all these unwanted components, washing indirectly increases the gel strength and quality of surimi (Amiza and Ain, 2012). Initially, washing was first introduced to maintain the shelf life of surimi during frozen storage by discarding sarcoplasmic protein present among other fish protein in myofibrillar protein of the fish (Jafarpour and Gorczyca, 2012). Washing approximately reduces ≈40% fat, ≈40% protein and ≈30% ash which altogether constitute ≈37% loss of total solids (Pippatsattayanuwong, 1996). This makes washing an important step in the surimi industry.

The surimi industries often adapt the 3-cycle washing (3:1 water/minced fish) to completely eliminate unwanted components (Park and Lin, 2005). The first washing cycle removes 50% of all water-soluble components and additional

washing concentrates the myofibrillar protein by removing blood and other impurities (Pippatsattayanuwong, 1996). Gel forming ability of surimi was found to increase with increased amount of washing cycle (Amiza and Ain, 2012; Park and Lin, 2005). It has been reported that the myosin heavy chain concentration for threadfin bream increased up to 3-washing cycle (Karthikeyan, Dileep and Shamasundar, 2006).

The amount of waste water produced by the surimi industry for washing is currently of major concern. With the 3:1 ratio, 1 kg of surimi will require 3 kg of water for washing. Furthermore, this 3 kg of washing water is already contaminated with several impurities and cannot be recycled directly, thus incurring more cost and pollution if remains untreated. Even though the surimi industries mostly adapt to the 3-cycle washing, washing for one cycle was also found to increase the water absorption, fat absorption and emulsion capacity of protein in threadfin bream (Karthikeyan, Dileep and Shamasundar, 2006). Priyadarshini *et al.* (2017) reported that single washing using alkaline saline water yielded higher gel strength than the conventional water. Other than that, reports have shown that over-washing can cause surimi hydration, myofibrillar degradation and finally affecting the gel strength of the surimi (Karthikeyan, Dileep and Shamasundar, 2006; Lin and Park, 1996). Continuous washing also increases protein solubility. It is therefore important that research to lower the amount of washing cycle and water used in the industry being done whilst maintaining a good quality surimi. In the present work, the effect of only using one washing cycle on fish paste with nanobubble water was investigated.

2.4 Effects of frozen storage

Surimi is an intermediate raw material which needs further processing into commercial products. In order to retain the quality of surimi during transportation, surimi is generally preserved in frozen temperature to inhibit microbial growth (Badii and Howell, 2002). However, freezing temperature has taken a toll on the texture and protein structure of surimi which represents surimi quality. A phenomenon called freeze denaturation causes changes in the protein functionality during frozen storage (Moosavi-Nasab, 2003). Freeze denaturation causes myofibrillar protein to lose water holding capacity, solubility and gel forming ability (Geirsdottir *et al.,* 2007; Park *et al.,* 1988; Scott *et al.,* 1988). The alteration of protein structure and functionality during frozen storage is mainly caused by the aggregation of protein and development of water crystal (Benjakul *et al.,* 2005; Badii and Howell, 2002). During frozen storage, some of the hydrophobic side of the protein chain is exposed to the surface which causes water to accumulate at the outer layer. Eventually, water molecules migrate out to form ice crystals and disrupt the hydrogen bonds and the protein network structure (Moosavi-Nasab, 2003).

Freezing also causes textural changes on surimi. Surimi was found to be more rubbery and hard after frozen storage (Sych *et al*., 1991A). Other than that, surimi toughness was found to increase due to formation of formaldehyde and higher amount of myofibrillar network formed during frozen storage (Kim, Park and Yoon, 2005; Pippatsattayanuwong, 1996). The changes in quality are dependent on many variables such as storage time, freezing temperature and chemical composition of fish (Park and Lin, 2005; Badii and Howell, 2002;

Pippatsattayanuwong, 1996). Fatty fish is reported to undergo denaturation and spoilage faster due to the presence of oxidative compound which is released by fat, whereas lean fish undergoes degradation due to protein aggregation (Badii and Howell, 2002). These changes cause a decrease in gel forming ability and water holding capacity (Carvajal, Lanier and MacDonald, 2005). Due to this, additives are added which complements the frozen storage thus preserving the quality of surimi for a longer period. A study on various combinations of additives and their effectiveness during prolonged frozen storage would greatly benefit the surimi industry.

2.5 Rheology

Rheology has been used extensively in the research area concerning the changes and properties of fluids and semi solids. Rheology is known as a study to understand the deformation and flow properties of a given material (Steffe, 1996; Ferry 1980). The knowledge of rheology and its application is very useful in understanding the characteristics of a material even at a molecular level. Its application in the food industry has been reported in many researches to predict the formation or breaking of bonds within a product without even using the microscope or other micro instruments (Tabilo-Munizaga and Barbosa-Cánovas, 2005A).

In the surimi industry, rheology has helped researchers to predict and determine the quality of the surimi. Surimi is unique as myofibrillar proteins display characteristics between viscous and elastic (Kim, Park and Yoon, 2005; Esturk, 2003). Due to this behaviour, rheology is deemed valuable and

indispensable in the surimi research arena as it does not destroy or alter the molecular structure of the sample while analysis is done (Fischer and Windhab, 2011; Tabilo-Munizaga and Barbosa-Cánovas, 2005A). There are two types of rheological testing namely the small strain testing and the large strain testing. The small strain testing is a non-fracture gel analysis which analyses the characteristics of the gel and causes deformation with minimal stress to prevent structural breakdown (Tabilo-Munizaga and Barbosa-Cánovas, 2005A). The large strain testing is *vice versa* in that its analyses cause deformation to the sample to a point where fracture and structural breakdown occurs. This test causes a permanent irreversible structural change (Kim, Park and Yoon, 2005; Esturk, 2003).

The rheological properties of surimi paste are seldom researched even though they influence the surimi end products. Many researchers are not keen on studying the rheological properties of surimi in paste form and their effects on the end product and consumers' perception due the changes of characteristics during thermal gelation. Kim, Park, and Yoon (2005) explained that by determining the rheological properties of fish paste, the surimi end products' characteristics can be predicted and also can be used to determine the flow property of surimi during extrusion thus determining the required energy for pumps and extruder. Understanding the rheological properties and behaviour of surimi seafood paste is also necessary for food manufacturers and food engineers to design equipment in order to maximise the production while maintaining the quality of foods. Majority of the manufacturers concerns are on the gelling ability and the rheological properties of a surimi gel rather than

surimi paste. However, researching and understanding the rheological properties of surimi paste can further help in designing food process equipment and flow, thus creating a much more efficient way to increase production and also quality (Park and Lin, 2005).

It is quite a challenge to measure the viscosity of the surimi paste as it is a semi-solid fluid with elastic properties due to the protein concentration (Kim, Park and Yoon 2005). Various kinds of tools have been used in order to measure the viscosity of a surimi in a paste form. Rheometer has been used by several researchers to determine the viscosity of fish paste. However, at high shear rate, it is very difficult to determine the viscosity of fish paste using a rheometer as the fish paste will slip and scattered. At low shear rate, the paste will be too viscous to be analysed. Rotational viscometer has been reported to be used as a rheological properties determination tool in the research of salmon fish paste (Baoraoui *et al*., 1997). Rotational viscometer consists of a concentric cylinder (bob) which is located inside a cup which is filled with the sample and measured using cone geometry (Kim, Park, and Yoon, 2006). Another tool used by Borderías *et al*. (1985) is the Brookfield viscometer to measure the viscosity of dilute extract at 10˚C but with several sample preparation steps to optimise the measurements. Another way to measure viscosity is by using a capillary viscometer (Kim, Park, and Yoon, 2006) which involves several complex mathematical equations.

Fish paste has been reported to have a shear thinning pseudoplastic behaviour with a yield stress (Baoraoui *et al*., 1997). Other than being

pseudoplastic, surimi fish paste is also thixotropic which means the viscosity changes with time of shearing (Kim, Park and Yoon, 2005). Yoon, Gunasekaran and Park (2004B) used the Carreau model in order to define the shear thinning behaviour of the Alaska pollock fish paste. In order to determine the rheological behaviour of Alaska pollock fish paste at high shear rates, a modified Cox-Merz rule with a frequency shift factor was developed. Apparent viscosity is usually being measured for surimi fish paste because it exhibits non-Newtonian fluid characteristics which will change with increase of shear rate (Kim, Park, and Yoon, 2005).

2.5.1 Dynamic rheology test

Dynamic rheology test is an analysis done by measuring the elastic (G') and viscous (G'') modulus of a given sample. It is used to understand the state (solid or fluid-like) of a sample at given temperature, frequency or time. It is also widely used to understand the viscoelastic behaviour and properties of the sample (Ferry, 1980). G' or the elastic modulus represents the measurement of energy stored and subsequently released per cycle of deformation per unit volume since the strain is recoverable in an elastic solid (Liu *et al*., 2014A; Gunasekaran and Ak, 2000). This modulus is also referred to as the storage modulus. On the other hand, G'' or the viscous modulus is called the loss modulus. It represents the viscous dissipation (loss) of energy as heat per cycle of deformation per unit volume (Campo-Deaño *et al.,* 2009B; Ferry, 1980).

Another parameter that is observed in a dynamic rheology test is the phase angle which is usually represented by delta (δ). The phase angle explains the relative effects of viscous and elastic modulus in a viscoelastic material which varies from 0° to 90° (Kim, Park and Yoon, 2005). The phase angle can represent the ratio of energy lost to energy stored per cycle and as the phase angle decreases, the material showed more elastic behaviour and *vice versa* for viscous material (Esturk, 2003). Stress sweep test, frequency sweep test and temperature sweep test are experiments done to determine the rheological properties of surimi.

Stress sweep is usually related to the linear viscoelastic region (LVR). It is a range of stress when applied does not deform or change the sample properties. It is also categorised as a small amplitude strain oscillation test (Esturk, 2003). This test provides information on the behaviour of the sample when subjected to a series of stress. By understanding the LVR of the sample, various tests could be done to further determine rheological properties of sample without causing any deformation (Liu *et al.,* 2014A; Steffe, 1996). LVR is important as it represents structural integrity and rigidity of sample. A wider LVR will signify a much stable and strong structural network of sample. Campo-Deaño and Tovar (2009A) uses the stress sweep to determine the behaviour of surimi gel added with egg albumen.

It has also been reported in various journals and literature that frequency sweep done in the LVR can determine the characteristics of a sample and its molecular structure. Frequency sweep is also defined as mechanical spectra

and it is used to determine the state of a sample at a given frequency and temperature. Frequency sweep done can determine the condition of the gel or sol by determining the dependent of the sample during frequency sweep. The strength of the sol or paste is usually determined by subjecting the sample to a range of frequencies within the linear viscoelastic stress. Campo-Deaño and Tovar (2009A) reported that by using the frequency sweep, the water holding capacity of surimi gel could be predicted. Solo-de-Zaldívar *et al.* (2014B) determined the shelf life stability of minced fish added with glucomannan during frozen storage by using frequency sweep. Yoon, Gunasekaran and Park (2004A) used frequency sweep as a test to differentiate the thermorheological properties of Pacific whiting and Alaska pollock at different moisture contents.

Temperature sweep can be used to determine various properties of surimi fish paste during heat treatments. Temperature sweep can also be used to determine the gelation point of surimi by analysing the storage and loss modulus of the curve when heat is applied. Other than that, temperature sweep can also predict the energy absorption or dissipation at different temperatures. Parameters which are usually observed during temperature sweep are storage modulus, loss modulus and tan delta. Kong *et al.* (2016) used temperature sweep to determine the effect of various starches on the gelation point of surimi. Cando *et al.* (2016) found that the gelation point of surimi added with lower amount of salt displayed more distinct peak in temperature sweep which subsequently indicated higher amount of energy needed to perform gelation.

2.5.2 Texture analysis

Another important characteristic in the surimi industry in determining the quality is texture. Food texture characteristics are also considered as rheological properties (Kim, Park, and Yoon, 2005; Yang, 1998). It is important that surimi displays desirable hardness (strength) and cohesiveness (deformability) that satisfy the processing requirement and consumer demand (Yang, 1998). Different combinations of hardness and cohesiveness will display different types of texture as illustrated in Figure 2.4.

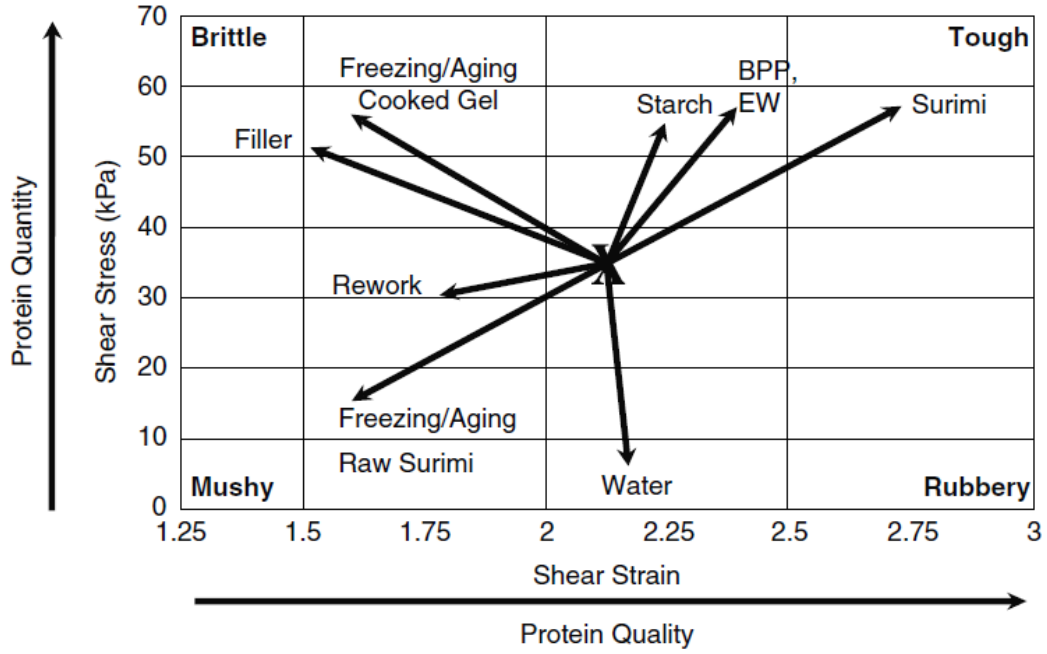


Figure 2.4: Texture map demonstrating effects of different stress and shear on gel texture (adapted from Park, 2005)

In analysing texture, a texture analyser (Figure 2.5) is an indispensable tool in the food industry. Texture analyser was initially designed by observing the mastication process of human being. It allows researcher to collect data of the sample as though it is being chewed and bitten by humans. Texture analyser produces texture profile which provides various information on the sample

characteristics such as chewiness, gumminess, and springiness. By allowing the sample to undergo a series of force depending on the probes and geometry, food researches can gather information on numerous food characteristics.

Figure 2.5: Texture analyser

Texture analyser uses deformation as its principle and records data through load cell attached to a software which detects the force, stress and strain applied during texture analysis. In order to determine the gel strength, two important parameters are observed under the puncture test namely the breaking force and the penetration depth (Tabilo-Munizaga and Barbosa-Cánovas, 2005A). The area under the curve is calculated as the gel strength as shown in Figure 2.6. This test involves applying stress or strain on the

sample until the sample is deformed (Esturk, 2003). The use of puncture test has been documented in numerous researches in determining the gel strength such as effects of modified starch on surimi, effect of various additives, effects of washing and also effects of different processing techniques (Kong *et al.,* 2016; Cando *et al.,* 2015; Kudre *et al.,* 2013; Benjakul *et al.,* 2005; Chaijian *et al.,* 2004; Scott *et al.,* 1988).

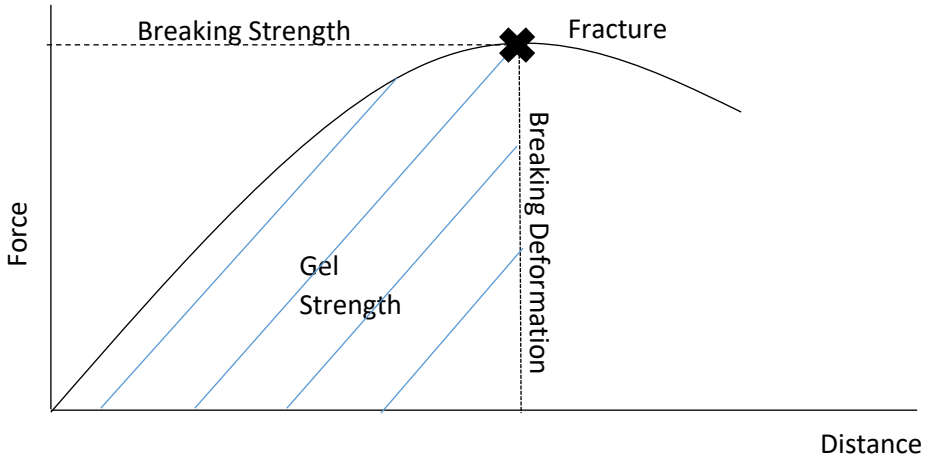


Figure 2.6: Example of data recorded from texture analyser obtained from puncture test

Another test is the torsion test which is among the standard measurement procedures designed to determine gel strength. It measures the shear stress and shear strain of the sample which represents gel strength and cohesiveness respectively. It involves twisting the sample up to 45° angle and records the angle where deformation occurs as stress and shear fail. However, there are some limitations to this test. The diameter of the sample used must be adjusted accurately to 1.0 cm and due to this fact, the sample preparation is tedious (Kim, Park, and Yoon, 2005; Tabilo-Munizaga and Barbosa-Cánovas, 2005A; Esturk, 2003).

A lot of researches have been mainly done on the gelation properties of the surimi with only scarce literature was found to analyse the properties of the surimi paste. Limited research has also been done to understand the rheological properties of the fish surimi in a paste state compared to the gel state (Kim, Park, and Yoon, 2005). Majority researches were done on the gelling and texture characteristics of the surimi to relate the product with the mouth feel during its consumption. However, understanding the fish paste rheological properties could invariably help food engineers to optimise and understand the fish product processing which uses fish surimi as a raw material (Tabilo-Munizaga and Barbosa-Cánovas, 2005A).

CHAPTER 3

Materials and Methods

3.0 MATERIALS AND METHODS

3.1 Fish paste preparation

Fresh Alaska Pollock fish obtained from the local market (Bullring Indoor Market, Birmingham, UK) was minced using a food processor to attain uniformity and homogeneity. The fish mince underwent washing with water to mince ratio 3:1 (w/w). Washing was done using chilled distilled water at 4°C. The mixture was stirred gently for 5 minutes and filtered using cheesecloth. The paste was then mixed with different formulation according to studies done on Chapter 4, 5, 6 and 7. The sample was then stored inside a freezer at -18°C overnight. Figure 3.1 shows the surimi in paste form.

Figure 3.1: Surimi in paste form

3.2 Fish gel preparation

For fish gel preparation, the frozen samples were thawed at room temperature for 2 hours. The samples were then blended using a food processor to

produce a homogenized fish paste. The samples were then inserted into an extruder and extruded into a polyvinilydine casing with a diameter of 25 mm. Both ends of the casing were tightly sealed. The samples were then boiled in water at 40°C for 30 min and 90°C for 20 min as described by Benjakul *et al*. (2002) two-step heating. The samples were cooled under running water and stored at -18°C overnight before analysis. Figure 3.2 shows the surimi in gel form.

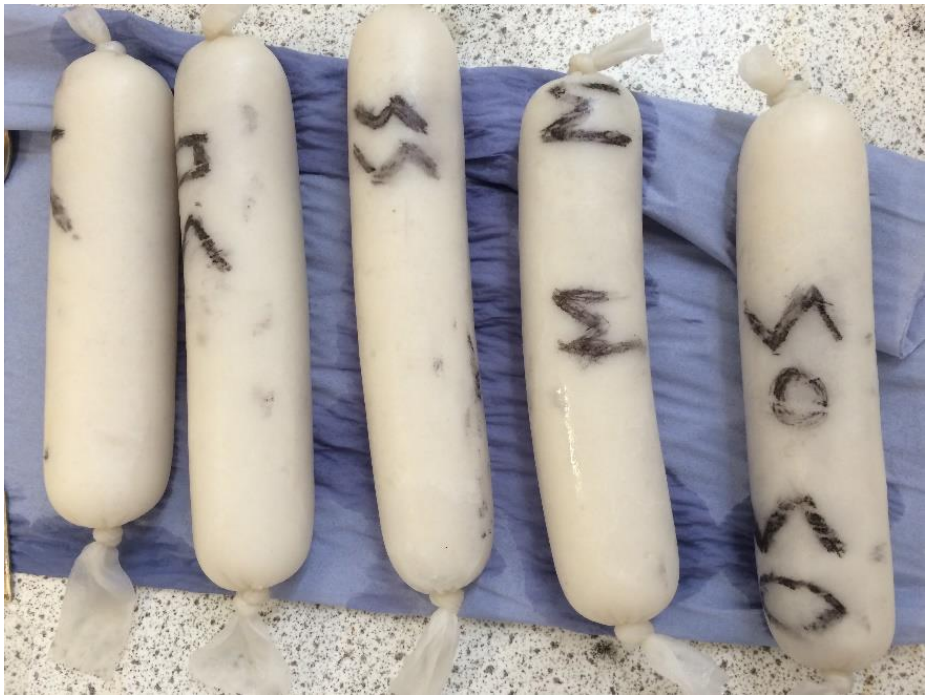


Figure 3.2: Surimi gel in casing

3.3 Frozen storage

Samples were frozen at -18°C for up to six months to investigate the effects of different formulation and additives on the shelf life of surimi gel. Samples were tested after one day, two months, four months and six months frozen storage.

3.4 Measurement of moisture content

Moisture content was measured by oven-drying 5 g fish paste at 120°. The sample was weighed every 15 minutes until the final weight of the sample remains constant. The initial moisture content and the moisture content of all samples after overnight freezing at -18°C were recorded. Moisture content measurement for each sample was determined in triplicate and averaged.

3.5 Expressible moisture content and water holding capacity

The expressible moisture content determination method was adapted from Kudre *et al*. (2013). Fish gel in a cylindrical shape with a thickness of 5 mm was weighed (A). The sample was then placed on top of three pieces of filter papers (Whatman No.1, Whatman International Ltd., Maidstone, England). Two more filter papers were placed on top of the sample and a standard weight of 5 kg was placed on top for 2 min as shown in Figure 3.3. The sample was then removed from the filter paper and weighed (B).

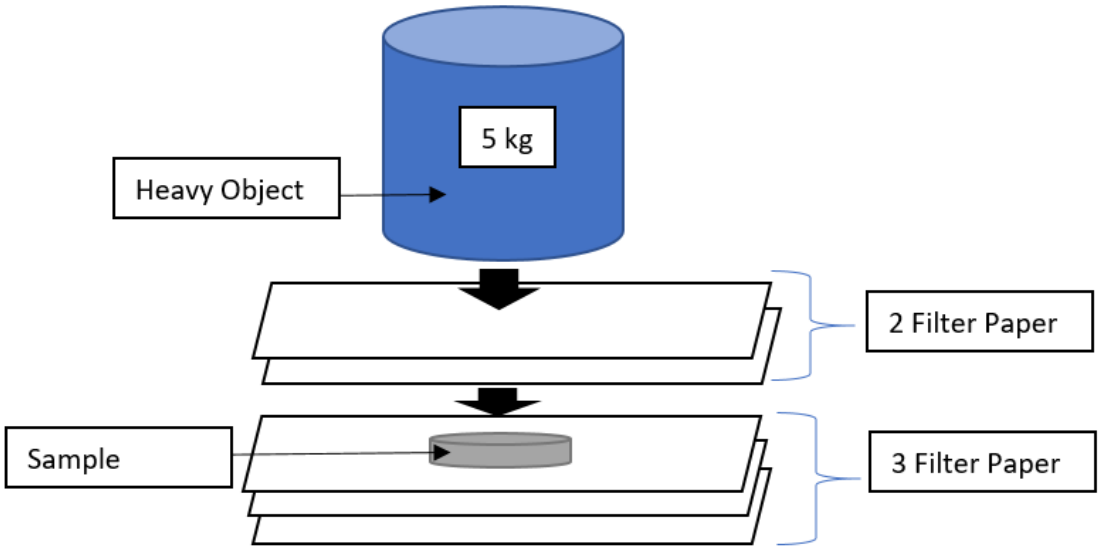


Figure 3.3: Illustration explaining the determination of expressible moisture content.

Calculation of expressible moisture content was carried out as follows:

Expressible moisture (%) = [A-B/A] × 100 **(3.1)**

The measurement of water holding capacity of fish gel was adapted from Alakhrash *et al*. (2016). It was calculated as below:

Water holding capacity (%)

$$= \frac{\text{[Moisture content (g) – Expressible moisture content (g)]}}{\text{Moisture content (g)]}} \times 100 \quad (3.2)$$

3.6 Measurement of rheological properties

Small amplitude oscillatory shear (SAOS) tests were performed using a Discovery HR-2 Hybrid Rheometer (TA Instruments, Waters Ltd, Elstree, Herts, UK) using a 40 mm 4° cone and a plate geometry with 59 µm truncation gap. The fish paste was thawed at room temperature prior to analysis. Fish paste was spread evenly on the lower plate and any excess sample was carefully removed. The sample was covered using a moisture trap during measurement to prevent moisture evaporation as illustrated in Figure 3.4.

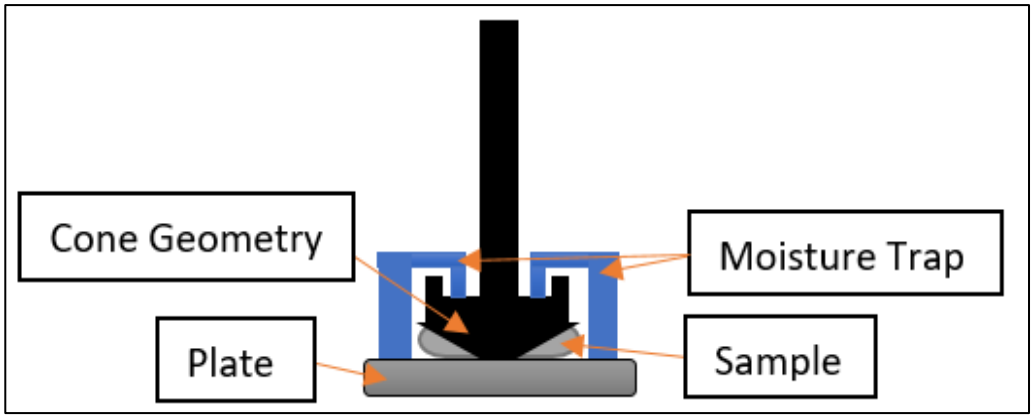


Figure 3.4: Schematic diagram of sample placed with moisture trap.

Stress sweep test was done to determine the linear viscoelastic region (LVR). Stress values ranging from 0.1 Pa to 1500 Pa were implemented on the fish paste at 1 Hz and 20°C. The storage modulus (G') and loss modulus (G'') were observed and recorded.

Frequency sweep test was done at 20°C using the cone and plate geometry in the range 1-10 Hz (0.1-100 rad/s). The gap was set at 59 µm. The frequency sweep was done at 0.5% strain which was within the Linear Viscoelastic Range.

Application of frequency sweep can be translated into the behaviour of product during storage and processing (Binsi *et al*., 2009). Campo-Deaño *et al*. (2010) stated that frequency sweep test can also be used to differentiate between a weak gel and a true gel. The frequency sweep data can also be expressed in terms of viscoelastic moduli which is storage modulus (G') and loss modulus (G'') as a function of angular frequency (ω) and index (n) by using a power law

relationship where n' is flow behaviour index for elasticity and n'' is the flow behaviour index for viscosity (Campo and Tovar, 2008):

$$G' = G'_o . \omega^{n'} \quad (3.3)$$

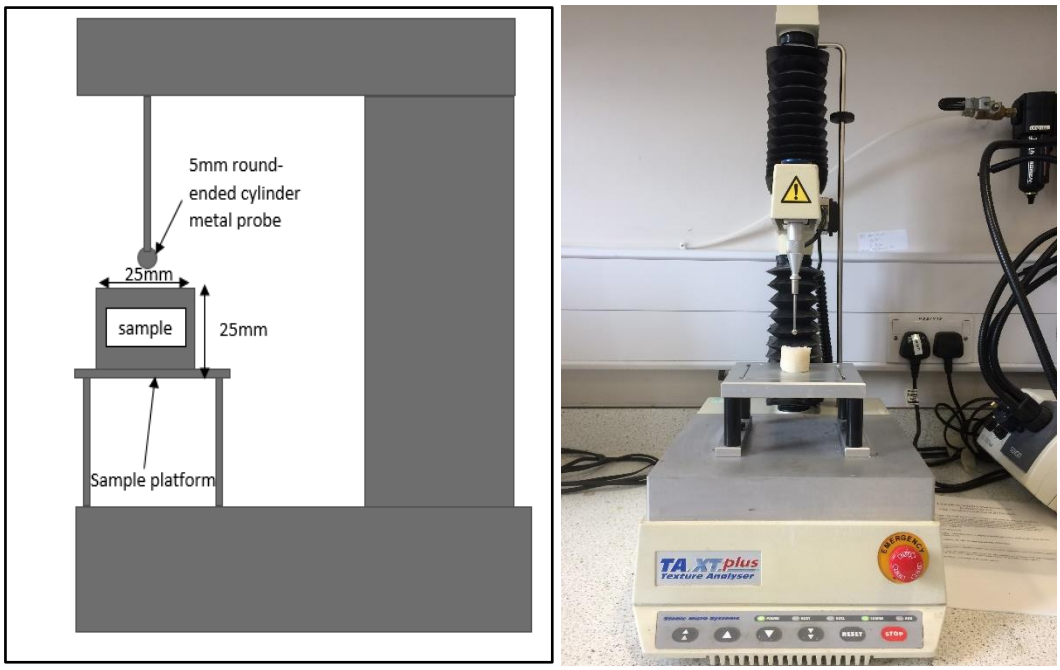
$$G'' = G''_o . \omega^{n''} \quad (3.4)$$

Temperature sweep test was performed to analyse the variations in storage modulus (G') and loss modulus (G'') when the temperatures changed. The temperature sweep was done at 0.5% strain with 1°C per minute increase from 10°C to 90°C.

3.7 Texture analysis

Puncture test was done using a TA-XT plus Texture Analyser (Stable Micro System Ltd., Surrey, UK). Samples with a diameter of 25 mm and a height of 25 mm were pierced to a breaking point using a 6.35 mm (1/4 inch) diameter round-ended cylindrical metal probe (P/0.25s) as shown in Figure 3.5. The crosshead speed was set at 1 mm/sec and a 5 kg load cell was used. The force required to cause deformation represents the breaking force (g) and the depth of penetration represents the breaking deformation (cm) as the gel loses its strength and ruptures. Gel strength was calculated using the equation by Huda, Leng and Nopianti (2011) as below:

Gel Strength (g/cm) = Breaking Strength (g) x Breaking Deformation (cm) **(3.5)**



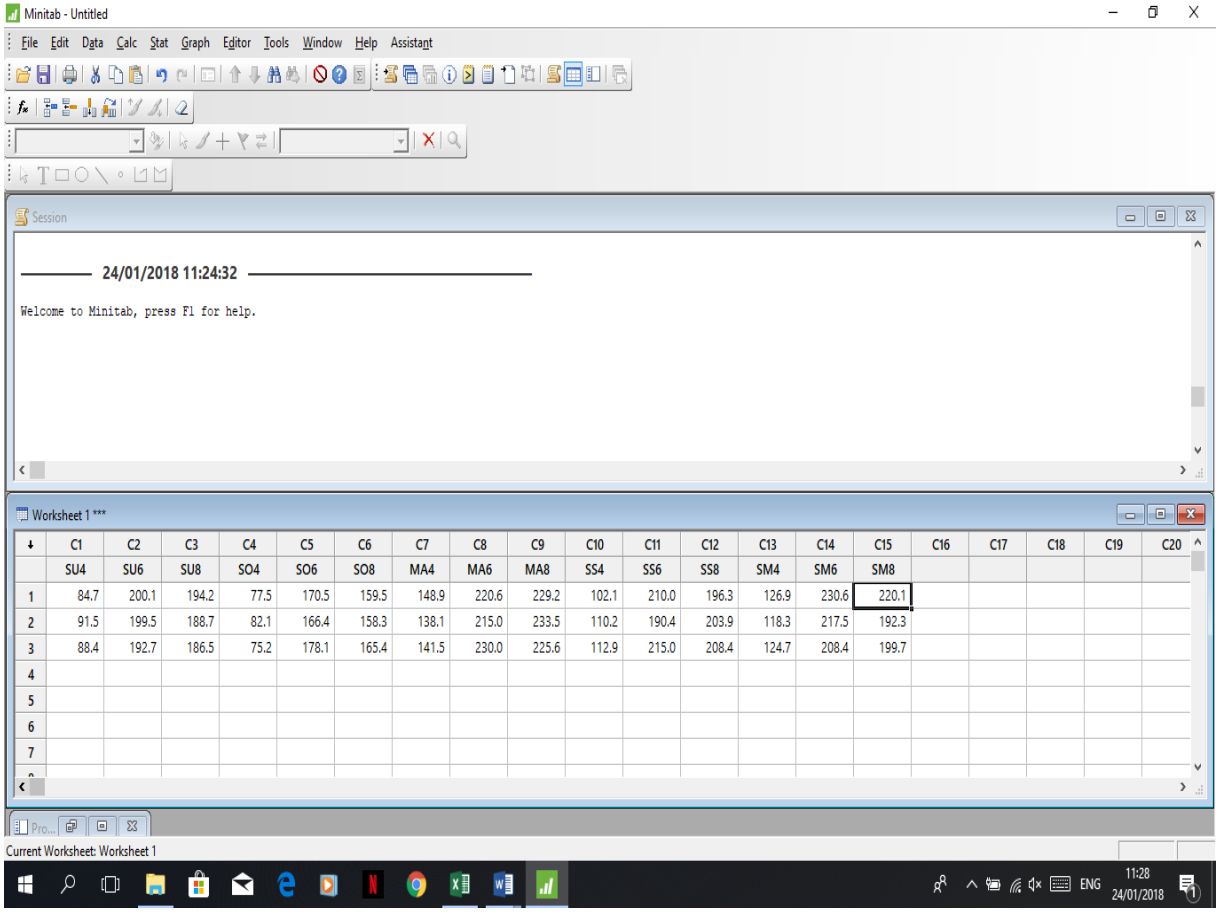
(a) **(b)**

Figure 3.5: Schematic diagram (a) and picture (b) of texture analysis performed on fish samples.

3.8 Statistical analysis

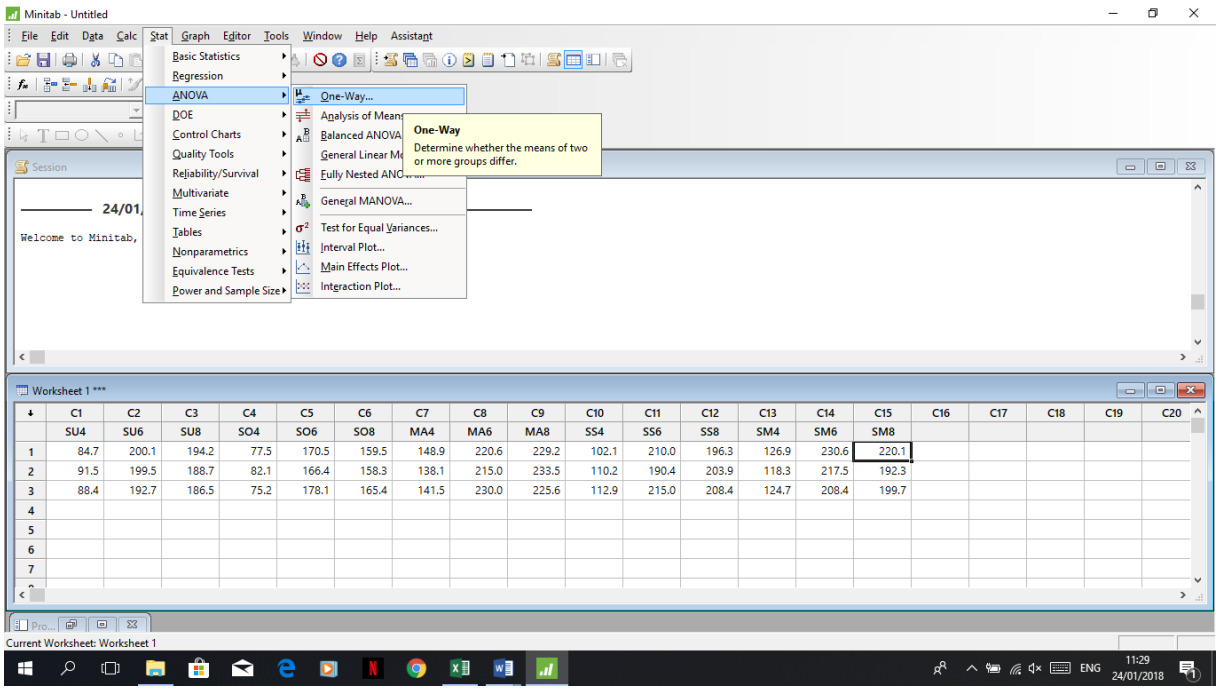
Statistical analysis was performed by using MINITAB 16 statistical software. Analysis of variance (ANOVA) was conducted to test the significant difference ($p < 0.05$) of the experimental results with Tukey test. Data are reported as mean values of triplicates ($n = 3$) ± standard deviation (SD). Data with significant difference between them ($p < 0.05$) will display difference letters (a,b,c and etc.). Figures below display the steps of using MINITAB statistical software for One-Way ANOVA analysis.

1) Data is inserted



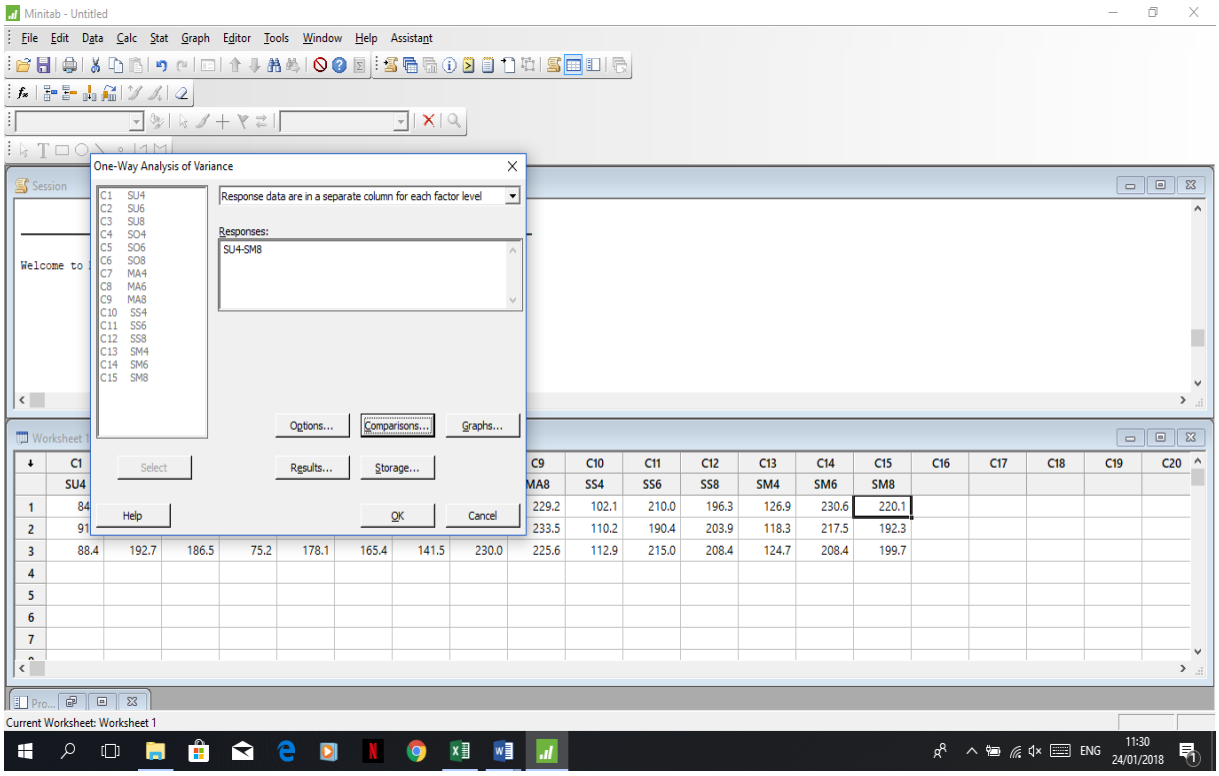
	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20
	SU4	SU6	SU8	SO4	SO6	SO8	MA4	MA6	MA8	SS4	SS6	SS8	SM4	SM6	SM8					
1	84.7	200.1	194.2	77.5	170.5	159.5	148.9	220.6	229.2	102.1	210.0	196.3	126.9	230.6	220.1					
2	91.5	199.5	188.7	82.1	166.4	158.3	138.1	215.0	233.5	110.2	190.4	203.9	118.3	217.5	192.3					
3	88.4	192.7	186.5	75.2	178.1	165.4	141.5	230.0	225.6	112.9	215.0	208.4	124.7	208.4	199.7					
4																				
5																				
6																				
7																				

2) One-Way ANOVA analysis is selected

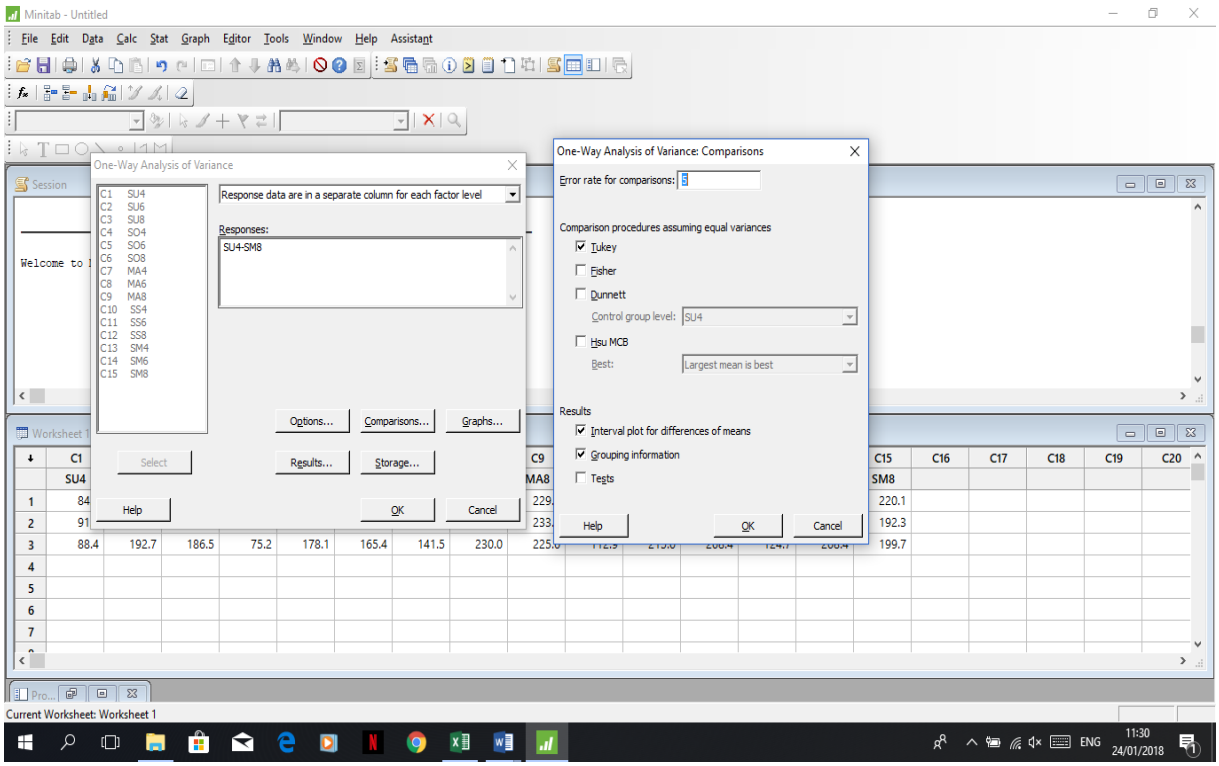


	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20
	SU4	SU6	SU8	SO4	SO6	SO8	MA4	MA6	MA8	SS4	SS6	SS8	SM4	SM6	SM8					
1	84.7	200.1	194.2	77.5	170.5	159.5	148.9	220.6	229.2	102.1	210.0	196.3	126.9	230.6	220.1					
2	91.5	199.5	188.7	82.1	166.4	158.3	138.1	215.0	233.5	110.2	190.4	203.9	118.3	217.5	192.3					
3	88.4	192.7	186.5	75.2	178.1	165.4	141.5	230.0	225.6	112.9	215.0	208.4	124.7	208.4	199.7					
4																				
5																				
6																				
7																				

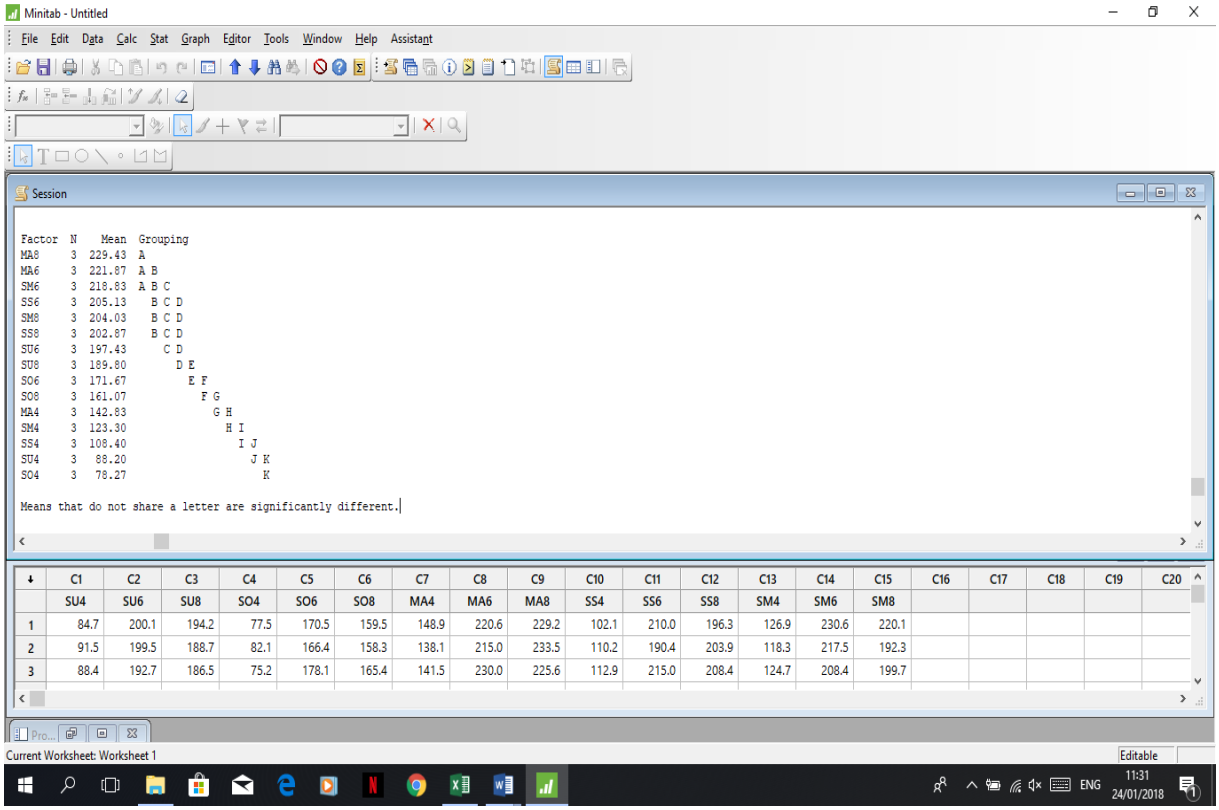
3) All data was selected to conduct ANOVA



4) Tukey test with 0.05% correction is selected



5) Data is calculated by MINITAB and displayed



	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20
	SU4	SU6	SU8	SO4	SO6	SO8	MA4	MA6	MA8	SS4	SS6	SS8	SM4	SM6	SM8					
1	84.7	200.1	194.2	77.5	170.5	159.5	148.9	220.6	229.2	102.1	210.0	196.3	126.9	230.6	220.1					
2	91.5	199.5	188.7	82.1	166.4	158.3	138.1	215.0	233.5	110.2	190.4	203.9	118.3	217.5	192.3					
3	88.4	192.7	186.5	75.2	178.1	165.4	141.5	230.0	225.6	112.9	215.0	208.4	124.7	208.4	199.7					

CHAPTER 4

Effects of Sucrose, Sorbitol and Mannitol on Rheology and Texture of Surimi

4.1 INTRODUCTION

Freezing has been used to preserve surimi during handling and transportation. However, denaturation occurs during frozen storage which promotes protein aggregation thus reducing gel forming ability (Zhou *et al*., 2006; Shenouda, 1980). Cryoprotectants were introduced as a solution to prevent denaturation during frozen storage (MacDonald and Lanier, 1994). Adding cryoprotectants was able to alter the surface hydrophobicity of surimi thus preventing oxidation and protein aggregation (Parvathy and George, 2014; Campo-Deaño, Tovar and Borderías, 2010; Zhou *et al*., 2006). The ability of cryoprotectants to increase the hydration of surimi also decreases the protein denaturation process (Nopianti *et al*., 2012: Yoon and Lee, 1990). Various low molecular sugars such as sucrose and sorbitol have been identified as cryoprotectants and have been added to surimi to prevent freezing damage (Campo-Deaño, *et al*., 2010; Carvajal, Lanier and MacDonald, 2005). These sugars are chosen because they are economical, easy to obtain and cause minimal Maillard browning reaction to surimi (Carvajal *et al*, 1999). However, due to its high calorie content and sweetness (sucrose), other sugars with lower calorie content and sweetness such as trehalose, polydextrose, maltodextrin have been researched to promote a much healthier product (Campo-Deaño *et al*., 2009B; Zhou *et al*., 2006; Sych *et al*., 1991A; Park *et al*., 1988). Sych *et al*. (1991A) reported that polydextrose, a branched polysaccharide with no sweetness could substitute sucrose/sorbitol as cryoprotectant on cod. However, a high concentration of polydextrose causes the natural actomyosin to possess higher viscosity thus making it harder to be processed (Herrera and Mackie, 2004).

In this research, mannitol was used to determine its suitability of substituting sucrose and sorbitol as cryoprotectant. Mannitol possesses the same molecular weight and structure as sorbitol but with a little difference in molecular arrangement. Nabors (2001) reported that mannitol in general is lower in calorie and produces a less sweet taste compared to sucrose and sorbitol. Mannitol is 50% less sweet compared to sucrose whereas sorbitol was reported to have 60% sweetness compared to sucrose. In terms of calories, sucrose has 4 calories per gram; sorbitol has 2.6 calories per gram whereas mannitol has only 1.6 calories per gram (Nabors, 2001). Addition of mannitol could therefore promote a healthier surimi if it turns out to be appropriate.

Food researchers have widely used rheological testing to understand the structures and characteristics of material properties at different stresses, temperatures and oscillation frequencies. Rheology has also been identified to predict the state of the sample especially the gelling state. Rheological application is vast in food industry thus using rheology to understand the effects of sugar additives to food samples is highly informational for food engineers. (Fischer and Windhab, 2011)

This study was done to understand the rheological and textural properties of Alaska Pollock surimi when sucrose, sorbitol and mannitol were added as the cryoprotectants. In addition, the feasibility of using mannitol as a cryoprotectant was also investigated. So far, only few reports on the use of mannitol as cryoprotectant exist. In conjunction to that, the effects of sugar

concentrations on surimi paste and gel were also investigated. Lowering sugar concentrations might produce a less sweet surimi with low calorie content. The rheological data obtained were compared with the commercial blend of 1:1 ratio sucrose and sorbitol (4% w/w). Results obtained from this research could be extended to understand the feasibility of using rheological properties to predict the gelling behaviour of fish paste.

4.2 MATERIALS

4.2.1 Sample preparation

Fish paste and fish gel samples were prepared as discussed in Chapter 3 using the formulation shown in Table 4.1.

Table 4.1: Fish sample formulations with different types of sugars and sugars combination.

SAMPLE	FORMULATION (w/w)
C	No additives
SU	8% Sucrose
SO	8% Sorbitol
MA	8% Mannitol
SS	4% Sucrose + 4% Sorbitol
SM	4% Sucrose + 4% Mannitol

4.3 EFFECTS OF DIFFERENT SUGAR CONCENTRATIONS

4.3.1 Sample preparation

Fresh Alaska Pollock fish sample was obtained from the local market (Bullring Indoor Market, Birmingham, UK). Samples was deboned, minced, and washed as described in Chapter 3. Five different types of sugar formulations were

used which were sucrose, sorbitol, mannitol, sucrose + sorbitol, and sucrose + mannitol. These sugar formulations were added to fish mince at three different concentrations which were 4%, 6%, and 8% concentrations (w/w). Samples were then stored overnight in a freezer at -18°C. Samples were thawed 2 h prior to testing. Samples were also prepared into fish gel for analysis.

4.4 RESULTS AND DISCUSSION

4.4.1 Moisture contents

Moisture content for each formulation was recorded after overnight storage. Table 4.2 records the moisture content for each sample. There was a significant difference in moisture contents between the different types of formulation ($p < 0.05$) after overnight frozen storage at -18°C with control displayed the highest value of moisture content. Nopianti *et al*. (2012) suggested that sugar causes dehydration within the system thus reducing the moisture content of the fish paste. This explains the highest moisture recorded in control because it did not contain any sugar. Surimi paste with addition of sorbitol (SO) displayed the highest value of moisture content followed by MA and SU when compared to other samples with sugar added. Sorbitol was reported to exhibit higher value of moisture content rather than sucrose (Nopianti *et al*., 2012). The moisture content of SS was found to be almost the same with the value reported by Sych *et al*., (1991A) which was 75.6%. The moisture content recorded after overnight freezing was within the range for good quality surimi as suggested by Park and Lin, (2005) which is between 72-77%.

Table 4.2: Moisture contents of different sugar formulations after subjected to overnight frozen storage at -18°C. Values are means ± SD of triplicates (n = 3). Different letters indicate significant differences ($p < 0.05$). C = control.

Sample	Formulation (w/w)	Moisture Content (%)
C	No additives	$78.37^{a} \pm 0.14$
SU	8% Sucrose	$76.56^{bd} \pm 0.46$
SO	8% Sorbitol	$77.56^{bc} \pm 0.47$
MA	8% Mannitol	$77.21^{c} \pm 0.15$
SS	4% Sucrose + 4% Sorbitol	$75.31^{e} \pm 0.08$
SM	4% Sucrose + 4% Mannitol	$76.64^{d} \pm 0.11$

4.4.2 Expressible moisture content and water holding capacity

The expressible moisture content and water holding capacity of fish paste are usually inter-related. Equations (3.1) and (3.2) show the relationship between these two parameters. The lower the expressible moisture content (EMC), the higher the water holding capacity (WHC) of a fish gel (Chaijian *et al*., 2006; Nopianti *et al*., 2012). Expressible moisture content is expressed as amount of water exuded when pressure is applied to the gel and it reflects the ability of the gel to withhold water (WHC). It is usually measured in gel form. WHC is important as it reflects the degree of protein denaturation within surimi (Nopianti *et al*., 2012).

WHC is known to decrease during frozen storage, thus, cryoprotectants added will lower the reduction rate by preventing protein denaturation and aggregation. Sugar added as cryoprotectant has been reported to reduce

surface tension and formation of large water crystals, thus, retaining WHC of surimi (Ohkuma *et al*., 2008; Zhou *et al*., 2006; Yoon and Lee, 1990). Unfolding and aggregation of protein during storage are considered the reason for poor gelation and WHC (Nopianti *et al*., 2012; Ohkuma *et al*., 2008; Sych *et al*., 1991A). WHC is important as it affects the texture quality of food such as tenderness and juiciness (Alakhrash *et al*., 2016). It can also be used as an indicator to determine the rate of protein denaturation (Nopianti *et al*., 2012). Low WHC and high expressible moisture cause a decrease in gelling ability of surimi (Nopianti *et al*., 2012; Benjakul *et al*., 2002). Mahawanich *et al*. (2010) reported the same findings which relate water holding capacity, expressible moisture and gel strength on gel properties of red tilapia surimi. Furthermore, fish gel which has lower expressible moisture content will display a much more rigid structure (Yoon and Lee, 1990).

Results displayed in Table 4.3 indicate that there was a significant difference ($p < 0.05$) in expressible moisture and water holding capacity with different types of sugars. This proves that different sugars react differently towards moisture which can be related to gelling quality of surimi. Even though the moisture content of control was the highest (Table 4.2), it could not retain the moisture within the gel protein network and yielded the lowest WHC value when compared in gel form (Table 4.3). Poor water-holding capacity can be associated with the formation of formaldehyde during frozen denaturation (Benjakul *et al*., 2005). Without any sugar added, formation of formaldehyde could not be prevented in control.

Table 4.3: Expressible moisture content and water holding capacity of different sugar formulations after subjected to overnight frozen storage at -18°C. Values are means ± SD of triplicates (n = 3). Different letters indicate significant differences ($p < 0.05$). C = control.

Sample	Expressible Moisture Content (%)	Water Holding Capacity (%)
C	$35.31^{a} \pm 0.227$	$54.87^{e} \pm 0.235$
SU	$20.46^{c} \pm 1.133$	$73.28^{c} \pm 1.451$
SO	$30.48^{b} \pm 0.964$	$60.70^{d} \pm 1.243$
MA	$11.19^{e} \pm 0.940$	$85.79^{a} \pm 1.220$
SS	$18.76^{cd} \pm 1.373$	$75.09^{bc} \pm 1.822$
SM	$17.80^{d} \pm 0.612$	$76.77^{b} \pm 0.801$

Sample MA displayed the highest value for water holding capacity. This suggests that mannitol showed a promising result as a good cryoprotectant after overnight frozen storage when compared to commercial blend (SS). A good cryoprotectant will prevent or reduce the rate of protein denaturation (unfolding and aggregation), thus allowing better quality gel network to form upon heating or gelation (Nopianti *et al*., 2012). This helps retain water and maintain good gelation properties for surimi gel.

4.4.3 Rheological tests

Rheological tests are done by researchers to understand the flow behaviour or deformation of a material. Storage modulus, G', presented from small amplitude oscillatory shear (SAOS) results is defined as the energy stored within each deformation cycle per unit, and represents the elastic nature of the material. The loss modulus, G'', on the other hand, is viscous dissipation loss

during each cycle per unit of deformation, and is associated with the viscous nature of the material (Liu *et al*., 2014A; Gunasekaran and Ak, 2000).

Stress sweep is a rheological test done to determine the linear viscoelastic region (LVR) which is used to analyse the rheological properties and structure of a sample without destroying or disrupting the structure of the sample. Figure 4.1 shows the differences in G' and LVR when fish paste was subjected to different types of sugar additives at 8% (w/w). It was found that G' did not overlap with G'' for all samples during stress sweep analysis indicating that all samples have a more elastic nature rather than viscous. This is shown in Figure 4.2a-f

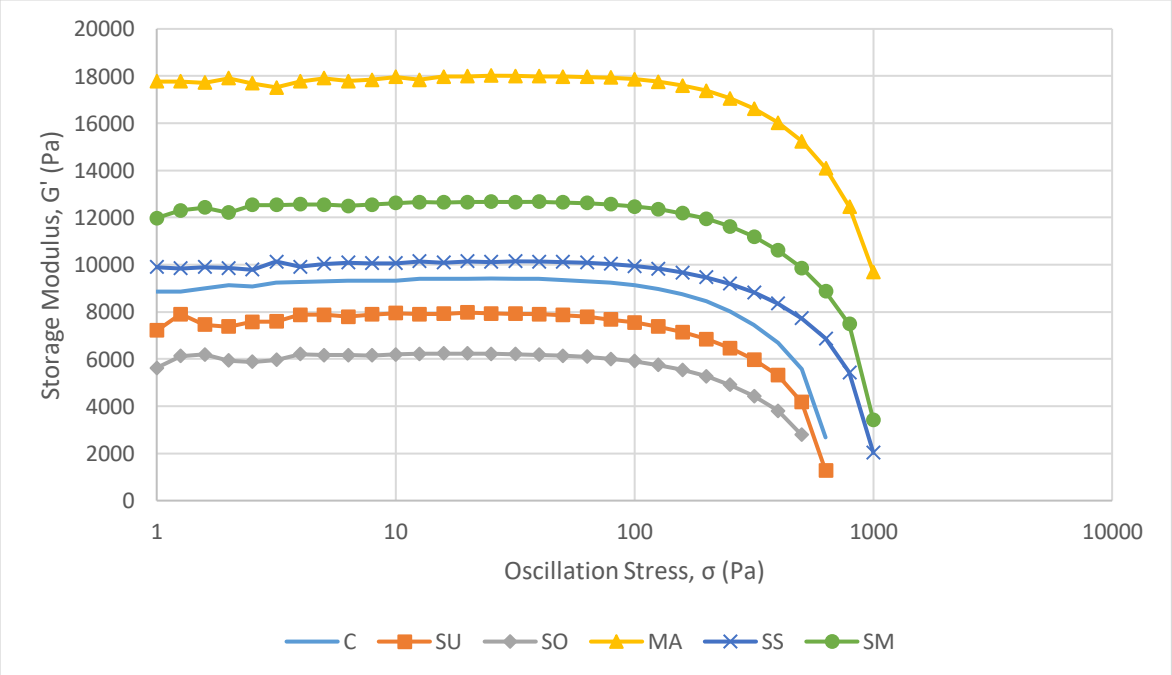


Figure 4.1: Linear viscoelastic range of sample with different types of sugar formulations subjected to stress sweep test from 1 Pa to 1000 Pa.

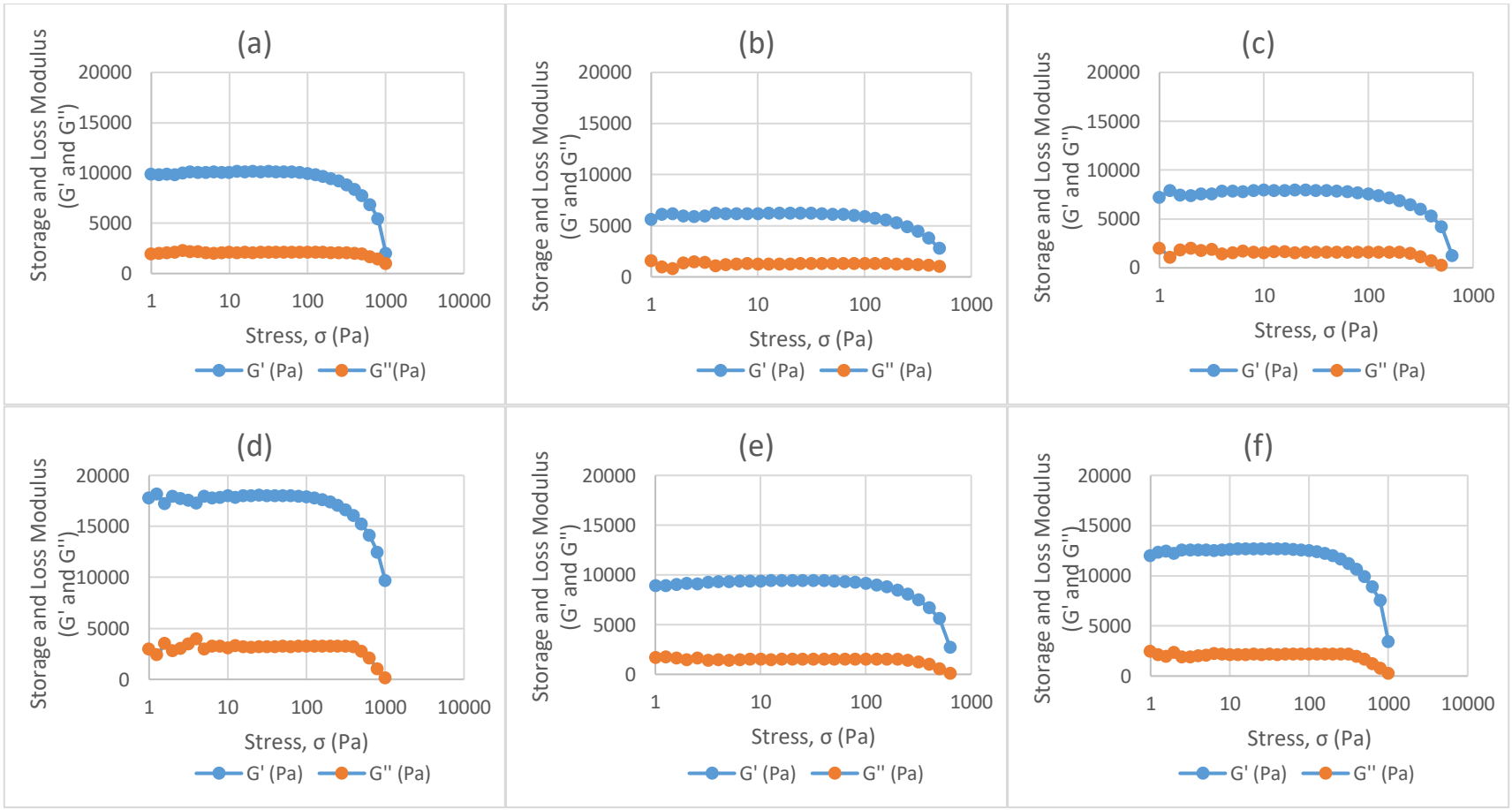


Figure 4.2: The storage modulus (G') and loss modulus (G'') for (a) control, (b) sucrose, (c) sorbitol, (d) mannitol, (e) sucrose + sorbitol and (f) sucrose + mannitol.

Figure 4.1 clearly shows that MA had value of G' whereas SO the lowest. This suggests that MA had a more rigid structure and might produce a stronger gel when compared to other samples (Campo-Deaño and Tovar, 2008). Although this was done while the surimi was in a paste form, the gel strength of MA was further measured with a texture analyser in gel form to further support this finding. SO on the other hand required the lowest stress to flow or deform. A lower G' can also be associated with low WHC. Lower WHC will promote ice crystals formation which causes structural damage (Campo-Deaño *et al*., 2010; Shenouda, 1980) thus interrupting gel formation network. This result corroborates the data presented in Table 4.3 in which SO recorded a significantly low value of WHC ($p < 0.05$).

Table 4.4 shows the limit in LVR range for stress (σ_{max}), strain (γ_{max}), and phase angle (δ_{max}) of each sample by adapting Campo-Deaño and Tovar (2009A) method which measured the maximum stress and maximum strain by plotting a stress *versus* strain curve from which a linear graph would be obtained. Stress max and strain max are identified when the linear curve starts to deviate. At this point, it is considered as σ_{max} and γ_{max}. The same method is used to obtain δ_{max} by plotting stress against phase angle. Phase angle represents the ratio of energy dissipate to energy stored in a cyclic deformation (Campo-Deaño *et al*. 2009B). From Table 4.4, MA yielded the highest value of maximum stress (σ_{max}) and strain (γ_{max}) in LVR. These values displayed a significant difference *(*$p < 0.05$) compared to other samples. Solo-de-Zaldívar *et al*., (2014*A*) stated that higher values of σ_{max} and γ_{max} indicated that the protein structure of the fish paste has higher flexibility and denser

network. This result is also supported by other researchers who reported that shear present within the surimi fish paste was due to the cross link and density of actomyosin, indicating that higher shear represents a more complex protein entanglement of fish (Chen and Huang, 2008; Yoon *et al*., 2004). Other than that, Table 4.4 also illustrates the same result (highest G' value) which further supports the data that MA contained denser protein network.

Table 4.4: Stress max, strain max, phase angle max and storage modulus max for sample with different types of sugar formulations. Values are means ± SD of triplicates (n = 3). Different letters indicate significant differences ($p < 0.05$). C = control.

Sample	σ_{Max}, (Pa)	γ_{Max}, (%)	δ_{Max}, (°)
C	$631.25^{b} \pm 0.27$	$9.27^{b} \pm 0.25$	$17.03^{a} \pm 0.32$
SU	$100.45^{d} \pm 0.39$	$2.77^{c} \pm 0.48$	$17.71^{a} \pm 0.23$
SO	$63.28^{f} \pm 0.03$	$2.00^{c} \pm 0.34$	$15.34^{b} \pm 0.50$
MA	$1500.88^{a} \pm 0.15$	$10.98^{a} \pm 0.63$	$17.47^{a} \pm 0.76$
SS	$79.47^{e} \pm 0.20$	$1.83^{c} \pm 0.21$	$15.18^{b} \pm 0.77$
SM	$126.14^{c} \pm 0.03$	$2.75^{c} \pm 0.33$	$16.51^{ab} \pm 0.41$

γ_{max} can also be used to assess the stability of a sample and its extensibility (Campo-Deaño *et al.,* 2009A). A high value of γ_{max} indicates a better stability of the sample. Better extensibility promotes better functional protein which produces a more efficient WHC (Nopianti *et al*., 2012) thus producing gel matrix which is more structured and denser (Campo-Deaño *et al.,* 2009A). This suggests that MA might produce a stronger gel compared to others. Table 4.3 further supports this result as MA displayed a significantly higher value of WHC compared to other samples ($p < 0.05$). Meanwhile SO displayed

the lowest value of σ_{max} and $\gamma_{max\ which}$ might indicate that it possessed a porous and more rigid structure.

Frequency sweep test is usually done to determine the state of the sample at a given temperature and frequency, *i.e*. whether it is sol or gel. The frequency sweep data can also be expressed in terms of viscoelastic moduli which is storage modulus (G') and loss modulus (G'') as a function of angular frequency (ω) and index (n) by using a power law relationship (Campo-Deaño and Tovar, 2008). This is presented in Equation 3.3 and 3.4

Comparison of different types of sugar added on the viscoelasticity of surimi paste is presented in Table 4.5. The viscoelastic parameter showed different and significant values ($p < 0.05$) when compared between each formulation. The highest to lowest value of viscoelastic moduli (G'_o and G''_o) is as follow, MA, C, SM, SS, SU and SO.

Table 4.5: Power law exponent for equations (3.3) and (3.4) as determined by the frequency sweep for different types of sugar formulations. Values are means ± SD of triplicates ($n = 3$). Different letters indicate significant differences ($p < 0.05$). C = control.

SAMPLE	G'_o (Pa)	G''_o (Pa)	n'	n''	n'-n''
C	18645.9 ± 16.0^{b}	2764.3 ± 50.8^{b}	0.12 ± 0.01^{b}	0.10 ± 0.00^{a}	0.02
SU	3575.6 ± 38.3^{e}	1050.3 ± 34.0^{e}	0.12 ± 0.01^{bc}	0.10 ± 0.01^{a}	0.02
SO	3165.3 ± 37.3^{f}	959.3 ± 58.4^{e}	0.11 ± 0.01^{c}	0.10 ± 0.00^{a}	0.01
MA	26650.7 ± 19.9^{a}	5667.9 ± 39.2^{a}	0.12 ± 0.00^{bc}	0.06 ± 0.00^{b}	0.06
SS	4637.0 ± 40.4^{d}	1458.1 ± 31.7^{d}	0.13 ± 0.00^{ab}	0.10 ± 0.00^{a}	0.03
SM	5498.2 ± 20.2^{c}	1893.6 ± 77.0^{c}	0.14 ± 0.00^{a}	0.10 ± 0.00^{a}	0.04

SO was found to display the lowest values of G'_o, G''_o, n' and n'' value when compared to others which indicates that it had a weak gel attribute. This result is consistent with result obtained from the stress sweep test in which SO displayed lowest value of G', σ_{max} and γ_{max}. This result also suggests that SO formed a weak and less stable protein network which agrees with result presented by Solo-de-Zaldívar *et. al*. (2014B) who reported the effects of freezing on fish mince added with glucomannan thus, characterising sorbitol as a less effective cryoprotectant compared to sucrose and mannitol. MA showed the most consistent result of G'_o and G''_o. Higher G'_o and G''_o values are associated with higher number of protein crosslinks which produce much denser protein network (Techarang and Apichartsrangkoon, 2015). These gel networks can only be formed consistently if protein denaturation during frozen storage is prevented. These statements suggest that MA formed the strongest gel when heated, and mannitol has the ability to decrease the protein denaturation process during freezing.

Cryoprotectants are added to stabilize the structure of water in the three-dimensional structure of protein during gelation (Yoon and Lee, 1990). They inhibit the freeze denaturation of protein by preventing the ice crystal formation and hydration thus protecting protein (Somjit *et. al,* 2005). It is reported that cryoprotectant also acts as a water binder which helps to retain moisture and hydration of surimi paste (Liu *et. al.,* 2014C). Among the various cryoprotectants used in this research, mannitol was found to be the most effective in preventing and protecting protein during frozen storage. This is suggested by the highest G'_o and G''_o values it displayed.

Another method to understand the gel strength of a material by using frequency sweep is to analyse the dependency of angular frequency (ω) by investigating the n' and n'' values. A higher value of n' and n'' denotes that the material is subject to frequency dependent thus more unstable during frozen storage (Campo-Deaño *et al*., 2010). As the difference (n'-n'') increases, the gelling characteristics of the fish gel improves (Campo-Deaño and Tovar, 2008). A positive value indicates a more elastic behaviour whereas a negative value indicates a more viscous behaviour. From Table 4.5, MA was found to have the biggest difference followed by SM, SS, C, SU and SO. Result displayed in Table 4.5 is consistent with data obtained from stress sweep and water holding capacity tests. These results further support the role of mannitol as cryoprotectant which is more effective when compared to sucrose and sorbitol.

Temperature sweep was done to observe the gelling ability and gelation point of fish paste subjected to different types of sugar addition. Yongsawatdigul and Park (2003) reported that dynamic oscillatory test specifically the temperature sweep test has been widely used by food researchers to understand the gelation profile of fish myofibrillar protein. Storage modulus (G') and loss modulus (G'') observed from the result represent the structure changes during food protein gelation (Liu *et al*., 2014).

Figure 4.3 shows the G' of fish paste containing different formulations of additives subjected to heating from 10°C to 90°C. The results show four different stages of gelation profile as reported by several researchers. The four

stages can be defined as softening, first heat gelation, resolution and second heat gelation (Cando *et al*., 2016; Campo-Deaño *et al.,* 2009B; Chen and Huang, 2008). At each stage, protein undergoes different types of process from unfolding to aggregation. These stages are important as they dictate the fish paste characteristics at any given temperature.

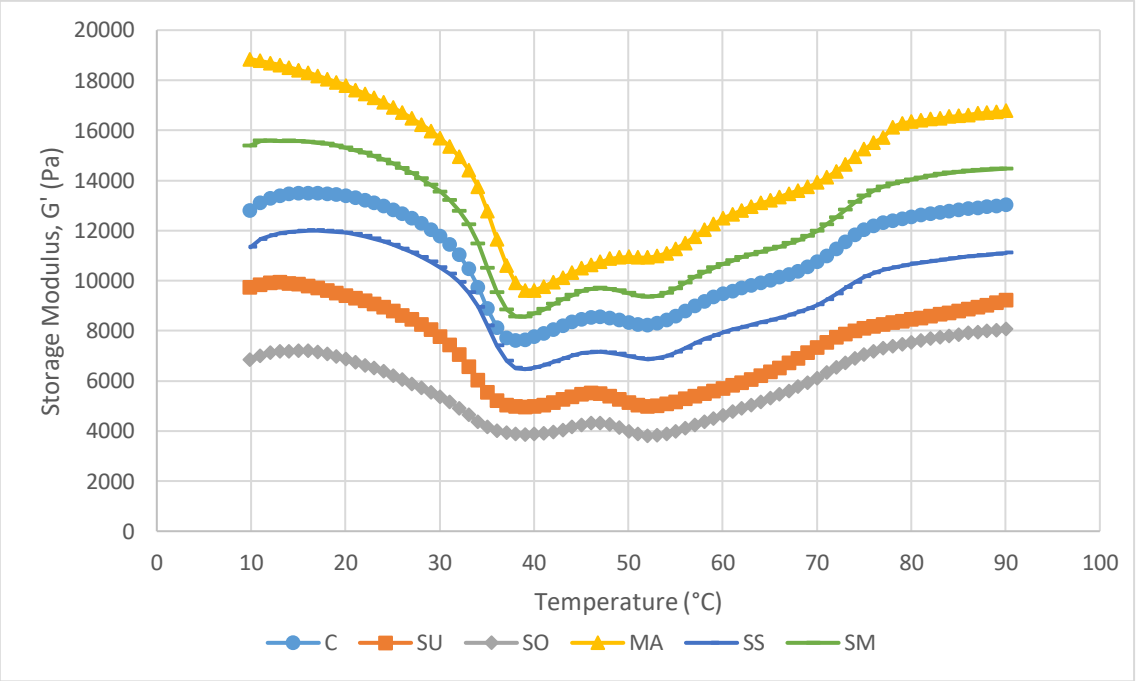


Figure 4.3: Storage modulus (G') of sample with different types of sugar formulations for temperature sweep done from 10°C to 90°C with heat rate of 1°C/min.

Regardless of the type of additives and concentrations, the G' value was the lowest at a temperature around 30°C to 40°C and displayed similar curve indicating gel softening. This is only visible through slow heating of surimi (Yin and Park, 2015). At this temperature, Esturk (2003) reported that a major structural change occurred which is the unfolding of actomyosin. An increase of G' value for the second time at temperatures above 52°C was reported as a result of permanent irreversible cross-linked myosin filament formation and

also a stable three-dimensional network structure (Chan *et al*., 1987). The three-dimensional networking structure is formed by hydrophobic interactions and also disulphide covalent bonds (Lanier, Carvajal and Yongsawatdigul, 2006). This can be observed as G' increased until 80°C and after that a small incline to indicate a stable gelling networking. Thermal gelation was completed at temperature where G' reached a maximum plateau (Esturk, 2003).

All samples displayed similar curves with two gelation points. However, the difference in G' at each gelation point indicates that there were differences in the initial gelation point. These differences suggest that the bonds between the type and concentration of sugars had an effect on the myofibrillar structure. It was reported that higher value of storage modulus during the onset of gelation is related to higher elasticity which can be translated into deformation in texture analysis (Poowakanjana *et al*., 2012). A similar trend of the G' value was recorded as the stress sweep data with MA having the highest value of G' even in temperature sweep, and SO have the lowest G' value. Increase in G' also indicates the increase in elastic gel network which causes fish gel to be more rigid (Yin and Park, 2015).

Figure 4.3 suggests that fish paste with mannitol added required more heat in order to induce gelling and form a gel structure. The amount of energy stored for gelation is due to the stability of the protein network which requires a higher amount of thermal energy to dissociate (Poowakanjana *et al*.,2012). This suggests that mannitol prevented protein denaturation during freezing thus preserving the protein in its prime state. Other than that, it indicates that

mannitol added promoted a complete protein-protein interaction, thus requiring higher energy to dissociate the bonds to cause deformation and aggregation of protein.

4.4.4 Texture analysis

Texture analysis was done to determine the effect of different types of sugar on texture more specifically on the gel quality. Generally, sugar is added to prevent protein denaturation during frozen storage (Nopianti *et al*., 2012). Changes in gelling structure are usually related to the myofibrillar proteins gel forming ability and amount of moisture present in the sample. By decreasing the rate of protein denaturation during freezing, the gel forming ability of myofibrillar protein can be prolonged (Matsumoto, 1980; Benjakul *et al*., 2005). Thus, cryoprotectants are introduced in order to retain protein functionality during freezing.

The breaking strength is defined as the maximum amount of force needed before the sample starts to deform. The breaking strength of the samples with different formulations is shown in Figure 4.4. MA showed a significant difference ($p < 0.05$) and the highest breaking strength when compared to other samples. SM recorded the second highest followed by SS which is the commercial blend. However, SM did not show a significant difference ($p < 0.05$) when compared to the commercial blend SS which indicates that substituting sorbitol with mannitol produces similar properties. This is a good indicator that mannitol can be used to substitute sorbitol as a cryoprotectant.

Although a high breaking strength does not necessary define a good gel strength property. A further analysis on texture is required.

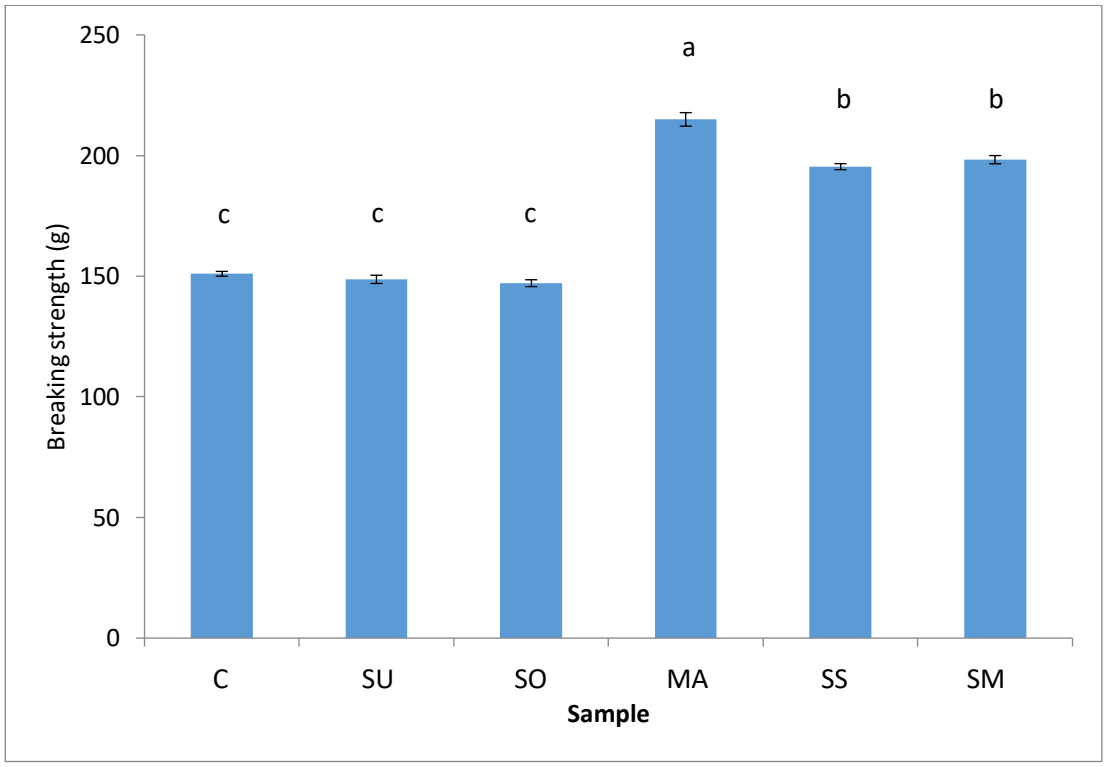


Figure 4.4: Breaking strength of sample with different types of sugar formulations. Values are means ± SD of triplicates (n = 3). Different letters indicate significant differences ($p < 0.05$). C = control.

Breaking deformation represents the maximum ability of the sample to withstand deformation when force is applied to the material. Another representation of breaking deformation is called penetration depth. It is measured as the maximum distance (mm) of probe penetration which causes sample deformation. Breaking deformation of samples with different formulations is illustrated in Figure 4.5. SU displayed the highest value for deformation followed by MA, SM, SS, C and SO. A high breaking deformation value is usually related to high elasticity. In surimi, too high of breaking deformation will cause the sample to be to gummy rather than the cohesive

attribute desired. All samples displayed significant difference ($p < 0.05$) except for C and SO.

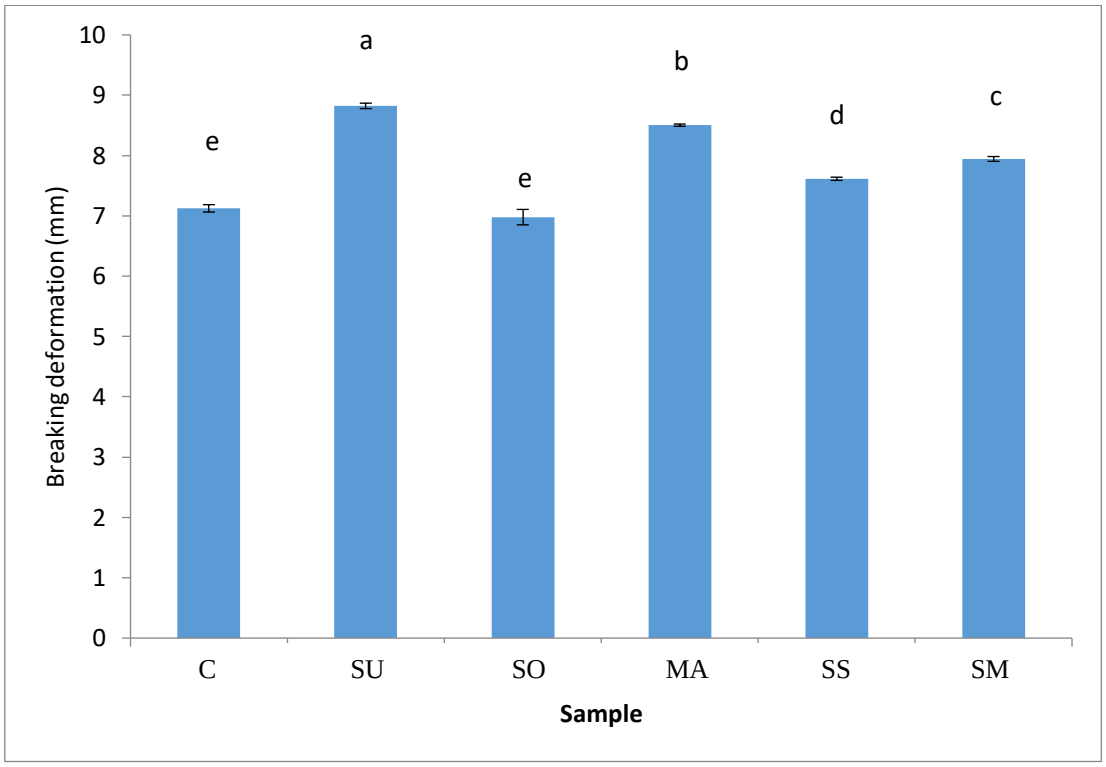


Figure 4.5: Breaking deformation of sample with different types of sugar formulations. Values are means ± SD of triplicates ($n = 3$). Different letters indicate significant differences ($p < 0.05$). C = control.

Figure 4.6 illustrates the gel strength of samples with different formulations in which MA showed a significantly high value of gel strength ($p < 0.05$) when compared to other samples. Yoon and Lee (1990) demonstrated in their experiment that there was a correlation between expressible moisture and gel-forming ability; increase in expressible moisture caused a decrease in gel-forming ability. The rheological tests done earlier also supported this result as MA displayed the highest value for stress (σ_{max}), strain (γ_{max}), and phase angle (δ_{max}) (Table 4.4). This result suggests that MA consisted of a denser gel network formation and mannitol has a good ability to prevent protein denaturation during overnight frozen storage. SO recorded the lowest gel

strength. This detrimental effect is associated with the protein denaturation process that occurred during freezing causing myosin and actomyosin being secluded during gel network formation when heat was applied (Sych *et al*., 1991A). Without myosin and actomyosin, the elasticity and water holding capacity of the gel is reduced (Liu *et al*., 2014B). Low gel strength is due to the formation of poor gel matrix networking and can be associated low WHC (Nopianti *et al*., 2012), thus, explaining why SO recorded a low gel strength due to low WHC as presented in Table 4.3.

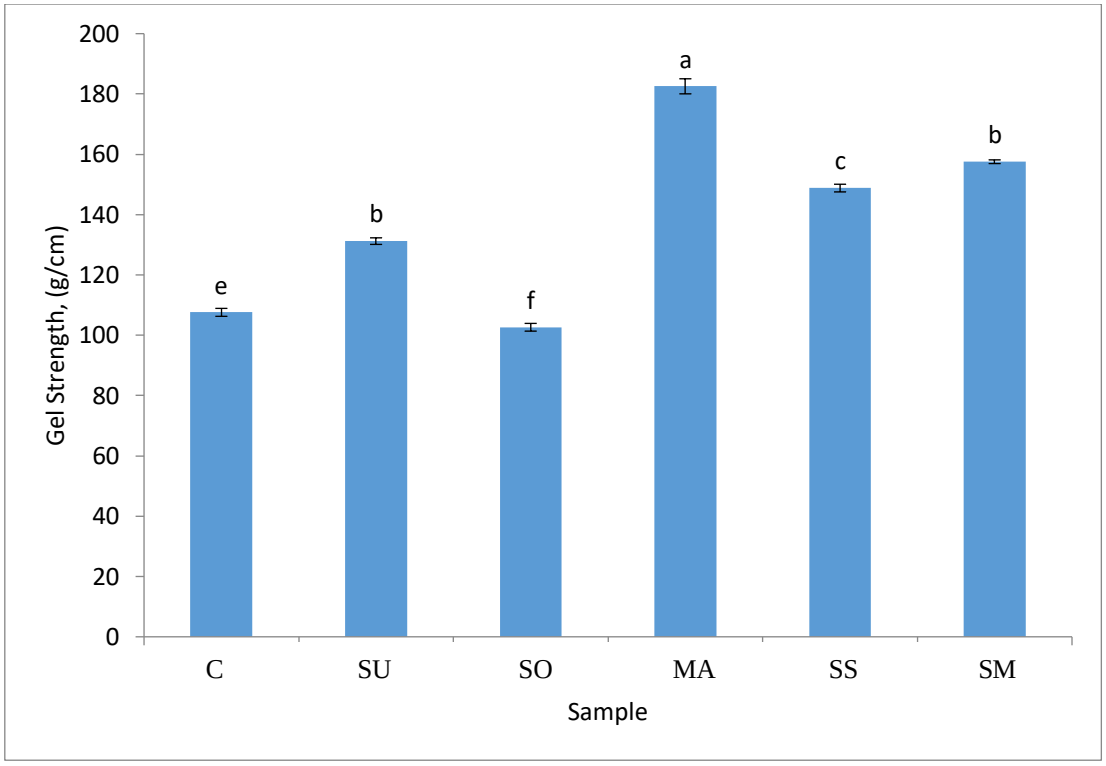


Figure 4.6: Gel strength of sample with different types of sugar formulations. Values are means ± SD of triplicates (n = 3). Different letters indicate significant differences ($p < 0.05$). C = control.

The gel forming ability recorded was found to differ from each other suggesting that each sugar has a different effect on the stability of protein during frozen storage (Benjakul *et al*., 2005). Interaction between the low

molecular weight sugar and protein is still vague and further analysis should be done to determine the actual effects. However, values obtained in the present work are slightly lower than reported in the literature; Alaska Pollock surimi was reported to have values of breaking force and deformation of approximately 250 g and 10.6 mm (Yin and Park, 2015); and another work on Alaska Pollock surimi was reported to have approximately 555 g/cm gel strength (Sultanbawa and Chan, 1999). The difference in value might be due to the amount of washing and additives used. Industrial surimi has a higher number of washing cycles and contains other additives (phosphates) which improve the gelling characteristics of the surimi.

Experiments in the present work only consisted of fish paste and sugar which was washed only with one cycle. However, the results obtained indicate that the addition of mannitol produced the highest values of breaking strength, breaking deformation and gel strength compared to other sugar combinations, thus, proving that mannitol can be considered as an alternative to replace sucrose and sorbitol in the surimi industry.

4.4.6 Effects of different sugar concentration

4.4.6.1 Rheological tests

Stress sweep tests were done to determine the effects of different concentration of sugar on its LVR. Figures 4.7 to 4.11 shows the effects of different types of sugar with 4%, 6% and 8% sugar concentrations (w/w) on the storage modulus (G').

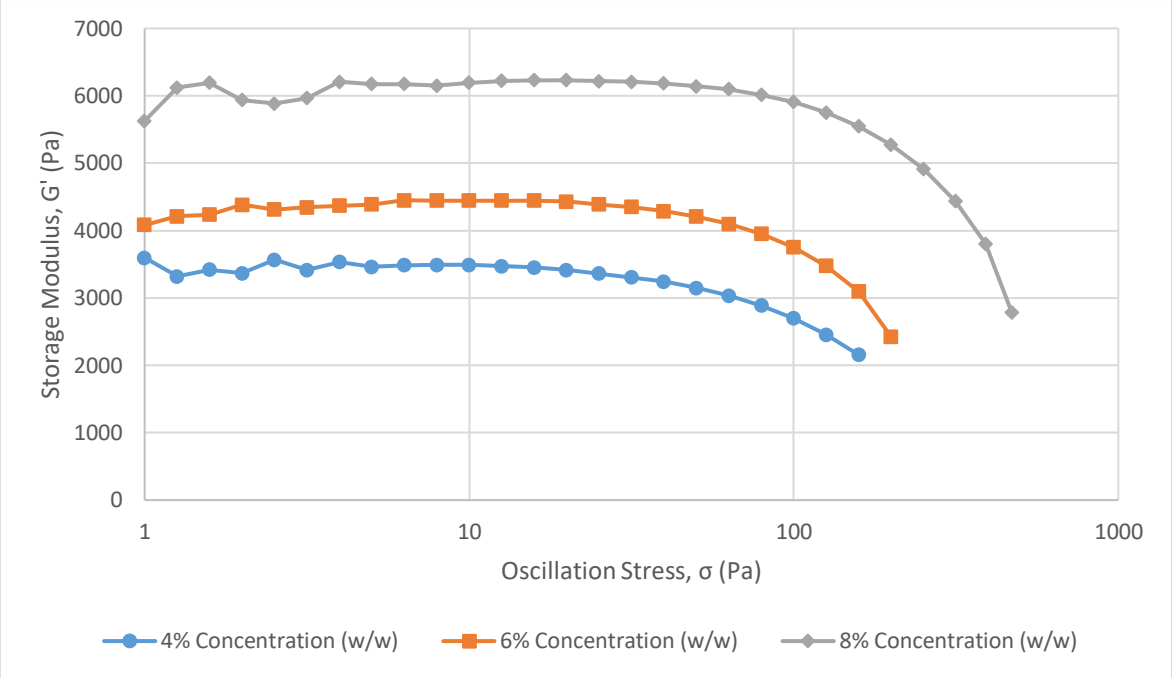


Figure 4.7: LVR of fish paste containing different concentrations of sucrose using stress sweep at shear 1 Pa to 1000 Pa.

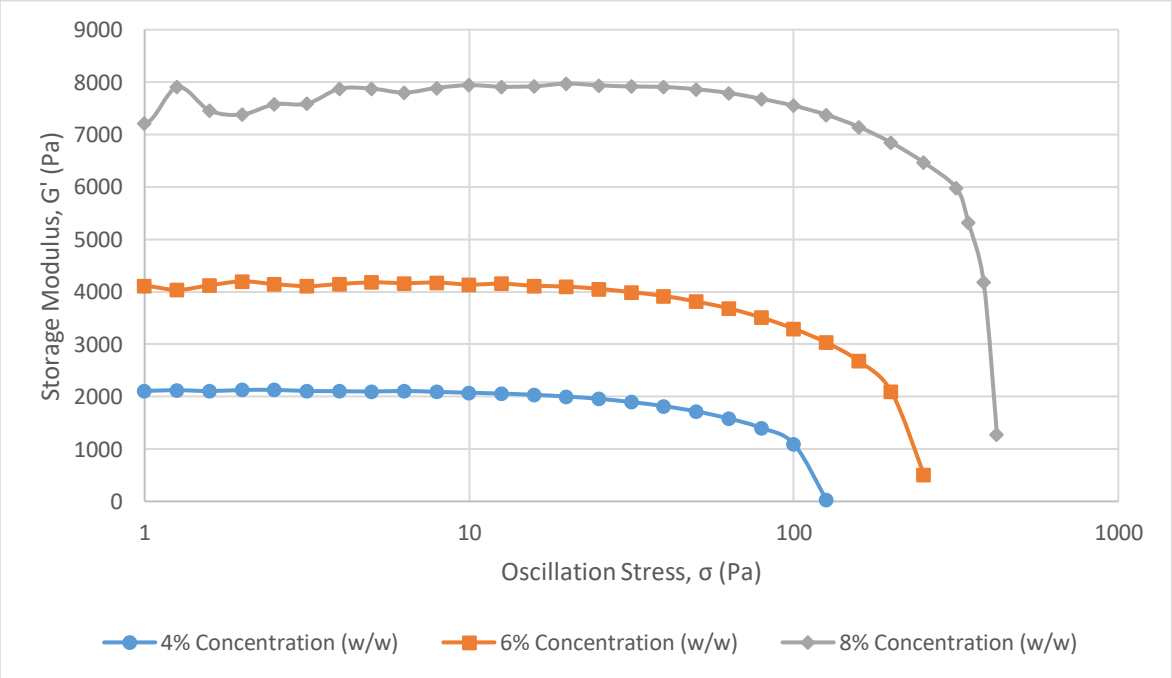


Figure 4.8: LVR of fish paste containing different concentrations of sorbitol using stress sweep at shear 1 Pa to 1000 Pa.

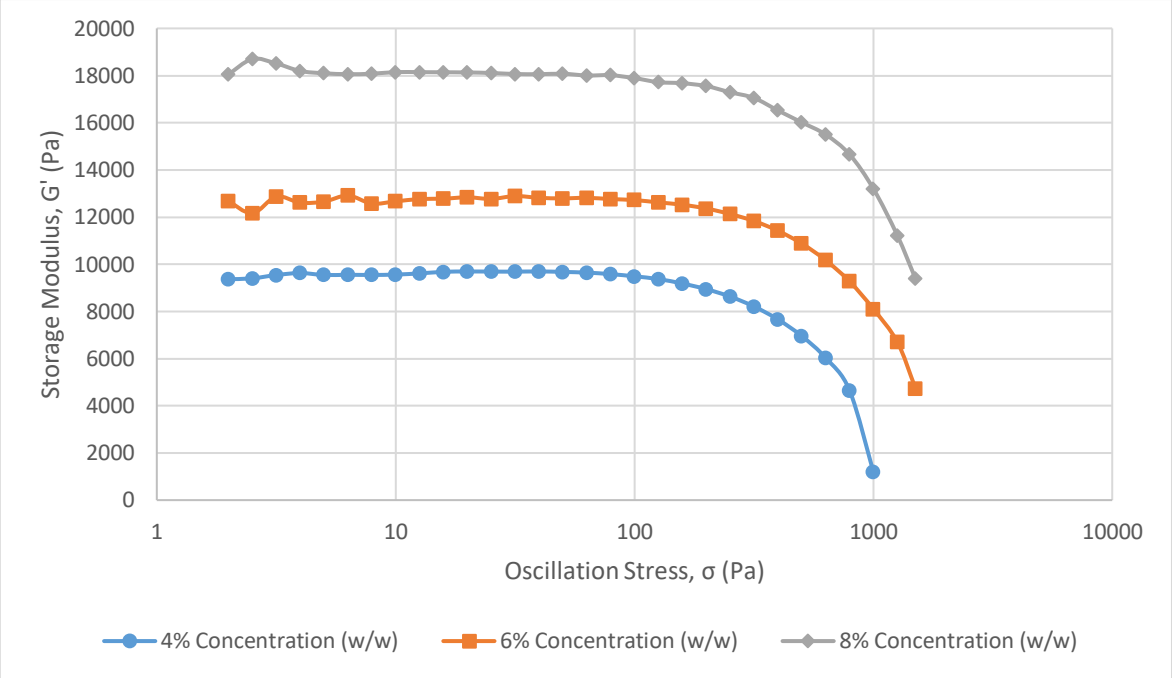


Figure 4.9: LVR of fish paste containing different concentrations of mannitol using stress sweep at shear 1 Pa to 1000 Pa.

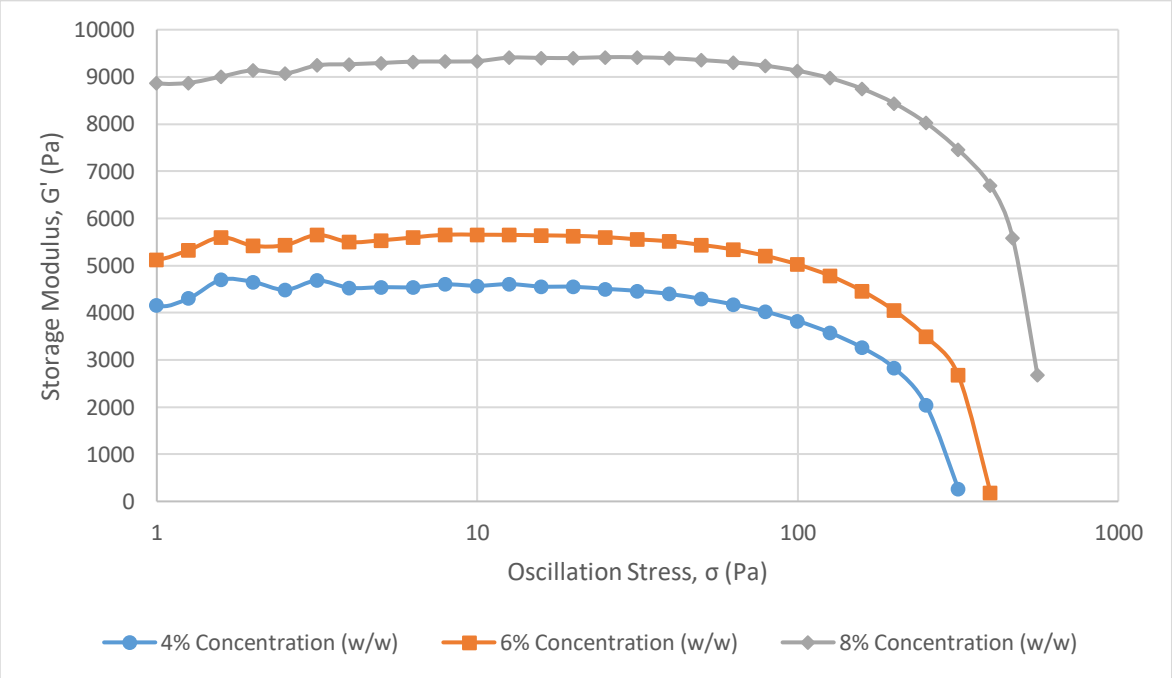


Figure 4.10: LVR of fish paste containing different concentrations of sucrose + sorbitol using stress sweep at shear 1 Pa to 1000 Pa.

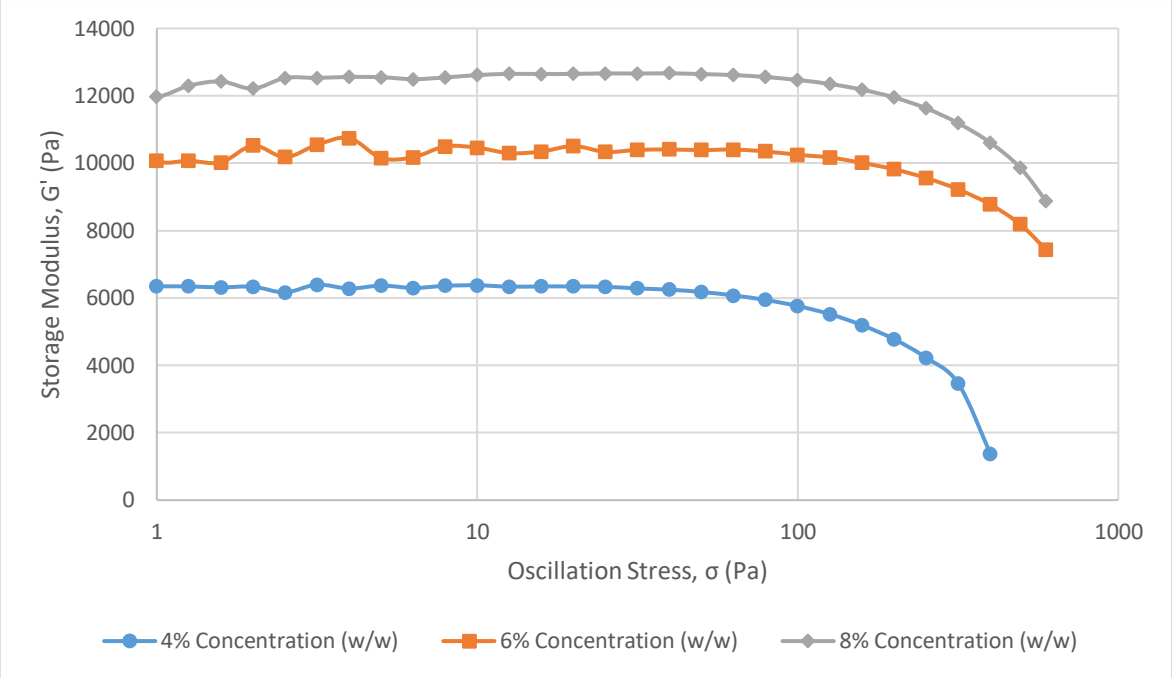


Figure 4.11: LVR of fish paste containing different concentrations of sucrose + mannitol using stress sweep at shear 1 Pa to 1000 Pa.

All samples in Figures 4.7 to 4.11 display similar results in which the G' and G'' did not overlap which in turn indicate that the samples were elastic. As the stress increased to a certain level, G' value started to decrease. The point where G' value started to decrease is considered as the maximum value of stress that can be applied to the sample before it deforms.

G' value of samples with different formulations displayed similar result. It was found that 4% (w/w) sugar concentration for all samples displayed the lowest value of G' and as concentration increased, the G' values also increased. Another observation that is apparent from Figures 4.7 to 4.11 is that as sugar concentration increased, the LVR became wider. Strong gels exhibit a much wider range of LVR compared to weak gels (Steffe, 1996) as evidenced when the sugar concentration increased, the gel strength of all samples increased.

Different types of sugar did not display any difference in trend and this implies that all types of sugars behaved similarly in which as concentration increased, the G' value for LVR increased. Results obtained also suggest that as sugar concentration increased, the structure of the fish paste became more intact and rigid which is represented by the G' value (Campo-Deaño *et al*., 2010). As a result, the fish paste is predicted to have better gel strength when compared to others. As sugar concentration increased, moisture content and water activity decreased (Chen *et al.,* 2002) thus, the cryoprotective quality was enhanced.

Temperature sweep tests were done to determine the effects of sugar concentration on the gelation profile of fish paste. Figures 4.12 to 4.16 display the effects of sugar concentration on the gelation profile of fish paste with different sugar combinations. All samples show the 4-stage gelation. However, 6% (w/w) sugar concentration showed a much more distinct peak between the first gelation point and second gelation point (between 40°C to 55°C). This suggests that between these two points, the protein-protein interaction was more complex and requires more energy to dissociate (Poowakanjana *et al*., 2012). Lower energy consumption to induce gelation will result in a much lower peak rather than a distinct peak. These are represented by peaks with 4% and 8% sugar concentrations when compared with 6%. The final gelation point of each sample was found to decrease as concentration increased. These points are defined as the temperature where the second G' value started to increase (50°C to 60°C range). From this point onwards, gel strengthening phase took place. The protein (actin) started to form a much

denser, complex and irreversible gel network with other proteins (Campo-Deaño *et al.,* 2010). The amount of protein networking at this stage increased thus reinforcing the gel matrix to form irreversible gel (Campo-Deaño *et al.,* 2009B). Lower gelation temperature is beneficial for food manufacturers as less energy is required to induce gelation.

The G' value was found to be similar for samples at 6% at 8% at the end of the temperature profile (80°C to 90°C). This temperature range is when the final structure of surimi is shaped and becomes permanent (Belibagli *et al.,* 2003). This suggests that the gelling strength of sugar concentration at 6% and 8% might have a similar value. However, this will be further discussed and proven with texture analysis.

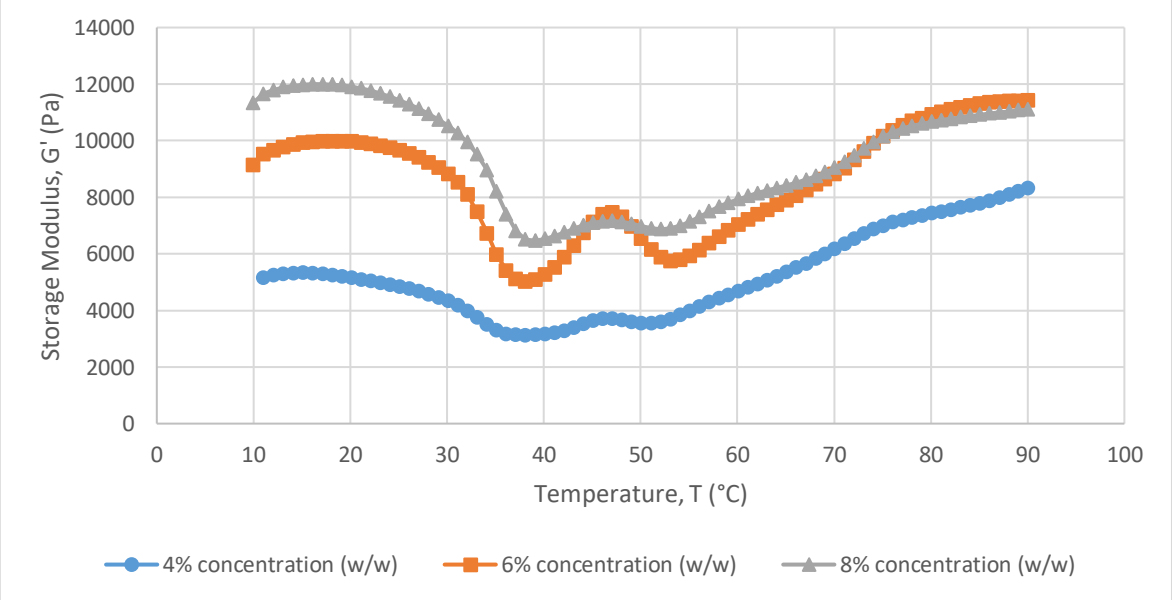


Figure 4.12: Temperature profile of fish paste added with sucrose at different concentrations using temperature sweep with a heat rate of 1°C/min.

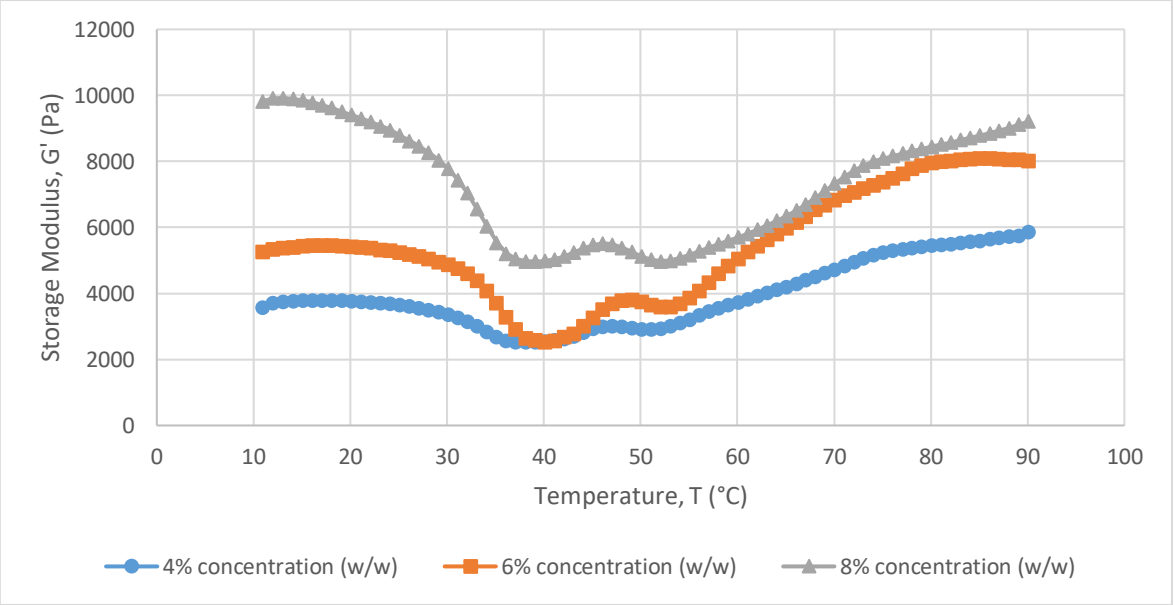


Figure 4.13: Temperature profile of fish paste added with sorbitol at different concentrations using temperature sweep with a heat rate of 1°C/min.

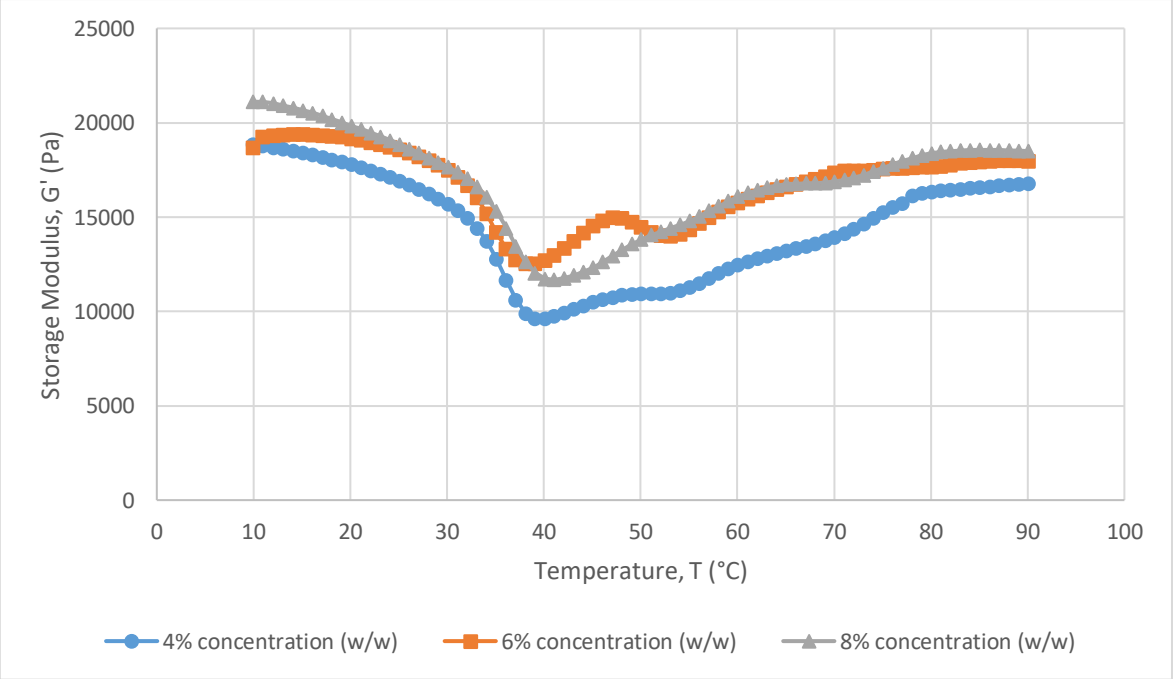


Figure 4.14: Temperature profile of fish paste added with mannitol at different concentrations using temperature sweep with a heat rate of 1°C/min.

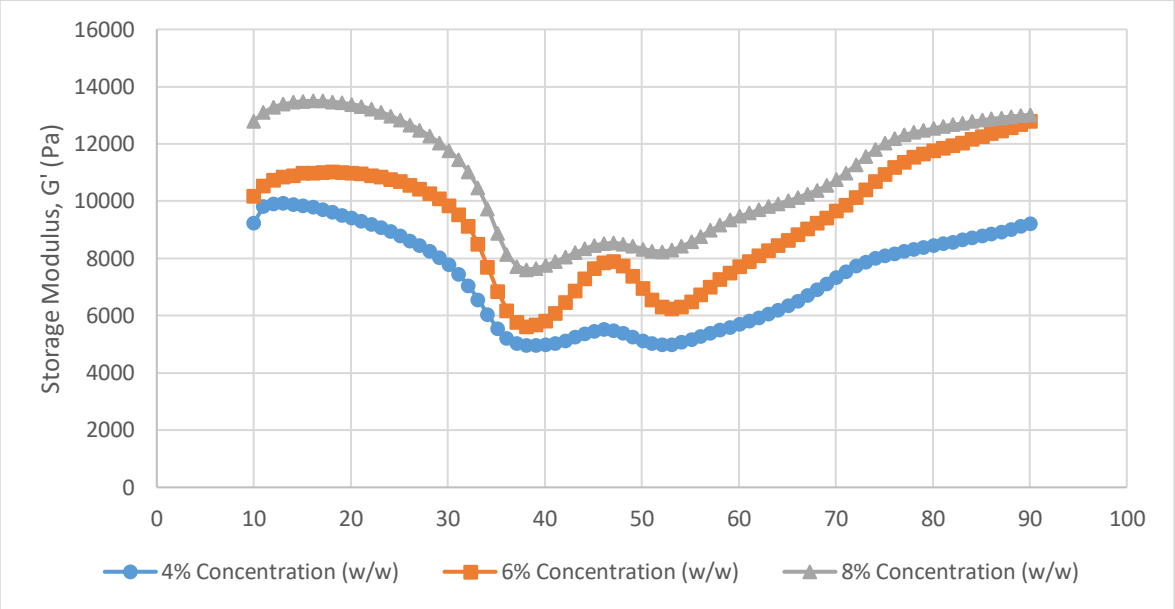


Figure 4.15: Temperature profile of fish paste added with sucrose + sorbitol at different concentrations using temperature sweep with a heat rate of 1°C/min.

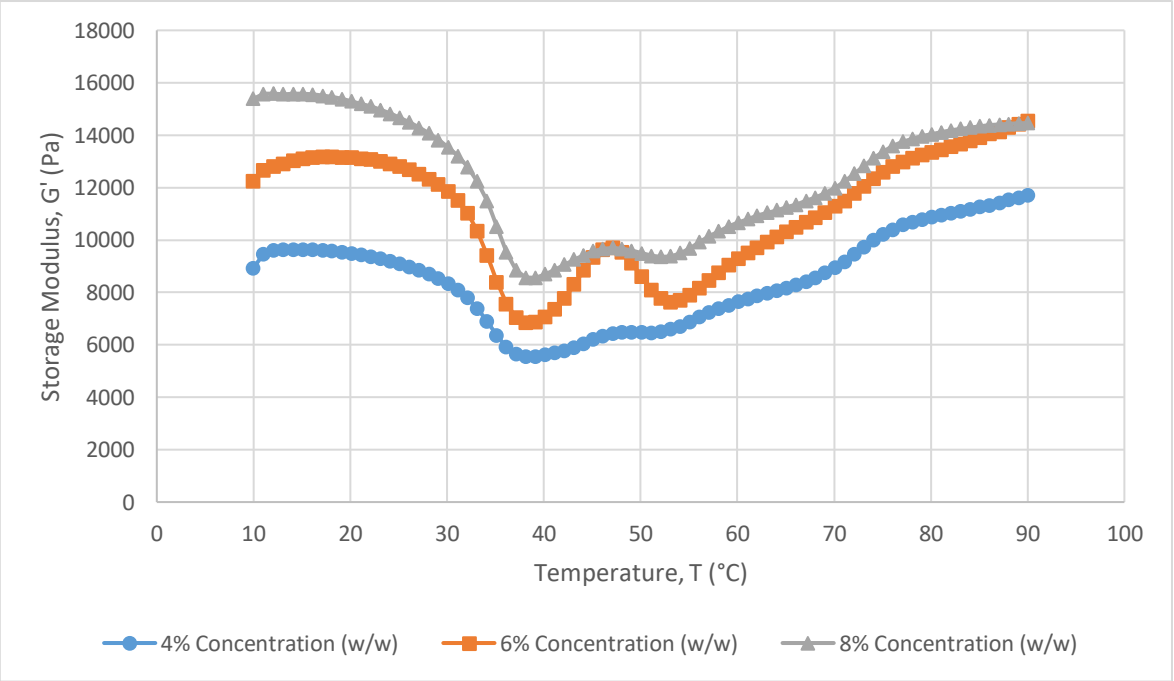


Figure 4.16: Temperature profile of fish paste added with sucrose + mannitol at different concentrations using temperature sweep with a heat rate of 1°C/min.

4.4.6.2 Texture analysis

The breaking force, breaking deformation and gel strength are determined and calculated for each sample at different sugar concentrations to determine the effects of different cryoprotective level following overnight frozen storage. Results obtained from these tests will further support the feasibility of using rheological tests to determine the gelling quality of surimi fish gel. Figure 4.17 display the breaking strength of sample at different sugar concentration.

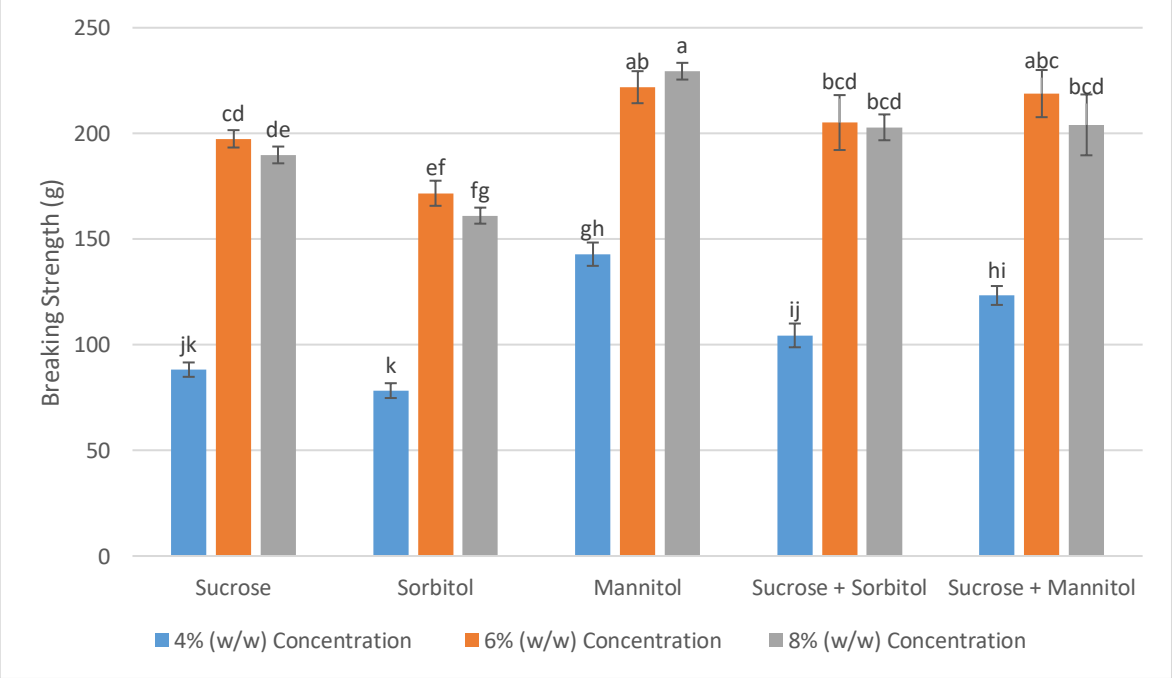


Figure 4.17: Effect of different sugar concentrations on the breaking strength of fish gel. Values are means ± SD of triplicates (n = 3). Different letters indicate significant differences ($p < 0.05$).

In Figure 4.17, the breaking strength of each sample displayed almost a 100% increase with addition from 4% (w/w) sugar concentration to 6% (w/w). However, 6% (w/w) sugar concentration did not show any significant difference when compared to 8% (w/w) sugar concentration ($p < 0.05$). This

suggests that the breaking strength does not vary when concentration changes from 6% to 8%. The stress required to produce surimi without destroying its structure is the same between these two concentrations. This implies that a lower sugar concentration could be used to produce similar quality but with lower calorie surimi.

Figure 4.18 depicts the breaking deformation of fish samples in which some samples did not show any significant effects of sugar concentration ($p > 0.05$). This suggests that these samples maintained flexibility even though sugar was added up to 8% concentration. There is however a significant difference between 4% and 8% concentration in breaking deformation for samples with sucrose and sample with mannitol ($p < 0.05$).

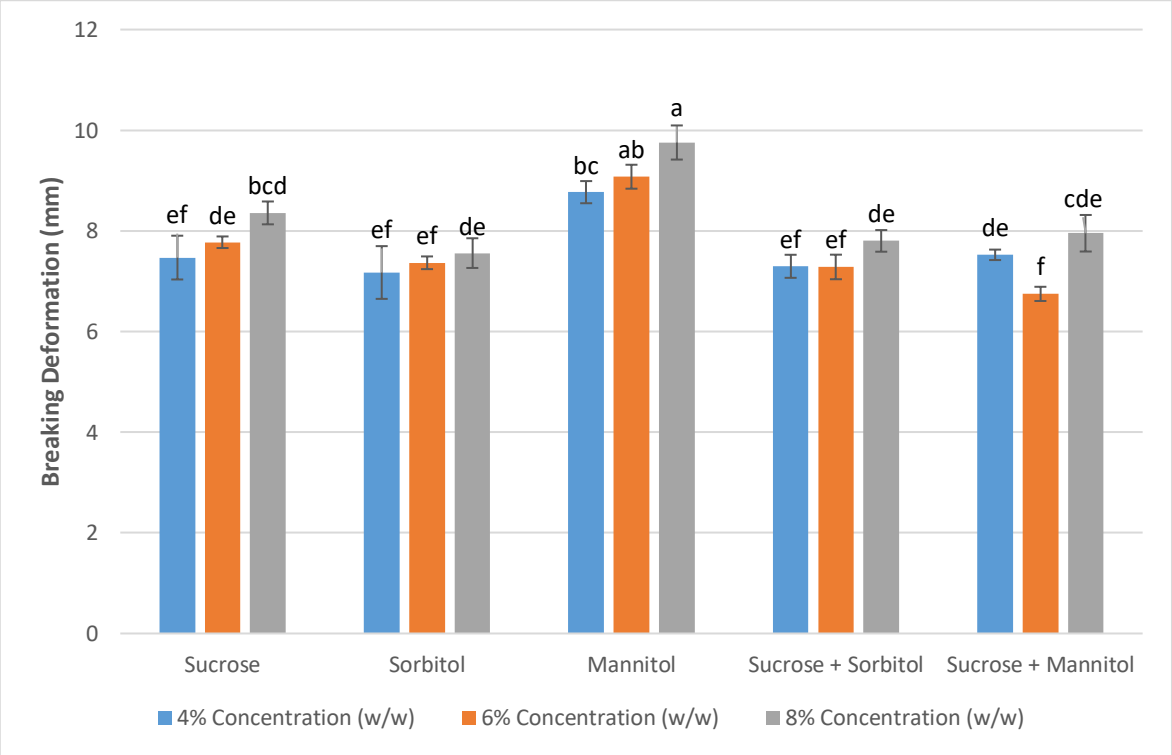


Figure 4.18: **Effect of different sugar concentrations on the breaking deformation of fish gel.** Values are means ± SD of triplicates (n = 3). Different letters indicate significant differences ($p < 0.05$).

Most of the sample did not show any significant difference ($p > 0.05$) when sugar concentration was increased from 6% to 8% except for sucrose + mannitol. Cryoprotectants have never been reported as additives to enhance the gel strength of surimi. They have always been used as a preservation technique to maintain the quality of protein and chemical structure of fish surimi during freezing. Results obtained from this experiment show the effectiveness of different types of cryoprotectant at different concentrations.

Figure 4.19 shows that the gel strength of each sample increased almost twice from 4% to 6% sugar concentration. A higher value in gel strength can be related to the cryoprotective effect. As the concentration increases, protein deformation is better prevented thus producing better gel quality (Huda *et al.,* 2011; Yoon and Lee, 1990). Even though all samples displayed similar trend, only mannitol displayed a significant difference at different mannitol concentrations ($p < 0.05$). However, 6% sugar concentration did not show any significant difference with 8% concentration ($p > 0.05$). Although initially the hypothesis was that 8% sugar concentration was to yield higher gel strength, the obtained data do not seem to support that hypothesis. Huda *et al.* (2011) also found that gel strength did not differ when cryoprotectant (polydextrose)'s concentration was increased from 6% to 9%. Another research on threadfin bream also showed a minimal increase of gel strength when concentration of sucrose:sorbitol (1:1 w/w) was increased from 6% to 8% (Parvathy and George, 2014). This indicates that the cryoprotective effect in this range (6% to 8%) might be similar.

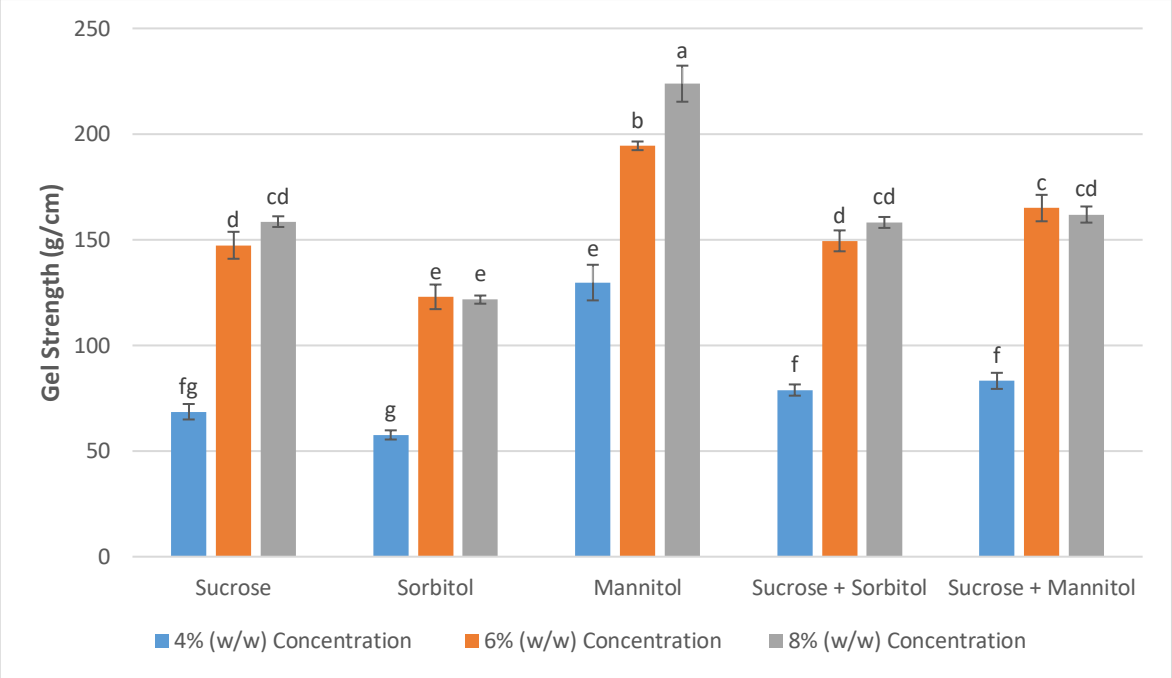


Figure 4.19: Effects of different sugar concentrations on the gel strength of fish gel. Values are means ± SD of triplicates (n = 3). Different letters indicate significant differences ($p < 0.05$).

Park *et al.* (1988) stated that certain sugar might inhibit gel formation. This might be true for the case of sugar at 4% concentration as all samples at this concentration appeared to display the lowest gel strength. Different types and concentrations of cryoprotectant differently affect the rate of protein denaturation, chemical structure and gelation properties of surimi (Huda *et al.,* 2011; Belibagli *et al.,* 2003; Sych *et al.,* 1991B). Overnight frozen storage was reported to induce protein (myosin) deformation which leads to lower gel strength (Nopianti *et al.,* 2012; Scott *et al.,* 1988). Without an effective cryoprotectant, the surimi gel quality decreases rapidly. However, when compared to the rheological tests done on the viscoelastic properties of samples, it was found that the results presented and recorded supported all the outcomes of texture analysis, thus, making rheology a feasible method to predict the gelling behaviour of fish paste.

4.5 CONCLUSIONS

In the present work, mannitol has showed promising results and could be considered as an alternative to sucrose and sorbitol which are commercially used in the surimi industry. Rheological tests and texture analysis showed similar trends for all sugar combinations. Results obtained from the present work show that each type of sugar has a different efficiency in terms of cryoprotective effect. Fish paste with mannitol showed the highest value of storage modulus (G') in rheological tests. Furthermore, results obtained from texture analysis are consistent with the results obtained from rheological tests. Sample with mannitol presented the highest value of gel strength which indicates high quality when compared with other fish gel and sugar combinations. This in turn indicates that mannitol could be considered as an alternative cryoprotectant in the surimi industry. Rheological testing of fish paste can also be used to predict the gel properties of surimi gel.

Effects of sugar concentrations on gelling strength showed only significant difference from 2% (w/w) to 4% (w/w). Increment from 6% to 8% did not show any significant difference on gelling strength except for mannitol. Thus, using 6% sugar concentration would be better in achieving a healthier low calorie surimi. A wider range with smaller increase (0.5% or 1%) in sugar concentration could be considered to determine the specific changing point of surimi properties influenced by concentration and to provide a better view of the trend. Results obtained could also be beneficial if shelf life study could be done to determine the prolonged effect of different concentration.

Mannitol, a sugar with lower calorie and less sweetness could be used to replace the higher calorie and more sweet sucrose and sorbitol as a cryoprotective agent. Another achievement in the present work is the success in reducing the amount of sugar concentration used in surimi whilst maintaining its quality similar to the amount of sugar concentration, 8% (w/w), used in the surimi industry.

CHAPTER 5

Effects of Salt Concentration and Different Sugar Combinations on Rheology and Texture of Surimi

5.1 INTRODUCTION

In order to be abreast with the current food trends and consumers' demand, a healthier approach on surimi processing is currently required. Reducing the salt concentration within the surimi products could be an initial step in producing a much healthier food compared to the conventional way of processing surimi (Tahergorabi and Jaczynski, 2012A). Gel properties are one of the main attributes in determining the quality of surimi. It gives body and texture to seafood commodities which translates human sensory perception to perceive the product as delicious and palatable. To further improve the gelling quality, salt (sodium chloride; NaCl) is added into the fish paste in order to solubilise the myofibrillar protein. This will then form a viscous protein paste which unfolds protein chain to create gel networks (Cando *et al.,* 2015; Greiff *et al.,* 2015). This is an important step to initiate protein gelation (myosin protein gelation). Myofibrillar protein which has been solubilised will then form a three-dimensional gel protein network when heat is applied (Esturk, 2003). Salt plays a significant role in gelling of surimi; thus, research on this important factor is required.

The extend of salt used in order to manufacture a stable and high-quality surimi gel is crucial as a low amount of salt (1-3%) might not be able to adequately solubilise the desired amount of myofibrillar in order to promote protein unfolding for further gelling (Cando *et al.,* 2016; Lanier, Carvajal and Yongsawatdigul, 2005). However, there are reports that higher amounts of salt concentration might also disrupt protein gelation due to salting out effect. Higher concentration of salt will produce much more protein-protein interaction

rather than the needed protein-solvent interaction. As a result, the protein develops aggregation and precipitates without forming gel network (Esturk, 2003). Therefore, a precise and considerable amount of salt concentration needs to be investigated.

Another beneficial property of salt is its ability to increase the water holding capacity within surimi (Belibagli *et al.,* 2003). This property is a key factor in producing a good gel. Water holding capacity has been reported to significantly affect the gel formation process (Yoon and Lee, 1990). Other than that, salt has also been reported to increase gel strength of fish gel by producing salt bridges and causing complete dissociation of myofibrillar protein (Grieff *et al.,* 2015; Fu *et al.,* 2012). However, there is still some dispute over the effects of salt to increase gel strength. Some researchers found that at certain level of salt concentration, the gel strength of fish gel was inhibited and lowered (Park and Lin, 2005; Esturk, 2003).

Addition of salt can be administered in several ways. However, the two most popular methods are adding salt directly during comminution, and incorporating salt solution into the washing water (Cando *et al.,* 2016; Park and Lin, 2005; Esturk 2003). Addition of salt during comminution will enhance the water holding capacity of myofibrillar proteins by exposing the negative charge anionic protein group. The chloride ion, on the other hand, will attach itself to the positively charged protein molecules and lower the isoelectric point, thus, increasing myofibrillar protein solubility (Lanier, Carvajal and Yongsawatdigul, 2005; Esturk, 2003).

Several researches have been done in this area due to the increase in health awareness among consumers. Tahergorabi and Jaczynski (2012A) introduced potassium chloride (KCl) in order to replace the ordinary salt due to the latter's antihypertensive properties. Cando *et al.* (2016) stated that three additives were selected to help increase gelling ability at low salt concentration. However, all these three additives have their disadvantages and flaws, thus, opening more areas of research into low salt concentration surimi. Other than additives, some researchers also applied other technology to increase gelling ability of surimi at low salt concentrations.

The work described in the present Chapter was done to determine the trend in rheological and textural properties of surimi fish paste and gel with increasing levels of salt concentration. Other than that, combinations of different salt concentrations and different sugar formulations were also studied to determine its ability to increase surimi gelling strength. The feasibility of using rheological tests to correlate the fish paste with the fish gel texture was also investigated. If successful, rheological tests could be introduced to the seafood industry as a crude method to predict the gelling capability of surimi before further processing.

5.2 MATERIALS

5.2.1 Sample preparation

Fish paste and fish gel samples were prepared as reported in Chapter 3 using the formulation shown in Table 5.1.

Table 5.1: Sample formulations for different types of sugar at different salt concentrations.

SALT CONCENTRATION (w/w)	FORMULATION (w/w)	CODE
1%	Control	CO1
	8% Sucrose	SU1
	8% Sorbitol	SO1
	8% Mannitol	MA1
	4% Sucrose + 4% Sorbitol	SS1
	4% Sucrose + 4% Mannitol	SM1
2%	Control	CO2
	8% Sucrose	SU2
	8% Sorbitol	SO2
	8% Mannitol	MA2
	4% Sucrose + 4% Sorbitol	SS2
	4% Sucrose + 4% Mannitol	SM2
3%	Control	CO3
	8% Sucrose	SU3
	8% Sorbitol	SO3
	8% Mannitol	MA3
	4% Sucrose + 4% Sorbitol	SS3
	4% Sucrose + 4% Mannitol	SM3
4%	Control	CO4
	8% Sucrose	SU4
	8% Sorbitol	SO4
	8% Mannitol	MA4
	4% Sucrose + 4% Sorbitol	SS4
	4% Sucrose + 4% Mannitol	SM4

5.3 RESULTS AND DISCUSSIONS

5.3.1 Moisture content

Table 5.2 shows the moisture content of samples with different salt concentrations and sugar combinations. SO1 significantly ($p < 0.05$) recorded the highest value of moisture content when compared to the other treatments. This might be due to the fact that sorbitol has always been known to retain water content, and this has also been discussed in Chapter 4. It is reported that at low concentration of salt, sorbitol itself possesses the ability to retain water due to its hydrogen and covalent bonds present in the fish protein itself (Nopianti *et al.,* 2012).

Table 5.2: Effect of salt concentrations on moisture contents of surimi fish paste with different formulations. Values are means ± SD of triplicates (n = 3). Capital letters indicate significant difference ($p < 0.05$) between columns. Small letters indicate significant difference ($p < 0.05$) between rows.

SAMPLE	Moisture content (%)			
	1% Salt (w/w)	2% Salt (w/w)	3% Salt (w/w)	4% Salt (w/w)
Control	$75.79^{Ba} \pm 0.3$	$75.89^{Aa} \pm 0.50$	$73.61^{ABb} \pm 0.56$	$71.84^{Cc} \pm 0.62$
Sucrose	$76.66^{Ba} \pm 0.63$	$75.42^{Aab} \pm 0.15$	$74.54^{Ab} \pm 0.55$	$74.24^{BCb} \pm 0.98$
Sorbitol	$78.99^{Aa} \pm 0.15$	$75.30^{Ab} \pm 0.30$	$71.63^{Cc} \pm 0.37$	$72.23^{Cc} \pm 1.74$
Mannitol	$76.73^{Ba} \pm 0.46$	$73.80^{Cb} \pm 0.22$	$72.24^{BCb} \pm 0.44$	$73.11^{Cb} \pm 1.22$
Sucrose + Sorbitol	$74.15^{Cb} \pm 0.26$	$74.34^{BCb} \pm 0.41$	$73.99^{Ab} \pm 0.71$	$76.78^{ABa} \pm 1.05$
Sucrose + Mannitol	$76.32^{Bb} \pm 0.43$	$75.10^{ABbc} \pm 0.28$	$73.83^{Ac} \pm 0.27$	$79.85^{Aa} \pm 1.78$

However, as salt concentration increased, especially at 4%, SS4 and SM4 displayed a significantly better moisture content value ($p < 0.05$). This shows

that the interaction between sugar combination and salt concentration had an influence on the moisture content of samples. In the present work, sucrose + sorbitol mix represents the commercial blend as it is the most popular combination of cryoprotectant used by seafood manufacturers worldwide.

As salt concentration increased, the moisture content of all samples began to change. Sorbitol showed a significant ($p < 0.05$) decrease of moisture content as salt concentration increased. Mannitol however, did not display any significant difference ($p > 0.05$) on moisture content as salt concentration increased. On the other hand, SS and SM displayed a different trend. SS and SM showed a decrease of moisture until 3% salt concentration and after that moisture content increased again significantly at 4% salt concentration ($p < 0.05$). Ultimately, the moisture content recorded after overnight freezing was within the range of good quality surimi (72 – 77%) suggested by Park and Lin (2006).

Choi *et al*. (2008) reported that moisture content of surimi samples increased significantly ($p < 0.05$) as salt concentration increased up to 3% (w/w). However, some results obtained in the present work are not in agreement with that of Choi *et al*. (2008). Only control sample showed this trend. This could be due to the addition of sugar. Nevertheless, the results in the present work are corroborated by Greiff *et al*. (2015) who reported that moisture content started to decrease with increasing salt concentrations.

Each sugar affected fish paste differently as salt concentration increased. This could be due to the different properties of sugars used and hence their different ability to retain water (Nopianti *et al.,* 2012). The difference in moisture content will affect the gelling texture of the fish sample. Moisture content is important in producing surimi as it affects the rheological and gelling properties of surimi (Yoon *et al*., 2004).

5.3.2 Expressible moisture content and water holding capacity

The measurement of EMC is usually done in gel form to determine its capability to retain water. Pressure is applied on the gel and the water that is squeezed out is measured as expressible moisture content, thus, relating EMC to the WHC of the protein gel network. A good WHC will produce a high-quality surimi with high gelling strength. WHC can also be used as an indicator of gel quality and protein denaturation that occurs within the protein network (Nopianti *et al*., 2012; Chaijian *et al*., 2006). Lower water holding capacity will allow the water inside the system to move around and clump together (Zhang *et al.,* 2013). This will form undesirable large water crystals which disrupt gel formation and cause protein deformation (Ohkuma *et al*., 2008; Zhou *et al*., 2006; Yoon and Lee, 1990). Thus, higher WHC will indicate better gel quality for surimi.

Tables 5.3 and 5.4 show the percentages of EMC and WHC after overnight frozen storage at -18°C. In Table 5.3, sorbitol significantly ($p < 0.05$) recorded the highest value for EMC regardless of salt concentrations when compared with the other treatments. This in turn suggests that sorbitol possesses the

lowest WHC when compared to the other sugar formulations. This is further supported by Table 5.4 in which sorbitol significantly ($p < 0.05$) yielded the lowest WHC at all salt concentrations tested. This also suggests that the fish paste added with sorbitol will produce a low-quality gel as mentioned earlier. On the other hand, fish paste added with SS and SM displayed the highest WHC at 1% salt concentration but without any significant difference ($p > 0.05$) between the two treatments. This suggests that both these samples might display the highest gel strength due to their high WHC. Tables 5.3 and 5.4 show that all samples displayed an increase in EMC and decrease in WHC as salt concentration increased from 1% to 3%.

Table 5.3: Effect of salt concentrations on expressible moisture contents of surimi fish paste with different formulations. Values are means ± SD of triplicates ($n = 3$). Capital letters indicate significant difference ($p < 0.05$) between columns. Small letters indicate significant difference ($p < 0.05$) between rows.

SAMPLE	Expressible Moisture Content (%)			
	1% Salt (w/w)	2% Salt (w/w)	3% Salt (w/w)	4% Salt (w/w)
Control	$17.19^{Cd} \pm 0.28$	$17.97^{Cc} \pm 0.13$	$24.76^{Aa} \pm 0.23$	$22.60^{Cb} \pm 0.10$
Sucrose	$20.09^{Bc} \pm 0.13$	$22.28^{Bb} \pm 0.25$	$22.55^{Cb} \pm 0.23$	$25.80^{Ba} \pm 0.10$
Sorbitol	$25.13^{Ac} \pm 0.21$	$26.88^{Ab} \pm 0.19$	$27.94^{Aa} \pm 0.25$	$26.34^{Ab} \pm 0.19$
Mannitol	$13.15^{Dd} \pm 0.05$	$14.19^{Dc} \pm 0.23$	$17.29^{Ea} \pm 0.23$	$15.29^{Db} \pm 0.21$
Sucrose + Sorbitol	$12.22^{Ed} \pm 0.12$	$14.04^{Dc} \pm 0.26$	$18.43^{Da} \pm 0.29$	$14.92^{DEb} \pm 0.19$
Sucrose + Mannitol	$12.62^{Ec} \pm 0.11$	$12.54^{Ec} \pm 0.38$	$17.65^{Ea} \pm 0.11$	$14.80^{Eb} \pm 0.10$

Almost all sample displayed the highest percentage of EMC and lowest WHC at 3% salt concentration ($p < 0.05$). Types of sugar added to the fish paste did not change this trend which concludes that types of sugar do not majorly influence EMC and WHC with addition of different salt concentration. Increased amount of sodium and chloride ions present within the system might change the isoelectric point, thus, lowering the water holding capacity of surimi (Esturk, 2003).

Table 5.4: Effect of salt concentrations on water holding capacity of surimi fish paste with different formulations. Values are means ± SD of triplicates ($n = 3$). Capital letters indicate significant difference ($p < 0.05$) between columns. Small letters indicate significant difference ($p < 0.05$) between rows.

SAMPLE	Water Holding Capacity (%)			
	1% Salt (w/w)	2% Salt (w/w)	3% Salt (w/w)	4% Salt (w/w)
Control	$77.32^{Ca} \pm 0.37$	$76.33^{Cb} \pm 0.16$	$65.54^{Dd} \pm 0.32$	$69.30^{Cc} \pm 0.13$
Sucrose	$73.79^{Da} \pm 0.17$	$70.46^{Db} \pm 0.33$	$69.63^{Cc} \pm 0.31$	$65.39^{Dd} \pm 0.09$
Sorbitol	$68.18^{Ea} \pm 0.27$	$64.31^{Eb} \pm 0.25$	$61.3^{Ed} \pm 0.35$	$63.23^{Ec} \pm 0.26$
Mannitol	$82.86^{Ba} \pm 0.07$	$80.77^{Bb} \pm 0.32$	$76.35^{Bd} \pm 0.32$	$78.84^{Bc} \pm 0.29$
Sucrose + Sorbitol	$83.51^{Aa} \pm 0.17$	$81.12^{Bb} \pm 0.35$	$76.00^{Bd} \pm 0.38$	$79.8^{Ac} \pm 0.25$
Sucrose + Mannitol	$83.47^{Aa} \pm 0.14$	$83.30^{Aa} \pm 0.51$	$77.90^{Ac} \pm 0.14$	$79.88^{Ab} \pm 0.11$

However, at 4% salt concentration, an increase in WHC value was observed. Higher concentration of salt produces salt bridges which combine with the peptide chains and ionic strength which in turn increase the WHC of surimi (Greiff *et al.,* 2015). Lower EMC and higher WHC will most likely produce a strong and rigid gel structure (Yoon and Lee, 1990). This suggests that

samples at 3% salt concentration are most likely to form the weakest gel. However, this prediction will be verified in the texture analysis further below.

Several works have reported that the addition of salt increases the WHC due to the unfolding of protein which exposes the anionic group of amino acid. When protein unfolds, the anionic group increases the strength of WHC by attaching and interacting with water molecules (Carvajal, Lanier and MacDonald 2005, Lanier, Carvajal and Yongsawatdigul, 2005; Esturk, 2003). This might be true when the samples showed an increase in WHC when subjected to 4% salt concentration in Table 5.4. An increase even at 1% salt concentration displayed a significant difference of EMC and WHC for surimi ($p < 0.05$). However, the correlation of EMC, WHC and gel strength when salt is added has yet to be proven and will be discussed further below.

5.3.3 Rheological tests

Stress sweep test was done to determine the LVR of samples. Figure 5.1a–f represents the storage modulus (G') of each sample as a function of salt concentration. From Figure 5.1, an increase in salt concentration from 1% to 3% displayed a significant change in the G' trend. G' was found to decrease with the increase in salt concentration. At 4% salt concentration, the G' value appeared to bounce back, reversing the trend. The same result was reported by Esturk (2003) when surimi from Pacific whiting was subjected to salt concentrations from 1% to 3%. This trend was also observed in Tables 5.2 and 5.4 where the MC and WHC of samples were found to decrease as salt concentration increased up to 3%. This suggests that lower MC and WHC had

an effect on the G' value of samples. Lower WHC will cause water to move freely and produces large water crystals upon freezing (Zhang *et al.*, 2013). This will disrupt formation of gel network and further produce a lower density gel protein network (Ohkuma *et al.*, 2008). This could relate to the low G' value obtained. As salt concentration increased from 1% to 3% (w/w), more protein is solubilize making it more viscous than elastic thus requiring lesser amount of energy to deform the sample. Sample becomes more liquid-like rather than gel like.

Damodoran (1997) reported that a high salt concentration is known to cause ion specific effects which cause proteins to lose their structural stability. Attracted ions increase ionic strength within the system which overcomes all other bonds such as protein-protein bonds and hydrophobic bonds. This effect thus reduces the stress needed for the structure to deform which can be measured and represented by the lower value of G' (Kim, Park and Yoon, 2005). Fish paste added with mannitol however did not exhibit similar result, in which G' value did not increase at 4% salt concentration. This indicates that 3% addition of salt concentration produced the lowest G' and addition of more salt caused a higher shift in G'. This result is consistent with the result observed in section 5.3.2. WHC was found to be the lowest at 3% salt concentration and continued to increase again at 4% salt concentration. The shift in G' with addition of more salt signifies that the paste became more rigid and elastic rather than viscous.

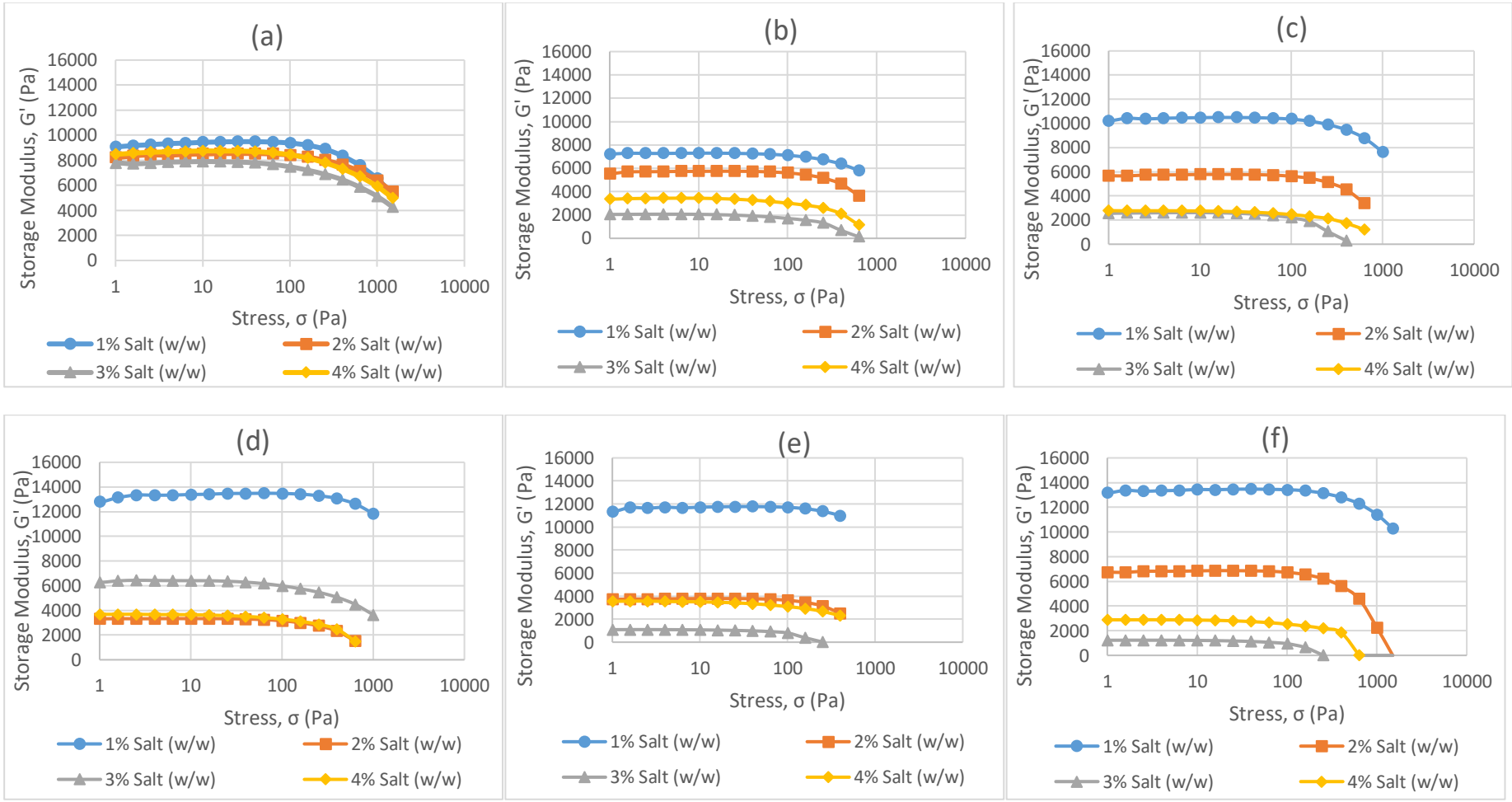


Figure 5.1: Variation of storage modulus (G') as a function of salt concentration for (a) control, (b) sucrose, (c) sorbitol, (d) mannitol, (e) sucrose + sorbitol and (f) sucrose + mannitol.

Tables 5.5 to 5.7 represent the maximum LVR value for stress (σ_{max}), strain (γ_{max}), and phase angle (δ_{max}) of each sample subjected to different concentrations of salt and sugar by adapting the methods of Solo-de-Zaldivar *et al*. (2014). These values are considered the threshold limits for stress and strain which a sample can withstand without altering its structure or destroying its natural state. These values are ideal for food engineers to determine the capability and characteristics of surimi paste for optimum processing condition.

Table 5.5: Data extracted from the stress sweep test to determine the stress max of different salt and sugar concentrations. Values are means ± SD of triplicates (n = 3). Capital letters indicate significant difference ($p < 0.05$) between columns. Small letters indicate significant difference ($p < 0.05$) between rows.

SAMPLE	Stress Max, σ_{max} (Pa)			
	1% Salt (w/w)	2% Salt (w/w)	3% Salt (w/w)	4% Salt (w/w)
Control	$631.19^{Aa} \pm 0.02$	$631.23^{Aa} \pm 0.01$	$631.24^{Aa} \pm 0.05$	$631.21^{Aa} \pm 0.04$
Sucrose	$631.12^{Aa} \pm 0.05$	$251.31^{Cb} \pm 0.08$	$199.94^{Bc} \pm 0.05$	$251.48^{Cb} \pm 0.05$
Sorbitol	$631.19^{Aa} \pm 0.05$	$251.36^{Cc} \pm 0.06$	$199.93^{Bd} \pm 0.04$	$251.59^{Bb} \pm 0.01$
Mannitol	$631.13^{Aa} \pm 0.08$	$251.50^{Bb} \pm 0.06$	$199.64^{Cc} \pm 0.05$	$251.50^{BCb} \pm 0.03$
Sucrose + Sorbitol	$631.17^{Aa} \pm 0.07$	$251.42^{BCb} \pm 0.02$	$100.44^{Dc} \pm 0.04$	$251.52^{BCb} \pm 0.02$
Sucrose + Mannitol	$631.12^{Aa} \pm 0.05$	$251.35^{Cc} \pm 0.02$	$100.41^{Dd} \pm 0.04$	$251.54^{BCb} \pm 0.05$

The σ_{max} value for each sample displayed a similar trend with a decrease in value as salt concentration increased up to 3%, and started to increase again at 4%. Combination of sugars (SS and SM) displayed a significantly lower ($p < 0.05$) σ_{max} value at 3% salt concentration when compared to other treatments.

This suggests that at 3% salt concentration, these samples might produce a softer texture when compared to other fish paste samples. This data agrees with previous findings which were discussed earlier on WHC and Figures 5.2 a–f (discussed below). Low WHC produces a weaker gel structure due to low amount of water present to bind protein networks to form gelation. Low σ_{max} suggests a less rigid structure with lower protein network density (Campo-Deaño and Tovar, 2008).

Table 5.5 also suggests that the control sample displayed no significant difference ($p > 0.05$) in σ_{max} regardless of the salt concentrations. This is a good indication that salt itself without any other substances does not alter the maximum stress limit of a surimi paste. This indicates that the stress max of control sample is not affected by salt concentration and at 1% salt concentration, types of sugar does not have an effect on the stress max. This also suggests that surimi without any sugar does not interact with salt to solubilize protein. Sugar might be needed as a precursor for protein to be solubilized by salt. The same observation can be seen at 1% salt concentration where 1% salt does not cause significant protein interaction even with different type of sugar.

This observation is important as it may suggest that surimi could be processed without sugar. The surimi commercial blend sample, SS, did not display any significant difference ($p > 0.05$) with SM at any salt concentration. This is also a significant finding as it shows that these two samples might produce similar

properties which could lead to the feasibility of using mannitol as a sugar alternative for surimi processing.

Table 5.6 depicts the strain max (γ_{max}) of different sugar formulations at different concentration. All samples displayed the highest value of strain max at 3% salt concentration indicating that the sample was the most flexible at this concentration. However, only two samples (S, SS) showed a significant increase ($p < 0.05$) at 3% salt concentration when compared with other salt concentrations.

Table 5.6: Data extracted from the stress sweep test to determine the strain max of different salt and sugar concentrations. Values are means ± SD of triplicates ($n = 3$). Capital letters indicate significant difference ($p < 0.05$) between columns. Small letters indicate significant difference ($p < 0.05$) between rows.

SAMPLE	Strain Max, γ_{max} (%)			
	1% Salt (w/w)	2% Salt (w/w)	3% Salt (w/w)	4% Salt (w/w)
Control	$7.45^{Ba} \pm 0.59$	$8.53^{Ba} \pm 0.22$	$8.92^{Ba} \pm 1.36$	$8.21^{Ba} \pm 0.90$
Sucrose	$10.52^{Aab} \pm 0.26$	$5.25^{Dc} \pm 0.07$	$11.55^{ABa} \pm 1.30$	$8.71^{Bb} \pm 1.13$
Sorbitol	$7.02^{BCc} \pm 0.09$	$5.04^{Dd} \pm 0.07$	$12.30^{Aa} \pm 0.73$	$11.15^{Ab} \pm 0.10$
Mannitol	$5.27^{Db} \pm 0.57$	$8.92^{Aa} \pm 0.10$	$4.46^{Cb} \pm 0.88$	$8.86^{ABa} \pm 0.73$
Sucrose + Sorbitol	$6.04^{CDc} \pm 0.41$	$6.62^{Cc} \pm 0.07$	$12.35^{Aa} \pm 1.12$	$9.47^{ABb} \pm 0.44$
Sucrose + Mannitol	$5.24^{Db} \pm 0.45$	$4.34^{Eb} \pm 0.11$	$10.92^{ABa} \pm 0.75$	$9.94^{ABa} \pm 1.24$

γ_{max} represents the amount of strain a sample can handle before it changes form. From data presented in Table 5.6, the highest strain max for each

sample was observed at 3% salt concentration. A higher value of strain represents a much more flexible structure. The commercial blend; SS, recorded the highest significant value of max strain at 3% salt concentration signifying that this sample has the highest tolerance on strain and flexibility.

Table 5.7 shows the phase angle max (δ_{max}) which is the ratio of energy loss to energy stored. This is also known as tangent loss. A higher phase angle number will represent a much more viscous-like structure (non-elastic) and *vice versa*.

Table 5.7: Data extracted from the stress sweep test to determine the phase angle max of different salt and sugar concentrations. Values are means ± SD of triplicates (n = 3). Capital letters indicate significant difference ($p < 0.05$) between columns. Small letters indicate significant difference ($p < 0.05$) between rows.

SAMPLE	Phase Angle Max, δ_{max} (^{o})			
	1% Salt (w/w)	**2% Salt (w/w)**	**3% Salt (w/w)**	**4% Salt (w/w)**
Control	$12.70^{Aab} \pm 0.09$	$12.21^{Cb} \pm 0.12$	$12.95^{Da} \pm 0.48$	$13.18^{Da} \pm 0.20$
Sucrose	$11.81^{ABc} \pm 0.27$	$12.64^{Cb} \pm 0.08$	$19.85^{BCa} \pm 0.13$	$19.90^{Aa} \pm 0.51$
Sorbitol	$11.57^{ABCc} \pm 0.57$	$12.67^{Cc} \pm 0.06$	$24.02^{Aa} \pm 1.91$	$18.44^{Bb} \pm 0.14$
Mannitol	$10.56^{CDc} \pm 0.50$	$14.90^{Ab} \pm 0.17$	$14.09^{Db} \pm 0.25$	$16.69^{Ca} \pm 0.39$
Sucrose + Sorbitol	$10.72^{BCDd} \pm 0.50$	$14.43^{Bc} \pm 0.16$	$21.81^{ABb} \pm 2.11$	$17.71^{BCa} \pm 0.53$
Sucrose + Mannitol	$10.40^{Dc} \pm 0.37$	$12.52^{Cb} \pm 0.30$	$18.28^{Ca} \pm 0.22$	$17.99^{Ba} \pm 0.54$

From the Table, SO3 recorded the highest value of δ_{max} when compared to other treatments. This suggests that SO3 was the weakest and most fluid-like.

It also indicates the formation of a weak gel. 3% salt concentration displayed the highest value of δ_{max} for most samples. This again supports the data obtained by previous tests that 3% salt concentration will cause a decrease in rigidity and structural integrity which is predicted to produce the weakest gel.

Frequency sweep or mechanical spectra is usually done to understand the stability and structure of a material during processing. Internal chemical reactions are prone to occur during a long period of storage; thus, this test also gives a crude indication of the stability or shelf life of material during long storage. Sample is subjected to different frequencies and if a material is frequency-dependent, the sample is categorised as less stable (Binsi *et al.,* 2009; Dileep *et al.,* 2005). Table 5.8 depicts the values of G'_o, G''_o, n' and n'' for each sample using the power law equation (3.3 and 3.4).

From the results, it is apparent that as salt concentration increased, G'_o decreased until 3% salt concentration. These data are also consistent with the results presented in the previous stress sweep test (Figure 5.1) and WHC (Table 5.4). The same trend can be observed where G'_o increased again at 3% salt concentration. From the results, 3% salt concentration is predicted to yield the softest and weakest gel texture compared to the other samples. The same inference was made when samples were subjected to stress sweep test described earlier. It is also found that the G'_o values were always higher than G''_o indicating that the samples were more elastic in nature instead of viscous. This result also suggests that 1% salt concentration and 4% salt concentration would yield high gel strength due to the high value of G'_o and G''_o. However,

high values of G'_o and G''_o does not affect the stability of the samples. It can just indicate a denser protein network creating a more strong and rigid gel.

Table 5.8: Effects of different salt and sugar concentrations on the frequency sweep parameter.

SAMPLE		G'_o (Pa)	G''_o (Pa)	n'	n''	n'-n''
Control	1% salt	12460.0	2230.0	0.136	0.081	0.055
	2% salt	6324.3	1093.5	0.119	0.057	0.063
	3% salt	7516.3	1359.7	0.127	0.056	0.071
	4% salt	9223.2	1707.9	0.135	0.068	0.067
Sucrose	1% salt	7758.5	1459.8	0.107	0.050	0.057
	2% salt	5047.0	1053	0.121	0.025	0.096
	3% salt	2630.6	645.9	0.173	0.096	0.077
	4% salt	2745.8	749.76	0.136	0.121	0.015
Sorbitol	1% salt	9422.9	1032.6	0.109	0.056	0.053
	2% salt	4582.8	805.6	0.130	0.024	0.107
	3% salt	2767.8	494.22	0.141	0.132	0.008
	4% salt	2776.0	584.69	0.166	0.137	0.029
Mannitol	1% salt	14712.0	2692.6	0.108	0.061	0.047
	2% salt	2713.8	570.92	0.126	0.067	0.059
	3% salt	6347.3	803.86	0.119	0116	0.002
	4% salt	3364.7	660.86	0.145	0.104	0.041
Sucrose + Sorbitol	1% salt	10456.0	1803.2	0.124	0.091	0.032
	2% salt	4857.5	1318.8	0.122	0.006	0.116
	3% salt	1432.8	338.3	0.155	0.128	0.027
	4% salt	3096.7	626.57	0.149	0.111	0.038
Sucrose + Mannitol	1% salt	13444.0	1974.1	0.112	0.082	0.030
	2% salt	5300.5	1182.4	0.122	0.033	0.089
	3% salt	1401.5	328.86	0.179	0.152	0.027
	4% salt	3431.0	948.65	0.155	0.128	0.027

The n’ value obtained can be related to the stability of the sample. A sample with a higher n’ value signifies lower stability over time due to its dependency on frequency. The more frequency-dependent the sample is, the more fluid-like rather than elastic behaviour it displays (Campo-Deaño *et al.,* 2009B). Samples at 4% salt concentration were found to be the most dependent on frequency when compared with other sample with lesser salt concentrations. This indicates instability of sample at 4% salt concentration. However, sample added with sucrose, SS and SM did not follow this trend. These samples displayed the lowest stability at 3% salt concentration. This suggests that addition of sucrose might affect the stability of surimi when added with salt. Majority of the samples indicated the best stability of surimi at 1% salt concentration. This information greatly increases the possibility of producing a healthier surimi with lower salt concentration.

Another indication of a strong gel networking is the difference between n’ and n’’. A wider gap between n’ and n’’ will represent a stronger gel networking (Campo-Deaño *et al.,* 2010). A stable surimi does not necessary possess a good gel network. SS2 was found to possess the strongest gel network based on the frequency sweep test results and power law equations. This might be the reason why SS2 is widely used around the world. The power law equation based on the frequency sweep test by Campo and Tovar (2008) is effective in understanding the rheological properties of a sample and determining its stability.

Temperature sweep test is done to understand the gelation profile or the thermorheological properties of fish paste. Protein denatures due to heat and establishes networks due to protein-protein interaction and other chemical interactions when heat is applied. The protein behaviour at increased temperature was investigated to determine the gelation point of sample which is commonly used by the industry to process surimi fish paste into various seafood analogues. Other than that, the rheological behaviour of the surimi paste was observed as temperature increased at 1°C per min from low temperature to high temperature. Figures 5.2 a–f shows the temperature sweep of each sample as affected by the salt concentrations from 10°C to 90°C.

Based on the Figure 5.2 a–f, a consistent decrease in the G' value was found with addition of salt until 3% concentration for all samples. This result is consistent with the G' value obtained from the stress sweep test and frequency sweep test performed earlier. A reduced G' value is an indication of a lesser viscosity surimi paste due to a higher degree of protein solubilisation (Carvajal, Lanier and MacDonald, 2005; Lanier, Carvajal and Yongsawatdigul, 2005; Esturk, 2003). Higher salt concentration will improve protein solubilisation thus explaining the result obtained up to 3% salt concentration. A lower G' value might also indicate a softer and less rigid gel. This observation has been discussed earlier when sample was subjected to stress sweep test and frequency sweep test.

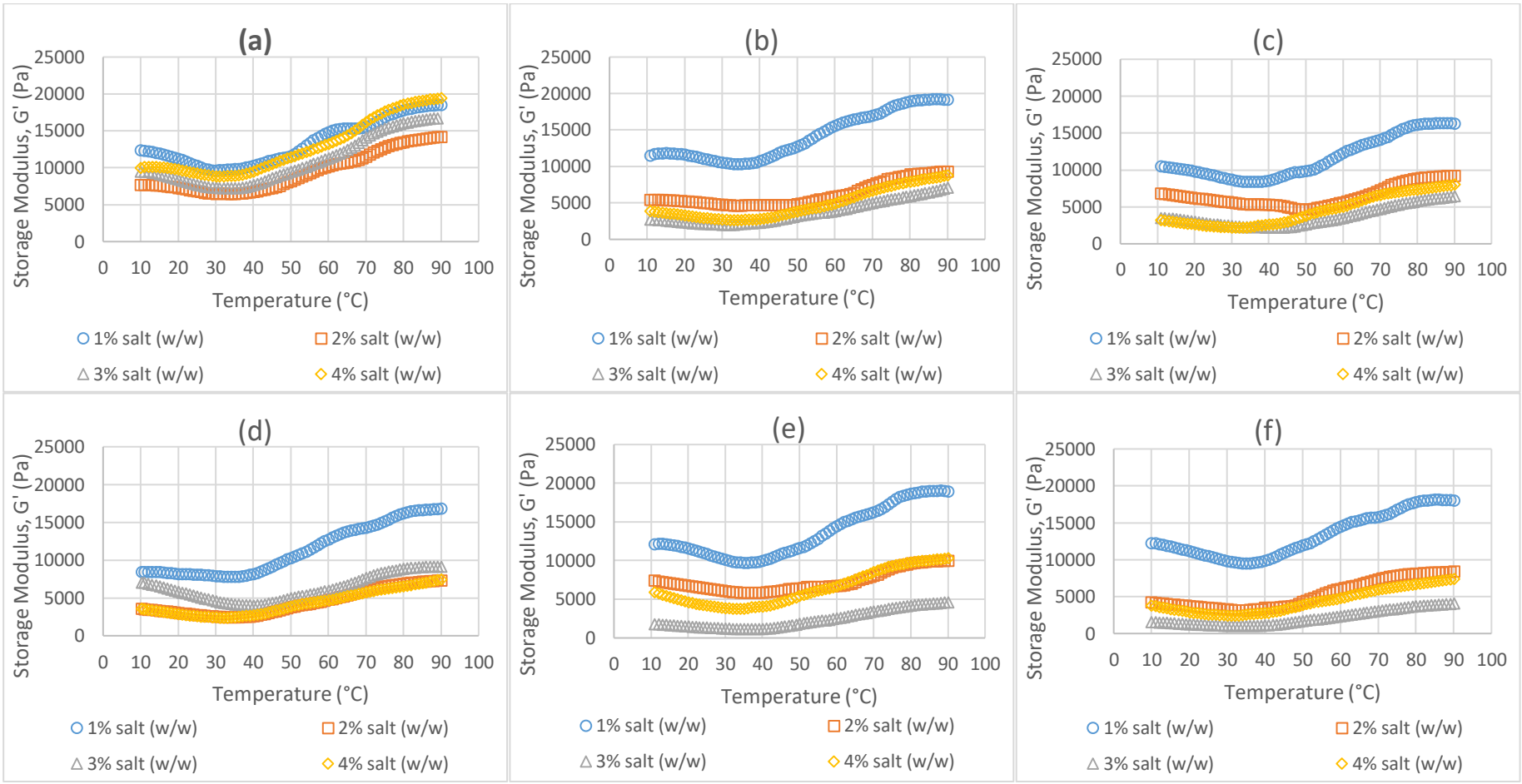


Figure 5.2: Storage modulus (G') for (a) control, (b) sucrose, (c) sorbitol, (d) mannitol, (e) sucrose + sorbitol and (f) sucrose + mannitol at different salt concentration when subjected to temperature increase from 10°C to 90°C.

The result obtained in Figure 5.2 showed the same four-stage gelation (softening, first heat gelation, gel weakening and second heat gelation) as discussed in Chapter 4. but the peaks here are not as distinctive. The peaks also are not as significant as displayed in Chapter 3. The changes are subtle and small but they can still be identified. This signifies that addition of salt did not change the gelation behaviour or thermorheological properties.

Lower salt concentration showed a more distinct curve which was due to a lower percentage of solubilised protein. The same finding was reported by Cando *et al.* (2016) who explained that higher salt concentration caused protein to solubilise more thus exposing a wider area of myosin to form networks. By exposing wider area of myosin, lesser energy is needed to break and form chemical bonds which eventually increases gel protein network. As the salt concentration increased, the gelation point shifted to a lower temperature (Table 5.9). This might due to the fact that addition of salt has been reported to decrease the enthalpy of heat denaturation which allows protein denaturation to occur at a lower temperature (Choi *et al.,* 2008; Park, 2006). Salt has the ability to reduce the heat stability of protein thus allowing gelation to take place at lower temperature (Esturk, 2003; Lanier, 1986).

Commonly, 2% to 3% salt is sufficient to solubilise protein and obtain the desired protein functionality. However, to achieve the desired gelation properties, the protein needs to be partially and not fully solubilised (Niwa, 1992). If it is fully solubilised, it will cause a salting effect where protein aggregates together rather than forming a gel protein network. Tahergorabi

and Jaczynski (2012) also reported that salt concentration influences the onset of myosin temperature and causes a larger myosin peak (transition enthalpy). Furthermore, salt addition has also been reported to cause a shift in the temperature for the protein aggregation phase (Choi *et al.*, 2008). However, the decrease in gelation point in the present work was minimal (±1.5°C). The samples did not display a vast difference suggesting that overnight storage might have not shown any noticeable effects on the gelation point. All samples displayed a similar trend with the G' curve except for control and mannitol sample (Figures 5.2a and 5.2d). The G' curve for these two samples are a bit different as salt increased. G' value for control sample was found to be the lowest at 2% salt and continued increasing to 4% salt concentration. This suggests that without sugar, 2% salt was able to solubilise protein much more effectively than 3% and 4% causing the texture to be less viscous.

From Figure 5.2a, prediction could be made that the gel strength of the control sample will be ranked as 4% salt, 3% salt, 1% salt and 2% salt from highest to lowest strength. Mannitol however displayed a similar G' curve at 3% and 4% indicating that the thermorheological characteristics were similar with 1% salt difference (Figure 5.2d). As salt concentration increased, the initial G' decreased indicating that the paste became less elastic and more viscous. Again, this is due to the solubilisation of protein caused by salt. However, the final gelation G' value depicts a different outcome. At 90°C, all sample displayed higher G' value which indicates an elastic behavior of gel. This further supported by results shown by tan delta in Table 5.9. Tan delta for all the samples decreased with the increase in temperature indicating that the

sample has turned from paste to elastic gel form. Table 5.9 records the initial G', transition G' transition temperature, tan delta initial and tan delta final for all samples at different salt concentration.

Table 5.9: Effect of sugar formulation and salt concentration on the gelation temperature.

Sample		Initial G' (Pa)	Transition G' (Pa)	Transition Temperature (°C)	Tan Delta Initial	Tan Delta Final
Control	**1% Salt**	12382.4	9624.3	30.146	0.199	0.093
	2% Salt	7702.0	6488.3	30.146	0.176	0.091
	3% Salt	9467.7	7239.6	33.157	0.183	0.098
	4% Salt	10017.9	8823.0	30.146	0.205	0.098
Sucrose	**1% Salt**	11522.5	10312.1	34.141	0.184	0.095
	2% Salt	5428.6	4623.1	34.141	0.206	0.087
	3% Salt	2730.7	2005.5	33.130	0.271	0.118
	4% Salt	3862.1	2633.8	32.146	0.251	0.102
Sorbitol	**1% Salt**	10486.4	8405.6	35.136	0.192	0.096
	2% Salt	6855.8	5339.9	35.136	0.205	0.087
	3% Salt	3501.5	2243.6	35.136	0.285	0.096
	4% Salt	3214.5	2206.7	33.125	0.275	0.100
Mannitol	**1% Salt**	8477.2	7795.3	34.128	0.197	0.092
	2% Salt	7057.7	4051.2	40.140	0.245	0.095
	3% Salt	3584.6	2432.8	34.128	0.261	0.102
	4% Salt	3576.4	2401.4	33.160	0.246	0.110
Sucrose + Sorbitol	**1% Salt**	12108.8	9739.7	36.140	0.174	0.094
	2% Salt	7420.4	5854.4	35.132	0.216	0.089
	3% Salt	1794.8	1170.8	35.132	0.294	0.097
	4% Salt	5934.8	3773.5	34.127	0.241	0.099
Sucrose + Mannitol	**1% Salt**	12281.8	9509.1	35.127	0.186	0.092
	2% Salt	4226.3	3172.1	35.127	0.228	0.091
	3% Salt	1546.3	1035.5	34.135	0.303	0.096
	4% Salt	3823.0	2439.9	32.162	0.259	0.104

5.3.4 Texture analysis

Texture analysis is done to determine the effects of different salt concentrations and sugar combinations on the texture of surimi. It is also important as texture depicts the quality of the surimi. Other than that, texture analysis is done to complement the data obtained by rheological tests on fish paste. If these two data complement each other, rheological tests could be done while the surimi is in paste form to predict its gel quality when it is processed to a gel form. Breaking strength, breaking deformation and gel strength of all samples were determined in texture analysis.

Figure 5.3 represents the breaking strength of all samples subjected to different salt concentrations. It is found that the breaking strength of all samples changes as salt concentration increased. From the Figure, it is apparent that the combination of sugar with 4% salt concentration enabled the surimi gel to increase its breaking strength hence allowing it to have a much more rigid and stronger gelling network. This is especially true for sample MA4 which showed a significantly high breaking strength value ($p < 0.05$). This might indicate that the mannitol reacted with the charged sodium and chloride ions to further increase ionic bonds thus increasing the breaking strength of the gel and producing a much more compact and denser protein network compared to the effects of other sugars. Most samples showed an increase in breaking strength up to 2% salt concentration but a constant or decrease at 3% salt concentration. This might be the reason why surimi manufacturers prefer to add only 2% salt concentration in surimi. A salting out effect might occur at 3% thus decreasing the breaking strength. SO1 displayed the lowest

value of breaking strength which is about 30% lesser than MA4. Sorbitol is known to produce softer structure and gel networking.

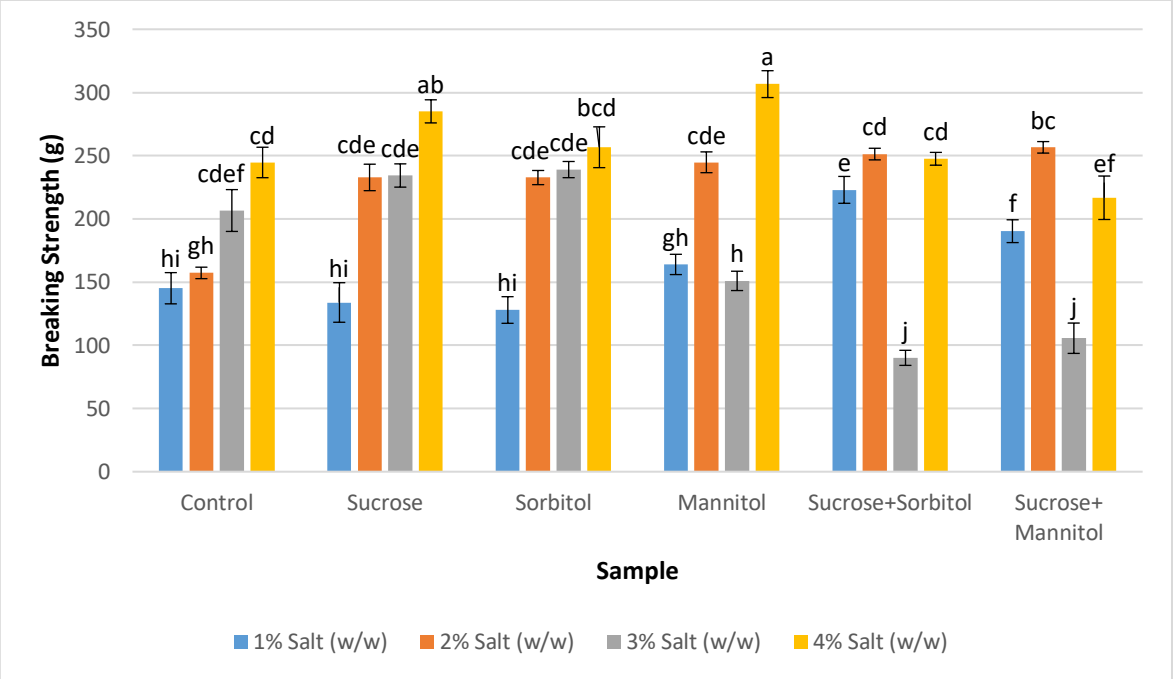


Figure 5.3: Breaking strength of samples with different salt concentrations. *Different letters represent significant difference of sugar with different salt concentrations ($p < 0.05$).*

Figure 5.4 shows the breaking deformation value (penetration depth) of each sample with different salt concentrations. This data represents the structure flexibility of the samples. Data obtained shows the distance of penetration before the sample started to deform and irreversibly changed. From the Figure, the highest resistance of penetration was shown by CO4 whilst the lowest value of deformation was presented by sample CO2. Both these data are significantly different ($p < 0.05$). However, SS and SM did not show any significant difference with addition of salt up to 3% ($p > 0.05$). Only 4% salt displayed a significant difference in deformation for both these sugar formulations.

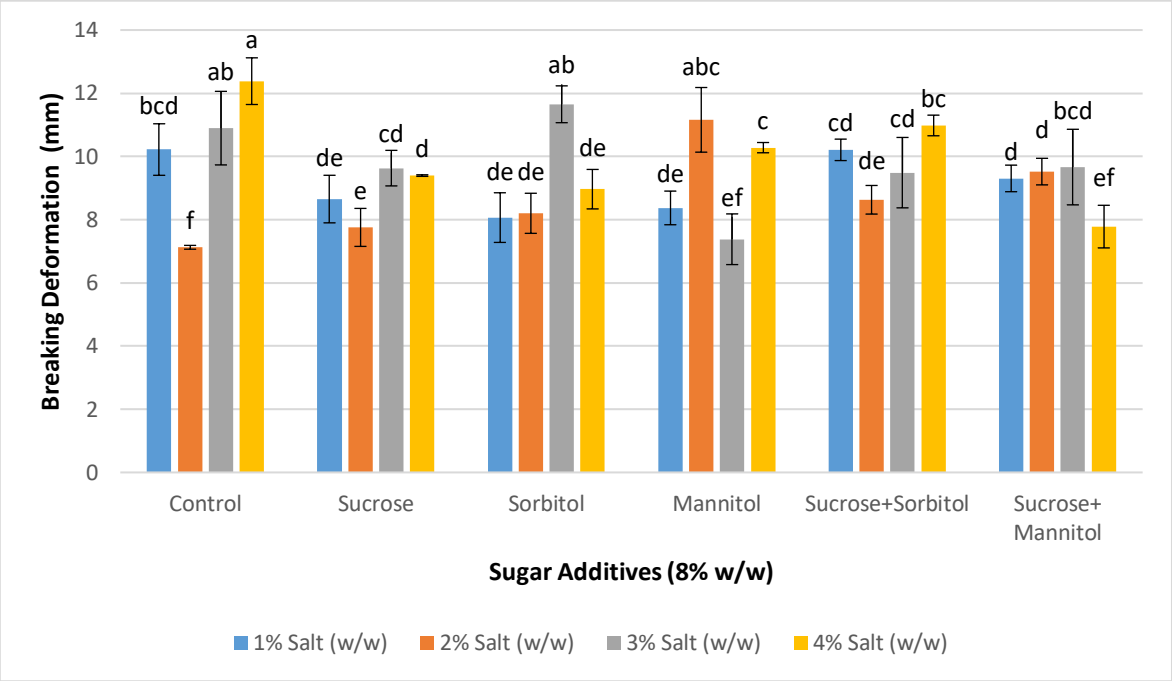


Figure 5.4: Breaking deformation of samples with different salt concentrations. *Different letters represent significant difference of sugar with different salt concentrations ($p < 0.05$).*

Figure 5.5 depicts that all the samples showed a significant difference with an increase in salt concentration ($p < 0.05$). However, there is no observable trend which relates the salt concentration and the gel strength with different formulations. Control, sucrose, sorbitol and mannitol samples showed a significant increase in gel strength with an increase in salt concentration ($p < 0.05$). Combination of sugar (SS and SM), however, showed a significant decrease in gel strength at 3% salt concentration and increased again at 4% salt concentration. This result is consistent with the rheological data discussed previously. MA4 displayed the highest gel strength value whilst SS3 showed the lowest value. Again, this might be the reason why commercial blend uses SS with 2% salt concentration in their surimi formulation since an increase in 3% salt concentration will deteriorate the gel structure when added with SS.

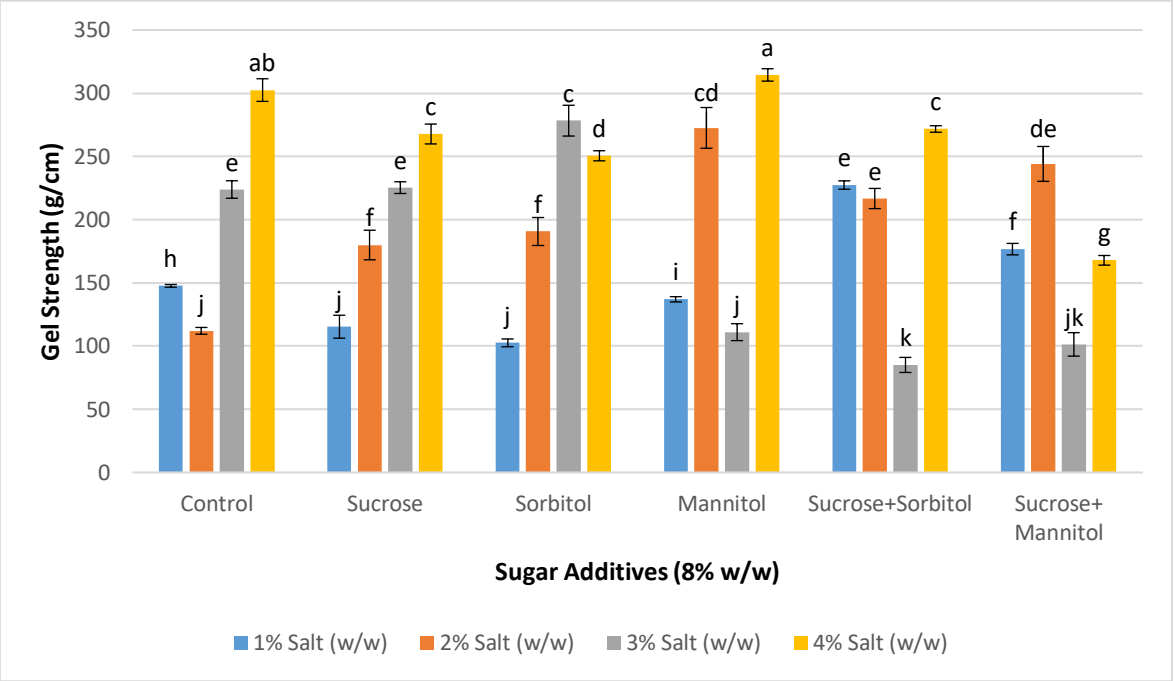


Figure 5.5: Gel strength of samples with different salt concentrations. *Different letters represent significant difference of sugar with different salt concentrations ($p < 0.05$).*

The aim of this study was to reduce the amount of salt concentration on surimi whilst maintaining its gel strength quality. From Figure 5.5, it is found that this is not feasible. The highest gel strength is recorded by using mannitol with addition of 4% salt concentration. However, if we compare the type of sugar formulation used, SM shows promising result as it yielded the highest gel strength at 2% salt concentration. This value is still higher than the commonly used SS sugar at 2% salt concentration. Mannitol at 2% salt concentration also yields higher gelling strength than SS2. This indicates that mannitol could be used as a sugar substitute for sucrose and sorbitol in the seafood industry.

Theoretically, an increase in salt concentration will produce a better gel strength for surimi gel (Park, 2006). Usually, a higher value of breaking force, breaking deformation and gel strength are translated into better gel product

quality. Sow and Yang (2015) reported that an addition of 1.5% salt caused a reduction in the gel strength of fish gelatine. However, there is a contradiction when comparing Atlantic menhaden (*Brevoortia tyrannus*) gel with and without salt; gel with salt showed a little increase in gelling strength when compared with without salt (Pérez-Mateos and Lanier, 2006). There are several factors reported which cause this decrease in gel strength. Most researchers relate this trend due to the ionic strength of sodium chloride and its reaction towards internal bond of protein (Sow and Yang, 2015; Haug *et al*., 2004; Choi and Regenstein, 2000; Sarabia *et al*., 2000). Sodium chloride has the ability to break the hydrogen bond which causes some disturbance towards the formation of hydrophobic bond (Choi and Regenstein, 2000). These phenomena then lead to the disruption of gelling network between proteins thus halting the formation of strong gel (Sarabia *et al*., 2000). Many reports and theories have been linked to gelling characteristics of fish protein. However, the definite mechanism affecting the gel formation within the fish protein is still vague and undetermined (Sánchez-González *et al*., 2008).

Choi *et al.* (2008) discovered that at 3% salt concentration, the concentration of protein network was more reduced compared to lower salt concentration at frozen storage. Higher amount of salt concentration causes higher number of protein solubility thus affecting the protein network formed. The more protein solubilized the weaker the protein network bond. Choi *et al.* (2008) also reported that NaCl at higher 3% causes inhibition to proteases which disrupts gel forming ability. At frozen storage, protein denaturation occurs thus disrupting the protein network formed explaining that at certain salt

concentration, the protein networks were reduced. As salt concentration increased, the shear force increased up to 2% (w/w) salt concentration added. Addition of salt above this point caused the shear force for fish gel to decrease. Hardness gradually decreased with increase in salt content. Gumminess and chewiness also decreased with increase in salt content (Tahergorabi *et al*., 2012).

Protein solubility is usually related to the myofibrillar proteins present. These salt-soluble proteins contain hydrophilic and hydrophobic bonds which affect the protein surface (Carvajal, Lanier and Yongsawatdigul, 2005). This characteristic is important for surimi gelation. Salt will further increase protein solubility by solubilising myofibrillar protein to produce gel network. Salt added during comminution or blending causes protein denaturation and reduces protein stability. When protein unfolds, the amino acids are exposed thus increasing the negative charge of the protein. These negative charge anions will create a bond with water present within the protein and increase the water binding capacity of myofibrillar proteins (Wu *et al*., 2006; Esturk 2003). Chloride ions that are released will neutralise free positive charge on protein molecules selectively (Esturk, 2003). This will consequently increase protein solubility.

Initially, the hypothesis is to relate the rheological result with the texture analysis as presented in the previous Chapter. However, when results are compared, it seems that the rheological results cannot be entirely relied upon to predict the textural properties for different salt concentrations. Even though

the rheological result supported each other; rheological data are not consistent with the data presented in texture analysis. Salt alters too many characteristics of the myofibrillar protein especially the viscoelastic properties during the paste form and gelling properties during the gel form. Therefore, no correlation or model can be made between rheological data during the fish paste and the texture analysis of the surimi gel. Formulation of different types of sugars also resulted in different value of breaking strength, breaking deformation and gel strength with no consistency. Thus, another key factor in producing a healthier low salt concentration surimi is to use a suitable sugar formulation.

5.4 EFFECTS OF FROZEN STORAGE ON TEXTURE OF FISH GEL

Samples with different salt concentrations and sugar combinations were stored in frozen temperature (-18°C) up to six months. These samples were tested for their texture and gel quality every two months. Breaking force, breaking deformation and gel strength of samples were determined, calculated and recorded every two months. This was done to understand the capability of different salt concentrations and sugar formulations in maintaining and increasing the shelf life of surimi. The highest gel strength value will indicate the best combination of sugar and salt concentration for preservation. Figures 5.6a–f depict the breaking strength of fish gel in frozen storage for a period of six months.

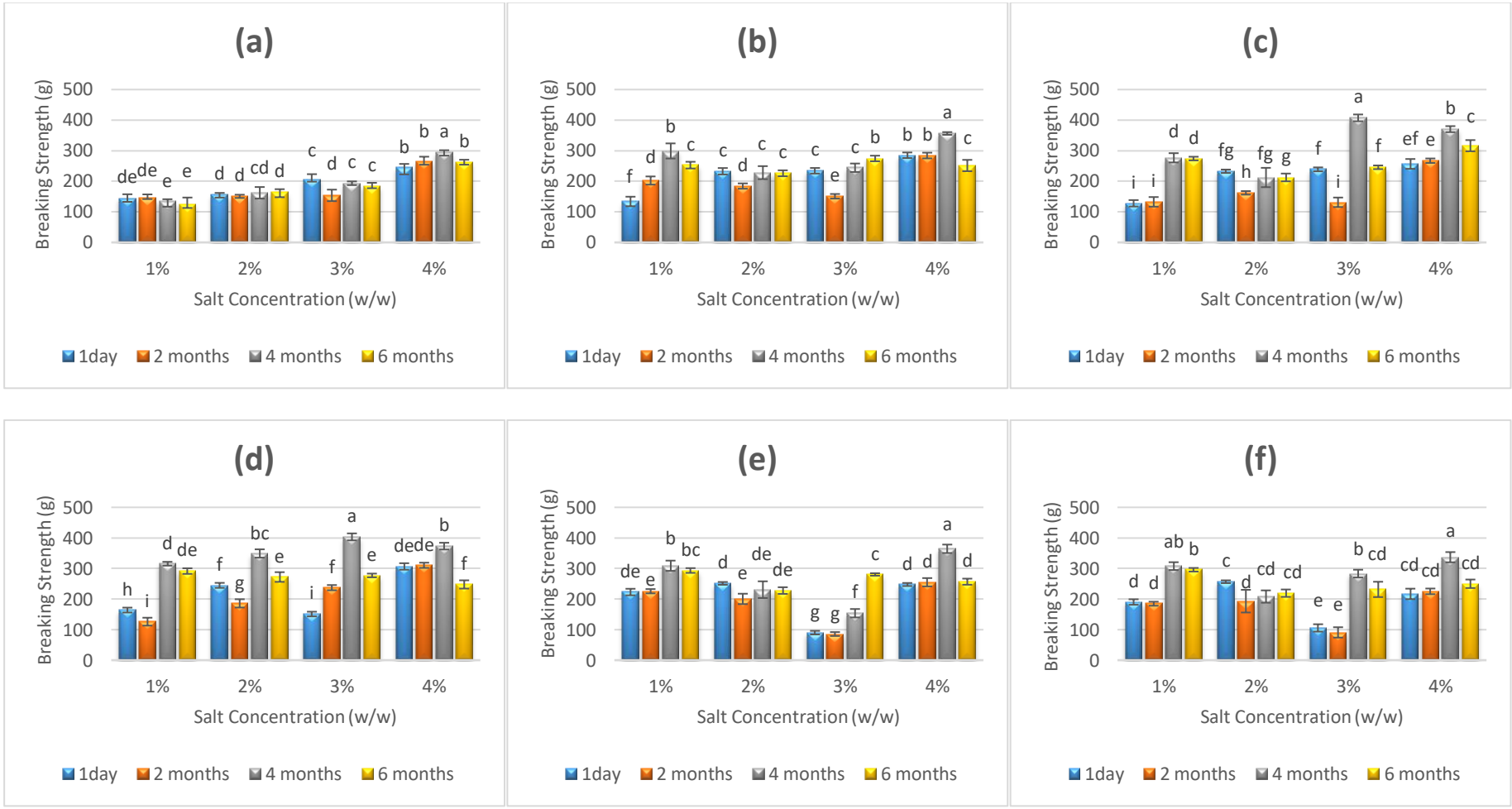


Figure 5.6: Effects of frozen storage on the breaking strength of (a) control, (b) sucrose, (c) sorbitol, (d) mannitol, (e) sucrose + sorbitol and (f) sucrose + mannitol at different salt concentrations. *Different letters represent significant difference of samples at different storage period ($p < 0.05$).*

From the Figure, control samples showed promising result in which salt as a preservative during frozen storage might be viable. Control sample did not display a significant difference in breaking strength after 6-month storage ($p > 0.05$). However, control samples still displayed the lowest breaking strength value despite their shelf life stability.

The least stable samples were sample added with sucrose and also sample added with mannitol at 4% salt concentration (Figures 5.6b and 5.6d). Even though these two samples possessed high breaking strength, the breaking strength showed a significant reduction after four months of frozen storage ($p < 0.05$). The gradual increase in breaking strength up to four months are usually associated with ice crystal growth and protein toughening at frozen temperature (Solo-de-Zaldívar *et al.,* 2014B). Figures 5.6e and 5.6f show that SS1 and SM1 displayed the best stability with high value of breaking strength up to six months without any significant difference ($p > 0.05$). Breaking strength represents the stress needed for the sample to deform thus describing the rigidity of each formulation when stress is applied. This characteristic indicates that prolonged frozen storage causes a deterioration within the chemical structure which changes the rigidity of sample. This is an indicator for food engineers to determine the processing parameters and life span of product to produce high quality surimi products.

As discussed previously, penetration depth represents the ability of the sample to withstand strain before it changes form. Figures 5.7a–f display the breaking deformation value of samples over a period of 6-month frozen storage.

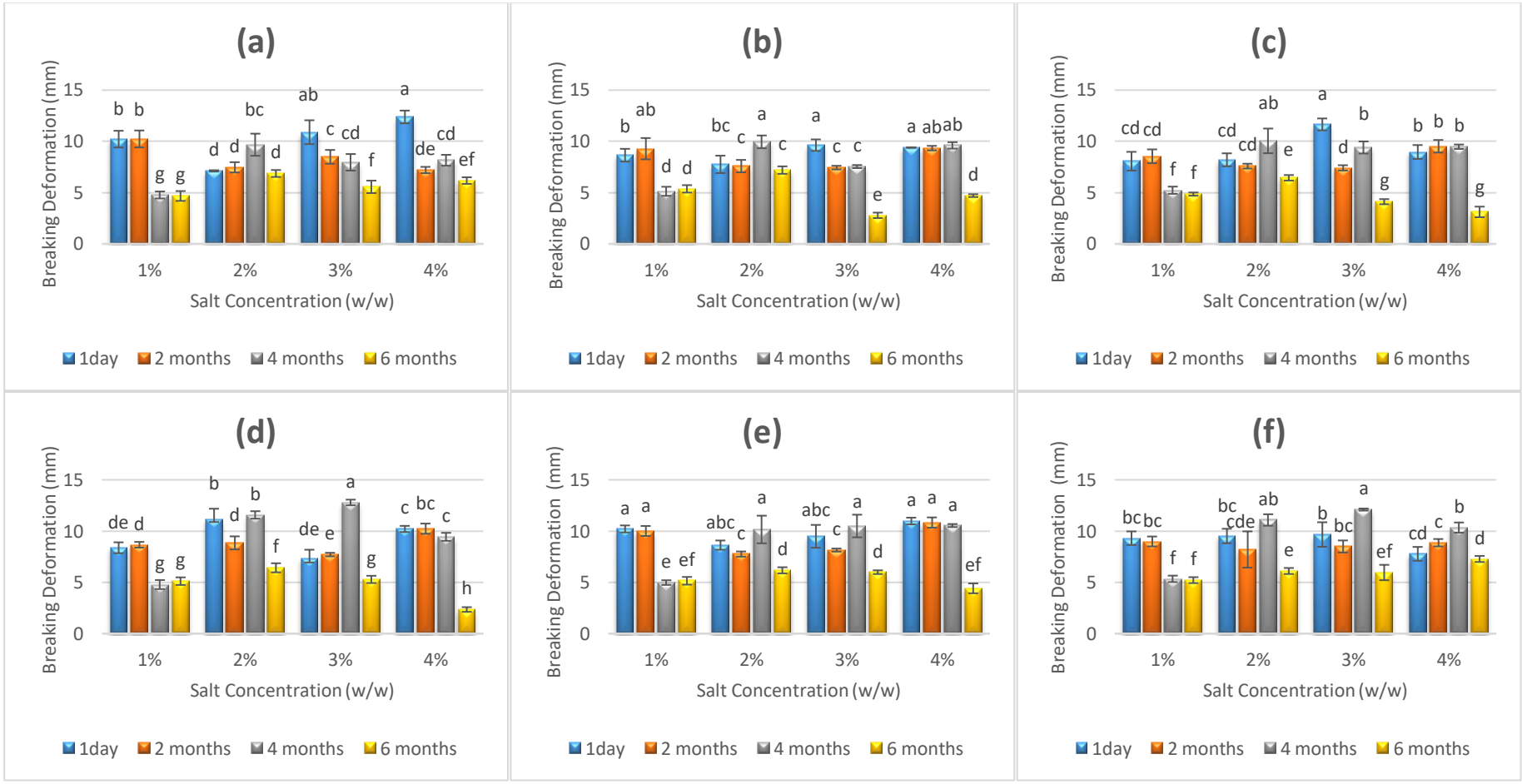


Figure 5.7: Effects of frozen storage on the breaking deformation of (a) control, (b) sucrose, (c) sorbitol, (d) mannitol, (e) sucrose+ sorbitol and (f) sucrose + mannitol at different salt concentrations. *Different letters represent significant difference of samples at different storage period ($p < 0.05$).*

From Figure 5.7, addition of 1% and 2% salt concentration could maintain the deformation of all samples up to two months without any significant difference ($p > 0.05$). However, 1% salt concentration showed a distinctive trend. After two months of frozen storage, the breaking deformation for samples with 1% salt concentration decreased and started to maintain up to six months without any significance difference ($p > 0.05$). The same trend could be observed for all samples. This suggests that 1% salt was consistent in preserving breaking deformation of all samples for a period of six month. Nevertheless, the value after six months storage was low. Control sample showed the least stable during frozen storage (Figure 5.7a) in which it showed a decrease in breaking deformation after only two months of frozen storage. Another factor why it was unstable is due to the absence of additives which prevents protein denaturation during frozen storage.

SM4 showed the highest stability of deformation as the breaking deformation value shown in Figure 5.7f for the first day and after six months did not show any significant difference ($p > 0.05$). Furthermore, when SM4 is compared to other samples at 4% salt concentration, all samples displayed a significant decrease in breaking deformation (up to 50% decrease) after 6-month storage. The decrease in breaking deformation was significant which indicates that 4% salt concentration is not suitable as a preservative. ($p < 0.05$). This shows that although higher salt concentration increases the value of breaking deformation, it does not have the ability to prolong the shelf life storage of surimi.

Another important textural property that was evaluated throughout six months frozen storage is the gelling strength. Gel strength is the prime quality indicator for surimi. Figures 5.8a–f represent the gel strength of samples with different formulations and salt concentrations after 6-month frozen storage. Reduction in gel strength is an indicator of protein deterioration and structural breakdown due to formation of ice crystal, hydration, and protein aggregation.

Most samples recorded the highest gelling strength value at four months frozen storage. Some showed a significant increase such as SO3, MA3, SM3 and SM4 (Figures 5.8c, e, f). Same result has been reported by Solo-de-Zaldívar *et al.* (2014B) where textural properties started to increase in the third to fourth month of storage. Increase values of gel strength could be caused by ice crystal growth and migration of water to produce large ice crystals. This causes re-aggregation of protein and various bonds that might strengthen the gel network due to re-arrangement of molecules (Solo-de-Zaldívar *et al.,* 2014B). Another inference is that the salt soluble protein decreases with increase of storage especially with sample with higher salt concentration. Greiff *et al.* (2015) reported that the myosin heavy chain protein was found to have the best quality during storage with 3% salt concentration. Cando *et al.* (2016) also found that some additives caused a change in protein network and amplified gel networking depending on types of additives as in this case different types of sugars might play a role.

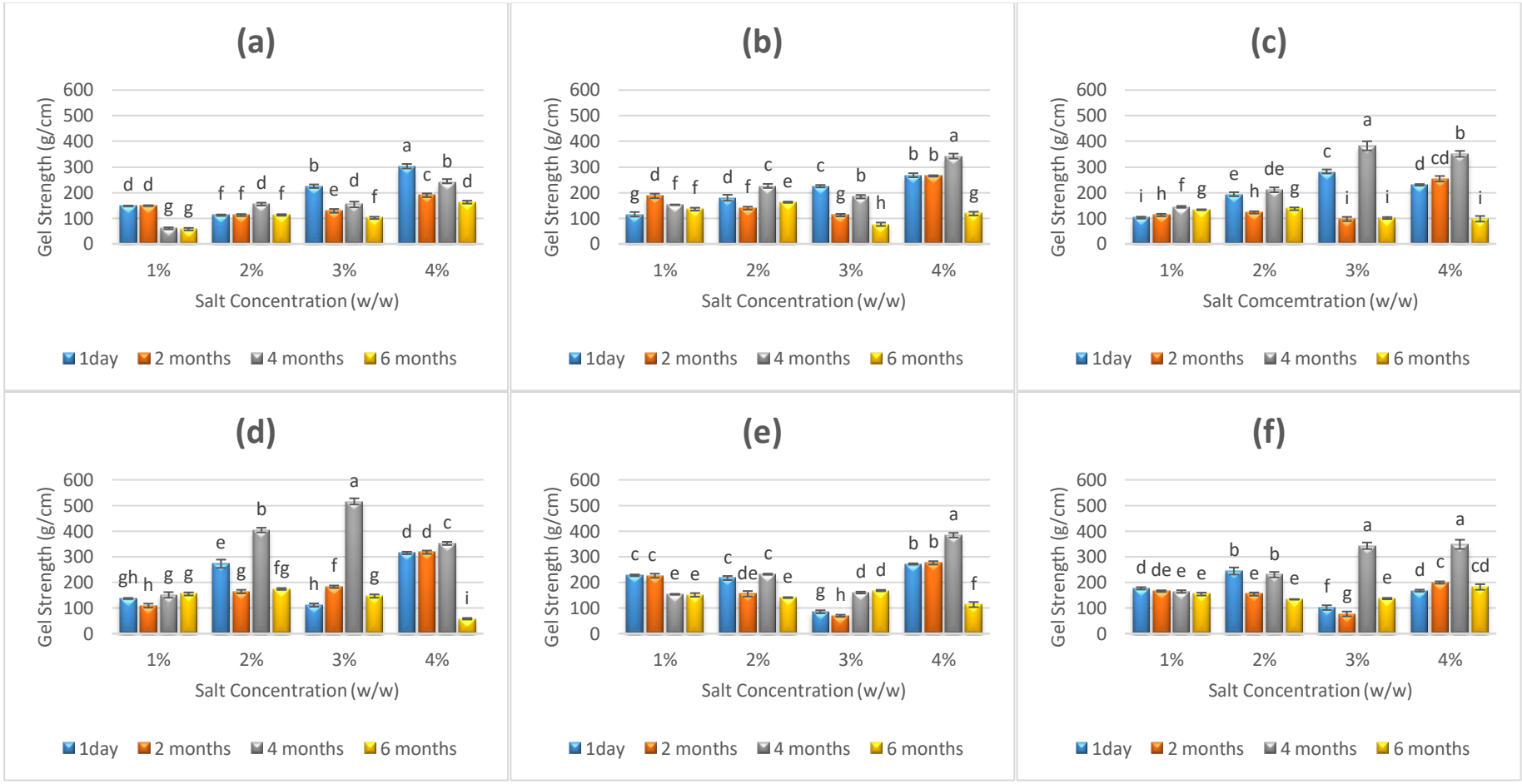


Figure 5.8: Effects of frozen storage on the gel strength of (a) control, (b) sucrose, (c) sorbitol, (d) mannitol, (e) sucrose + sorbitol and (f) sucrose + mannitol at different salt concentrations. *Different letters represent significant difference of samples at different storage period ($p < 0.05$)*.

Salt has the ability to inhibit proteases which reduces the protein denaturation due to enzymatic reaction. The lesser the myosin is solubilised, the lower the protein unfolding rate is. Some ingredients react with salt to cause myosin solubilisation and protein unfolding (Cando *et al.,* 2016), whilst some additives have negative effect on the surimi. Figure 5.8d shows that MA3 possessed the least frozen stability when the gel strength reduced significantly up to 83% followed by MA4 with 70% reduction from the fourth month to the sixth month of frozen storage ($p < 0.05$). Although MA3 in the fourth month showed the highest gel strength when compared to all samples, its instability after four months was the most significant when compared to the others. These results suggest that mannitol does not possess good preservation properties.

Figure 5.8 shows that most samples with 1% salt concentration maintained the gelling strength up to 6-month frozen storage. Only CO1 and SM1 showed significant reduction ($p < 0.05$) after 6-month frozen storage (Figures 5.9a and 5.9e). This indicates that 1% salt concentration is sufficient in preserving the quality of surimi up to six months. However, the gel strength was lower than samples with higher concentration. This provides options for food manufacturers whether they prefer a surimi with longer shelf life but lower gel strength or shorter shelf life but higher gel strength. By reducing salt concentration in surimi production, a healthier surimi can be introduced to the surimi industry.

5.5 CONCLUSIONS

Salt has been known to change the protein solubility of surimi and has been debated over its ability to produce better gelling quality. In this research, different concentrations of salt were tested to understand their effect on the rheological and textural properties of surimi paste and gel. Addition of salt from 1% concentration to 3% concentration caused a decrease in G' value which in turn indicated a lower rigidity on the surimi paste structure. This result was consistent throughout all the rheological tests done which were stress sweep test, frequency sweep test and temperature sweep test. This is also a clear indication that as salt concentration increased, more myofibrillar protein was solubilised which compromised the structure and rigidity. Higher salt concentration compromises the gel strength but increases the frozen shelf life stability.

On the other hand, sugars used showed a significant difference in the G' value when compared with the same salt concentration. Mannitol showed promising result and can be considered as an alternative to sucrose and sorbitol. This is because mannitol added to the surimi paste presented similar results with the fish paste added with the commercial blend (SS). By replacing mannitol, a healthier surimi can be achieved as it contains lesser calorie and sweetness compared to sucrose and sorbitol. Other than that, it was found that, salt alone without any addition of cryoprotectant could produce the same texture with surimi added with sugar which indicates that sugar could be eliminated if salt is added to surimi, hence, reducing the calorie and achieving a healthier surimi.

Result obtained from the experiment agrees with other researchers that as salt concentration increased, so did the gel strength increase. However, with the addition of different sugar formulations, the increase of the gel strength varied and some showed significant difference. A healthier surimi with high gel quality is attainable by selecting the best cryoprotectant combination. MA4 presented the highest gel strength whilst the commercial blend SS3 presented the lowest value. From the results, 4% salt concentration displayed the highest gel quality. This outcome is not desirable as higher amount of salt increases health risk. The research objective was to produce a healthier surimi option by reducing the salt concentration added. Therefore, future research and a different approach on producing low salt surimi should be done in the future.

Although 4% salt concentration displayed the highest value of gel quality, shelf life study shows that the gel and protein networking was the least stable when compared to other salt concentrations. 1% salt concentration was found to yield the most stable samples which suggests that this concentration is the best preservation formulation. Enlightened by this shelf life study, food manufacturers now have the option to choose the best sugar and salt concentration to produce a healthier and improve the surimi quality.

Even though the rheological result supported each other, rheological data are not consistent with textural data. G' value decreased when salt was added due to the solubilisation of salt in the fish paste. However, this step is important to produce a much stronger gel. Another reason is the salting out effect and the competition for water. Different sugars possess different water holding capacity

thus affecting the amount of free water present. Salt and protein compete for water in order to produce gel networks. Therefore, no correlation or prediction model can be designed from the rheological and textural data.

CHAPTER 6

Effects of Sago Starch on the Rheological and Textural Properties of Surimi

6.1 INTRODUCTION

Starch is known for its ability to act as filler in the surimi industry which promotes better gelling, firmness and higher elasticity (Elgadir *et al*., 2012). When it is combined with myofibrillar protein gel network, swelling of starch granules within the system acts as a support to increase surimi-starch gel strength. Starch added will alter the texture of surimi and also increase the freeze-thaw stability of the surimi (Kim, Yoon and Park, 2005; Yang, 1998). Starch granules are made of amylose and amylopectin which influence the ability of starch granules to expand or swell (Fu *et al.,* 2005; Ahmad *et al.,* 1999). Amylose is a linear polysaccharide chain whereas amylopectin is a branched polysaccharide chain (Park, 2005). Higher amount of amylose is usually associated with higher gel quality (Ahmad *et al.,* 1999).

Granule swelling occurs by absorbing water within the system which causes expansion of starch granules. This expansion will push outwards and further support the myofibrillar gel network. Formation of these elastic globules will cause protein matrix density to increase, thus fortifying and increasing gel strength (Zhang *et al.,* 2013; Elgadir *et al.,* 2012). Due to its ability to swell within the protein system, gel strength of surimi can be maintained even though lesser amount of fish proteins were used (Teng *et al*., 2011; Campo and Tovar, 2007). However, different types of starch have different influence on gelling strength of surimi depending on their gelatinisation temperature and granule size (Park, 2005).

A lot of researches have been done on wheat starch, corn starch, potato starch and tapioca starch. Each type of starch possesses different characteristics and properties depending on its amylose and amylopectin composition. Corn starch and wheat starch produce hard starch gel when heated, contain high lipid and produce high volume of syneresis upon freeze-thaw cycle (Teng, Chin and Yusof, 2013). Potato and sago starches have lower amount of syneresis and exhibit good cohesive gel characteristics when undergoing freeze-thaw cycle. Table 6.1 lists the proximate composition of different types of starches.

Table 6.1: Proximate compositions of different types of starches.
(adapted from Park, 2005, and Ahmad *et al.*, 1999)

	VALUE PER 100 g					
	Sago	**Wheat**	**Corn**	**Potato**	**Waxy Maize**	**Tapioca**
Amylose	24.00 - 31.00	25.00	26.00	20.00	1.00	17.00
Amylopectin	68.20 – 72.70	73.80	72.80	79.50	97.50	82.60
Protein	0.13 - 0.25	0.40	0.60	0.06	0.15	0.10
Fat	0.10 - 0.13	0.80	0.44	0.05	0.15	0.10
Ash	0.06 - 0.43	0.20	0.10	0.40	1.10	0.20

Potato starch was reported by Park (2005) to possess the highest gel strength due to its largest granule size. However, Ahmad *et al.* (1999) reported that sago possesses higher gel strength than potato starch in starch/water gelation. Effects of these starches on surimi-starch gel have also been reported. However, studies on Asian native starch as a functional ingredient in surimi processing has not been reported.

Sago starch has been known as 'starch crop of the 21st century' from South East Asia (Teng *et al*., 2011) and Malaysia has been known as one of the biggest producers of sago starch in the world (Widodo and Hassan, 2015). Sago starch is extracted from sago palm (*Metroxylon* spp.). The stem is cut, crushed and kneaded to extract the starch (Figure 6.1). Further processing of sago starch will produce sago flour which is useful in food applications.

Sago starch is considered as a tuber starch. Its application in food industry has been increasing and gaining interest from food developers around the world. In South East Asia, sago starch has been used to produce vermicelli, biscuit and crackers. In Malaysia, sago starch has been widely used to improve the texture of fish crackers. It increases the crunchiness and chewiness that suit the textural perception of the locals. Other than that, local vendors and Small and Medium Industries (SMI's) have been using sago starch to produce various desserts and fish products in Malaysia.

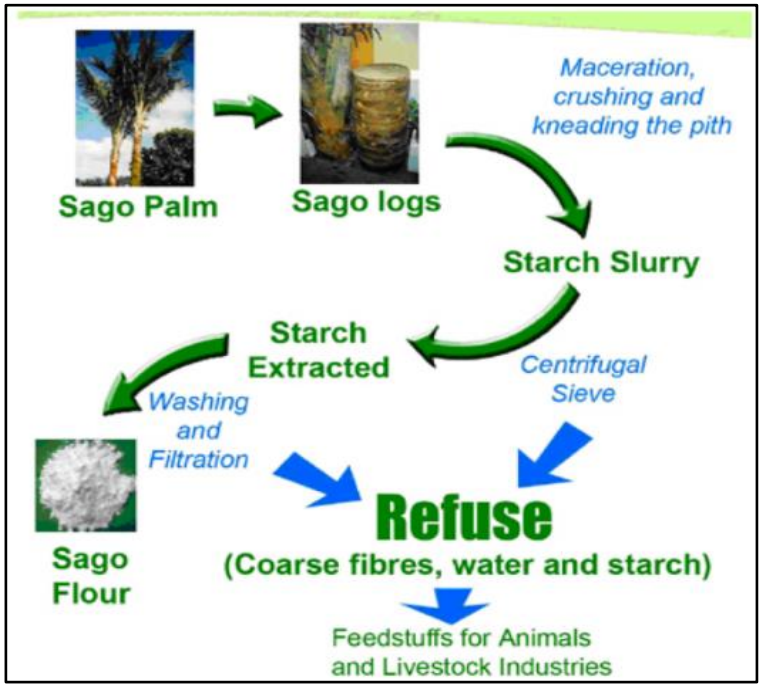


Figure 6.1: Sago starch and flour extraction process.

Sago starch possesses physio-chemical properties similar to other types of starches. It has high content of amylose and also gelatinisation temperature similar to corn starch which is ±65°C (Teng, Chin and Yusof, 2013; Ahmad *et al.,* 1999). In addition, it also displays similar viscosity profile to potato and tapioca starches (Teng *et al*., 2011). It is also easily gelatinised, cheap and undergoes little syneresis when compared to other starches (Ahmad *et al*., 1999). Being equipped with these properties allows sago starch the possibility of acting as a functional ingredient such as gelling agent, thickener or filler in the food industry. However, sago starch has not been introduced into the surimi industry. Its effectiveness in the surimi industry should be researched and tested to further increase its functionality in various kinds of food products.

Rheological tests have been used as a tool to determine the quality of fish gel surimi. Dynamic rheological measurement is often done in order to observe the viscoelastic gelling characteristics of the surimi gel and also understand the sol-gel transition during heating (Zhang *et al.,* 2013; Liu *et al.,* 2007). Rheological measurement has been found to be beneficial in characterising the behaviour of fish gelation during heating. Some researchers adopt rheology as a predication method to understand the stability and behaviour of material during prolong storage (Tabilo-Munizaga and Barbosa-Cánovas, 2005A).

Rheological tests have also been done to determine the effects of freezing storage on surimi-starch gel (Kim, Park and Yoon, 2005). Surimi gel quality is often being related to hardness (strength) and cohesiveness (deformability) (Yang, 1998). These two parameters are used because they simulate the

human mastication process and sensory perception. A perfect combination of these two parameters represents the gel strength of the surimi gel.

The objective of this study is to promote sago starch in the surimi industry. In conjunction to that, rheological and textural effects of different sago starch concentration on surimi were investigated. The shelf life storage of surimi-starch gel was also studied. Positive outcome from this research is hoped to promote sago starch as a functional surimi ingredient in the surimi industry.

6.2 MATERIALS

6.2.1 Sample preparation

Fish paste and fish gel samples were prepared as discussed in Chapter 3 using the formulation shown in Table 6.2.

Table 6.2: Sample formulations for surimi with different sago concentrations.

SAMPLE	SAGO STARCH CONCENTRATION (w/w)
C0	0%
C10	10%
C16	15%
C20	20%

6.3 RESULTS AND DISCUSSION

6.3.1 Moisture content

Moisture content of fish paste was recorded before the addition of sago starch. The initial moisture content was 90.21% ± 0.52%. Fish paste was then added with different concentrations of sago starch and stored overnight in frozen storage (-18°C). Table 6.3 records the moisture content of each sample after the overnight (24 hours) frozen storage.

Table 6.3: Effects of different sago concentrations on moisture contents of surimi samples following overnight frozen storage (-18°C). *Different letters represent significant difference ($p < 0.05$) of moisture contents at different sago concentrations.*

SAMPLE	MOISTURE CONTENT (%)
C0	$78.37^{b} \pm 0.14$
C10	$80.92^{a} \pm 0.33$
C15	$77.65^{b} \pm 0.68$
C20	$76.07^{c} \pm 0.33$

From the Table, it can be concluded that moisture contents decreased as starch concentrations increased. C20 recorded the lowest moisture content and was found to display a significant difference ($p < 0.05$) with 15.47% reduction after overnight storage. This is a clear indication that starch granules present within the system influenced moisture content. Starch granules are well known for their water absorption characteristics which explain the lower moisture content in higher concentration of starch (Tabilo-Munizaga and Barbosa-Cánovas, 2005B). However, in the present work, another trend was observed in

that addition of sago actually retained the moisture content of the surimi sample. This was observed when C0 and C10 were compared. C10 displayed a significantly higher percentage of moisture than C0 ($p < 0.05$).

Kim, Park and Yoon (2005) reported that surimi grade decreases as moisture content increases. The ideal moisture content of a surimi is 72% to 77% (Park and Lin, 2005). Yang (1998) reported that at cold temperature, starch absorbs water and swells slightly due to shear and time. Homogenisation and frozen storage duration might induce the changes in moisture, thus suggesting that sago starch at certain concentration might be used as an additive to prevent moisture loss during frozen storage.

6.3.2 Expressible moisture content and water holding capacity

Expressible moisture content (EMC) and water holding capacity (WHC) can also be used as an indicator of gel quality and protein denaturation that occurs within a protein network (Chaijian *et al*., 2006; Nopianti *et al*., 2012). A lower WHC will allow the water inside the system to move freely and bound together (Zhang *et al.,* 2013). This will form undesirable large water crystals during frozen storage which will disrupt gel formation and cause protein deformation (Ohkuma *et al*., 2008; Zhou *et al*., 2006, Yoon and Lee, 1990). Protein will lose its functionality and gel network building capability. Preservatives are usually added to prevent/inhibit deterioration process such as sugar and phosphates.

Table 6.4 shows the result of adding sago at different concentrations on the EMC and WHC of surimi samples. EMC and WHC of samples were significantly

affected by the presence of sago starch. However, it was also observed that addition of 10% sago starch did not display a significant difference on the EMC and WHC ($p > 0.05$) as compared to control (C0) which suggests that a little amount of sago does not have an effect on WHC and EMC of surimi. Another inference which can be made is that an overnight storage might not have a significant effect on protein deformation.

Table 6.4: Effect of different sago concentrations on the expressible moisture content (EMC) and water holding capacity (WHC) of surimi samples following overnight frozen storage (-18°C). *Different letters represent significant difference ($p < 0.05$) of parameters at different sago concentrations.*

SAMPLE	EXPRESSIBLE MOISTURE CONTENT (%)	WATER HOLDING CAPACITY (%)
C0	$31.21^{a} \pm 0.227$	$61.463^{c} \pm 1.176$
C10	$30.20^{a} \pm 0.923$	$62.677^{c} \pm 1.142$
C15	$20.37^{b} \pm 0.738$	$73.767^{b} \pm 0.953$
C20	$11.24^{c} \pm 0.333$	$85.230^{a} \pm 0.439$

As sago starch concentrations increased, WHC of the samples also increased. Addition of 5% sago starch increased the WHC from C10 to C15 by 17.7% and from C15 to C20 by 15.5%. Results obtained are also in agreement with the results presented previously on moisture content (Table 6.3). Moisture content was found to decrease as sago concentration increased. Moisture content is defined as free water in the system. The stronger the WHC, the lesser the amount of moisture present in the system. This result suggests that sago starch has the ability to improve the WHC of surimi fish paste. As starch granules numbers increased, surimi samples were found to have better WHC.

One of the properties of a starch is its ability to absorb water when heat is applied to gelatinise (Park, 2005; Ahmad *et al.,* 1999; Yang, 1998). When this happens, starch granules will swell and expand. Addition of sago starch increased the water intake and improved the ability to retain water within the system (Kong *et al.,* 2016). Water is retained by starch network developed during formation of gel (Tabilo-Munizaga, and Barbosa-Cánovas, 2005B). However, all these processes happen with the presence of heat. Even so, in cold temperature, starch imbibes water and swells slightly which causes changes in EMC and WHC (Yang, 1998). Homogenisation and frozen storage duration also induce changes in EMC and WHC.

6.3.3 Rheological tests

Stress sweep was done to determine the Linear Viscoelastic Region (LVR) of a sample without destroying its structure. LVR is expressed as region where viscoelastic moduli are independent of stress (Teng *et al*., 2011). Viscoelastic moduli determine the behaviour of samples whether they display elastic characteristics or more viscous characteristics (Ferry, 1980).

Figure 6.2 represents the storage moduli of all samples when subjected to a range of stresses. The stress sweep test done showed that G' did not overlap with G'' for all samples during stress sweep analysis indicating that all samples had a more elastic nature rather than viscous (Figure 6.3). A trend can be observed as the amount of sago concentration increased. G' values increased as sago concentration increased. Other than that, C20 displayed the highest G' value when compared with other samples. This suggests that C20 had the

firmest structure. A high value of G' value also suggests an increase in myofibrillar protein matrix due to the migration of water from the matrix to starch granules (Dileep *et al.,* 2005). This result is in agreement with the data shown in moisture content (Table 6.3) and WHC (Table 6.4) of C20. In addition, an increase in G' indicates increasing rigidity of the samples associated with the formation of elastic gel structure (Teng *et al*., 2011; Campo-Deaño *et al*., 2009). This result suggests that C20 will produce the highest gelling strength value.

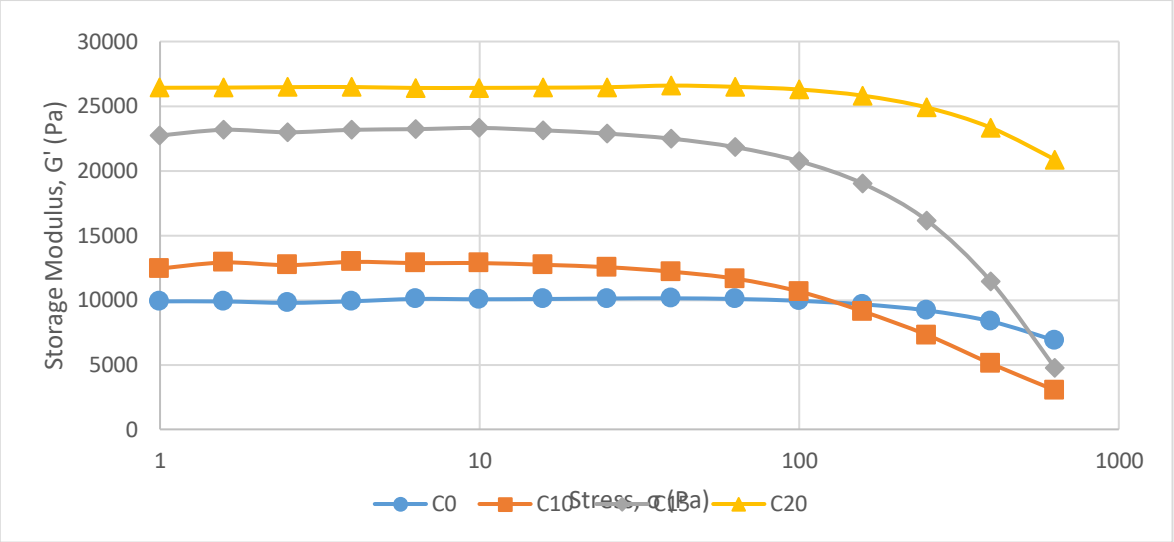


Figure 6.2: Linear viscoelastic range of surimi samples with different sago concentrations subjected to stress sweep test from 1 Pa to 1000 Pa.

Another observation that can be made is the length of LVR. C0 displayed a much longer LVR range when compared to samples with sago starch added. This indicates that C0 had higher stability when compared to other samples with starch even though C0 possessed lower G' value. As sago concentration increased, the LVR range became wider. A wider LVR range suggests a stronger protein and starch network as more stress is needed to cause structural deformation (Kim, Park and Yoon, 2005).

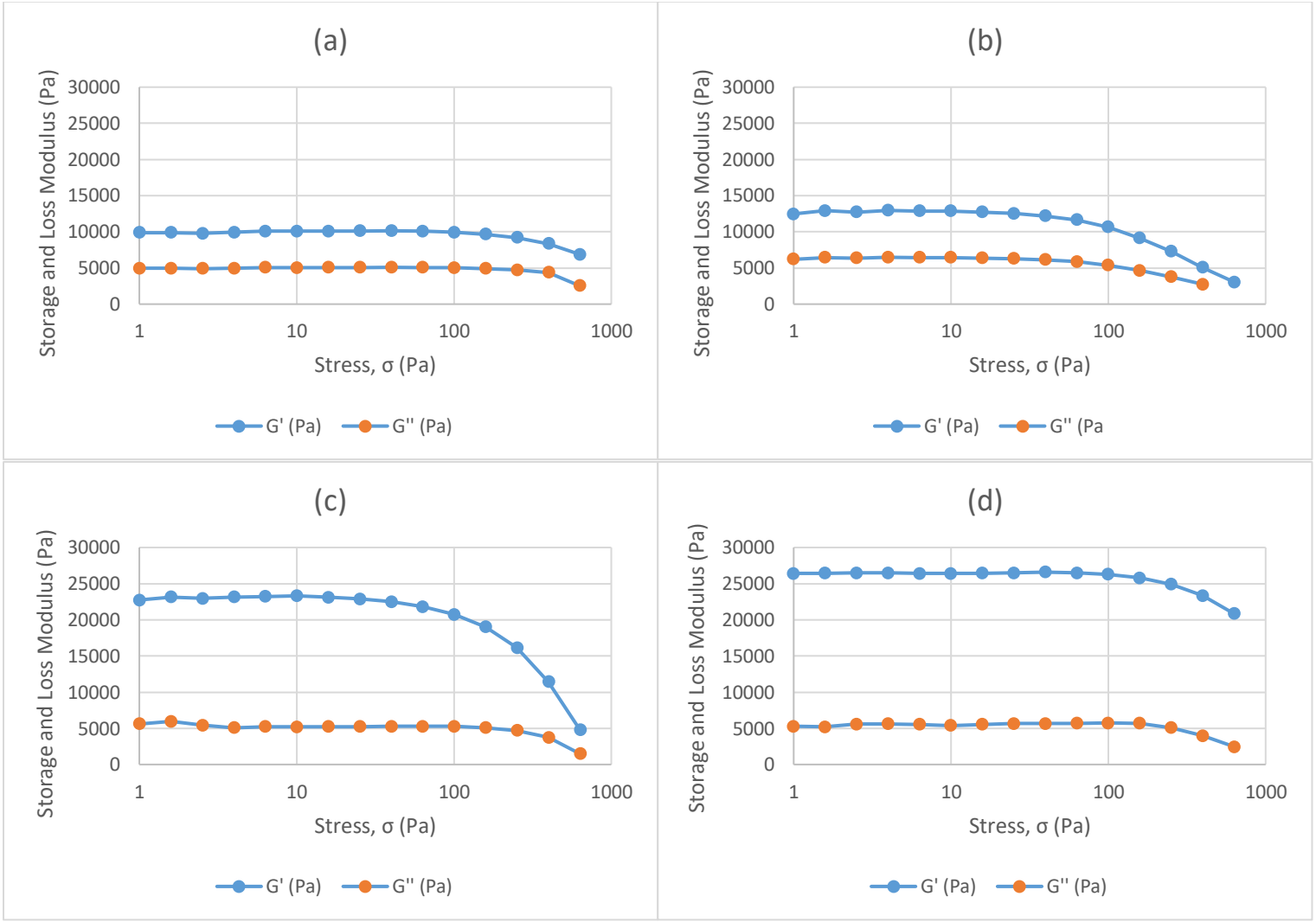


Figure 6.3: The storage modulus (G') and loss modulus (G'') for (a) C0, (b) C10, (c) C15, and (d) C20

Table 6.5 represents the maximum LVR limit values for stress max (σ_{max}), strain max (γ_{max}), and phase angle max (δ_{max}) of each sample subjected to different concentrations of sago formulation by adapting Solo-de-Zaldivar *et al*. (2014) methods. σ_{max} or critical shear stress is used as an index parameter for stability and γ_{max} is used an index for extensibility. δ_{max} is defined as ratio of energy loss/ energy stored (G'/G'') for each oscillation (Teng *et al*., 2011; Campo-Deaño *et al.,* 2009; Dileep *et al.,* 2005).

Table 6.5: Stress max, strain max, and phase angle max for surimi samples with different sago concentrations. *Different letters indicate a significant difference within column ($p < 0.05$)*.

Sample	Sago Starch (%)	Stress Max, σ_{max} (Pa)	Strain Max, γ_{max} (1/s)	Phase Angle Max, δ_{max} (^{0})
C0	0	$631.25^{b} \pm 0.27$	$6.10^{b} \pm 0.40$	$20.60^{a} \pm 0.79$
C10	10	$251.18^{c} \pm 0.56$	$1.08^{d} \pm 0.36$	$21.39^{a} \pm 1.24$
C15	15	$631.15^{b} \pm 0.11$	$1.38^{c} \pm 0.11$	$18.07^{b} \pm 0.47$
C20	20	$1500.08^{a} \pm 0.30$	$9.27^{a} \pm 0.25$	$17.03^{c} \pm 0.32$

From Table 6.5, it is apparent that C20 displayed the highest value of σ_{max} followed by C0, C15 and C10. Addition of sago starch at certain concentration caused the surimi to become stiffer and more rigid. High γ_{max} is related to better protein functionality (Campo-Deaño *et al.,* 2009). This is relevant when C0 displayed a high value of both. C0 which consists of only myofibrillar protein without any starch did not need to compete for water thus allowing optimum protein functionality. C0 also contains the highest amount of myofibrillar protein which indicates that protein network is strong without any additives or adulteration. However, C20 also showed the highest value of γ_{max} when

compared with the other samples ($p < 0.05$). This indicates that combination of gel protein network and starch gelatinisation network surpasses the extensibility of pure myofibrillar protein network (C0).

C20 also recorded the lowest σ_{max} value followed by C15, C0 and C10. No significant difference was found between C0 and C10. Results obtained from the stress sweep test were higher than results reported by Campo and Tovar (2008) on the effects of using wheat starch. In that work, σ_{max} of surimi added with 15% wheat starch was reported at 500 Pa whereas sago starch recorded 631.15 Pa in the present work. Similar trend is obtained when comparing the γ_{max} value of wheat starch and sago starch at 15% concentration (w/w). Sago starch recorded a higher value of γ_{max} which was 1.38 compared to wheat starch which was 1.26. This indicates that sago starch displayed a much positive result than wheat starch. A higher value of γ_{max} indicates higher extensibility and resistance to deformation. A characteristic that is required for a good gelling strength. A textural analysis on the surimi-starch gel will be done to further verify this result.

Frequency sweep is also known as mechanical spectra. Many food technologists use this rheological test to crudely determine the stability of a material during processing or storage. Information obtained from this rheological test allows food technologists to develop suitable processing conditions to cater the materials stability. Furthermore, the effects of functional ingredients on shelf life stability of a product can also be determined. This test is beneficial as it has a wide application on food characteristics.

Figure 6.4 plots the frequency sweep test of surimi samples with different sago concentrations. From the Figure, all samples displayed frequency dependence. However, at a certain frequency, the dependency of the sample increased. Dependency increase suggests that as product is stored longer the samples will become more unstable, thus, indicating a short shelf life. C20 yielded the highest G' value whereas C10 the lowest. This indicates a much rigid structure for C20 and *vice versa* for C10. This result is similar and consistent with the result obtained previously on the σ_{max} and the γ_{max} values (Table 6.5). Figure 6.4 also shows that an increase in starch concentrations caused an increase in G' values which might indicate that starch influenced the structure of surimi network.

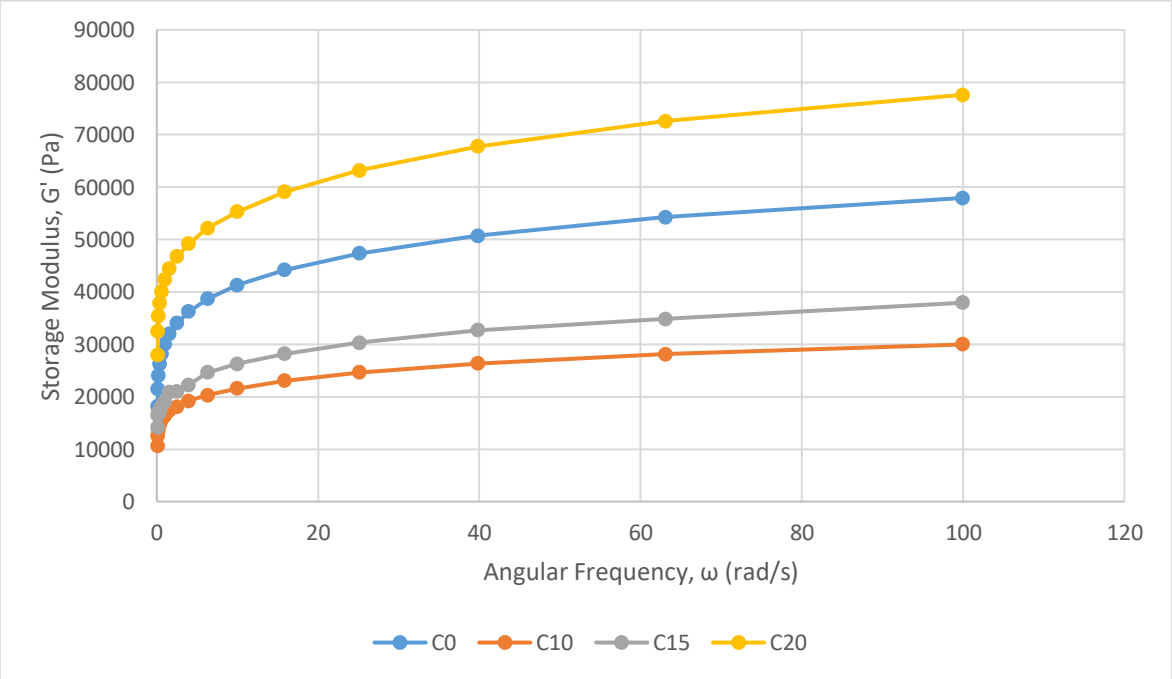


Figure 6.4: Storage modulus (G') of surimi samples with different sago concentrations subjected to different frequencies.

Another observation made is the slope of the samples. C10 showed a lesser incline than other samples especially C20 which showed a steep incline. This indicates that C10 was much more stable than C20. Therefore, C20 can be characterised as rigid and unstable. Frozen storage might influence the functionality of myofibrillar protein and starch granules due to the formation of large ice crystals (Badii and Howell, 2002). During frozen storage, large ice crystals can rupture starch granules and proteins. Ruptured starch granule will cause water to leech out and causes hydration. This will affect WHC and also starch gelatinisation (Yang, 1998). The same characteristics can also be referred to C0 as it displayed similar trend as C20. This is predicted as C0 did not contain any additives to prevent denaturation during frozen storage.

Table 6.6 displays the values of G'_0, G''_0, n' and n'' for each sample based on the power law equation by Campo and Tovar (2008). From the Table, C20 showed the highest value of G'_o, G''_o, n' and n''. High viscoelastic parameters suggest that C20 had a more rigid structure. High n' and n'' values show that C20 was unstable and frequency-dependent. As time increased, the characteristics of C20 became more unstable. Therefore, C20 could have produced a good gel quality surimi but could not withstand prolong storage.

Another inference can be made is that C20 might possess a good gel attribute but is not stable during processing. Addition of sago starch was found to significantly increase the viscoelastic moduli but decreased the n' value. Addition of starch could promote better gelling strength but compromises the stability of the surimi. The stability might decrease to the rupture of starch

granules with higher shearing or stress. This changes the chemical structure of starch and its interaction with myofibrillar proteins. The difference between n' and n'' was also observed. A wider gap between n' and n'' represents a better gel attribute (Campo-Deaño *et al.,* 2010). Table 6.6 dictates that C20 had the best gel attribute followed by C0, C15 and C10. Gel network and structure improved as starch concentration increased.

Table 6.6: Power law exponent for equations (3.3) and (3.4) as determined by the frequency sweep test on surimi samples of different sago concentrations.

SAMPLE	G'_o (Pa)	G''_o (Pa)	n'	n''	n' – n''
C0	29068	7694.5	0.1437	0.1265	0.0172
C10	15994	4489.4	0.1335	0.1218	0.0117
C15	19627	6094.0	0.1362	0.1202	0.0160
C20	41360	11743.0	0.1540	0.1309	0.0231

However, it is worth to point that the result obtained in Table 6.6 contradicts with the result displayed by stress sweep test (Table 6.5). Stress sweep test indicates that increasing amount of sago starch increased the stability and extensibility of surimi. Presumably, the stress sweep test might have just displayed the short-term structure stability and which was only measured after 24 hours of frozen storage. On the other hand, frequency sweep test indicates stability during processing and long-term structure. Verification on textural analysis is needed to further prove this inference.

Temperature sweep is done to understand the structural changes of a sample when heat is applied. Food technologists use this test to understand the gelation behaviour of a material. Figure 6.5 depicts the effect of heating on surimi samples with different sago concentrations. Initially, a slight increase in G' values was observed from 10°C to 20°C. At this temperature range, myosin began to aggregate and form initial protein network structure through hydrogen bond (Kong *et al.,* 2016; Esturk, 2003).

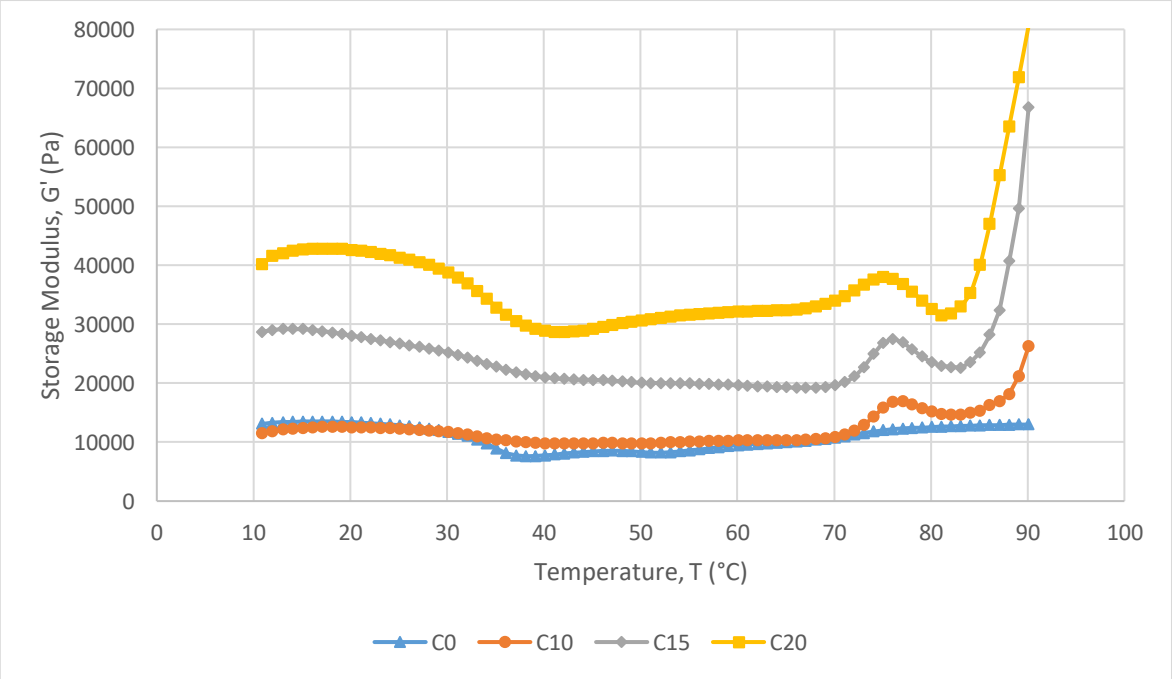


Figure 6.5: Storage modulus (G') of surimi samples with different sago concentrations subjected to different temperatures.

All samples showed a minimum G' value at temperature range between 35°C and 40°C. At this temperature, protein underwent denaturation and protein chains were further exposed for aggregation. This was caused by the dissociation and unfolding of myofibrillar protein to form more dense protein network or gelation (Campo-Deaño *et al.,* 2009). At this temperature range, actomyosin started to unfold which triggered the light meromyosin to promote

protein-protein interaction and gel network formation (Poowakanjana *et al*., 2012; Esturk, 2003). At this temperature range, only myofibrillar protein was reacting with heat.

As temperature increased (40°C – 60°C), G' values also increased due to the protein-protein interaction, hydrophobic bonds and ionic bonds to form gel networks (Lanier, Carvajal and Yongsawatdigul, 2005). The increase in G' is related to ordered aggregation of protein and formation of a three-dimensional network, with entrapment of water in the matrix (Campo-Deaño *et al.,* 2009; Dileep *et al.,* 2005). At this temperature range, highly elastic three-dimensional protein network was formed (Kong *et al*., 2016). The three-dimensional networking structure was formed by hydrophobic interactions and also disulphide covalent bonds (Kong *et al.,* 2016; Carvajal, Lanier and MacDonald, 2005).

Another trend that can be observed was the maximum G' values which were recorded between 70°C and 80°C for all samples. This can be related to the starch gelatinisation temperature. Although starch gelatinisation temperature is usually between 60°C and 70°C, the presence of protein within the system causes a shift in the gelatinisation temperature (Dileep *et al.,* 2005). Starch gelatinisation causes various chemical changes to the starch itself such as granule fragmentation, granule swelling and increased viscosity (Tabilo-Munizaga and Barbosa-Cánovas, 2005B). At this temperature range, starch granules start to swell due to the heat treatment. Expanded granule will further fortify and reinforce the myofibrillar gel network thus producing a much compact

gel (Park, 2006). This is further supported by the temperature profile shown by C0. Sago starch was not added to C0; therefore, no peak was visible at temperature range of 70°C to 80°C. C0, C15 and C20 recorded increasing G' values after gelatinisation up to 90°C. Dileep *et al.* (2005) reported similar findings when heat was induced to ribbonfish meat added with corn starch. Gelatinisation produces large number of elastic starch globules which cause a rise in G' value (Dileep *et al.,* 2005). However, C20 showed a significant increase compared to C15.

A difference in G' value is also related to the different types of bonds present within the surimi (Dileep *et al.,* 2005). C20 might possess a different protein gel network to starch gelatinisation network ratio. Higher density of starch gel network might be present in C20 when compared to other sample thus causing this effect. Kong *et al.* (2016) stated that when two gelation processes occur within a system but another one is overwhelmed by the other, the textural properties of the surimi-starch gel will be dictated by the stronger structured gel formed. When all samples were heated up to 90°C, C20 displayed the highest G' value at the end of the heat treatment indicating that starch gel network was more dominant than myofibrillar protein network. Other than that, C20 consisted of lesser amount of myofibrillar protein. This allowed C20 to form better starch gelatinisation network. Starch which acted as filler rapidly increased the G' value when optimum gelatinisation was reached by increasing the pressure within the protein gel matrix (Kong *et al.,* 2016). This also supports the fact that surimi with lower protein content can obtain high gelling strength with addition of starch.

6.3.4 Texture analysis

Breaking strength, breaking deformation and gel strength of each sample were tested and presented in Figures 6.6, 6.6 and 6.7 respectively. Figure 6.6 represents the breaking strength of surimi samples added with sago starch at different concentrations following overnight frozen storage. Based on the Figure, it is apparent that C15 and C20 significantly yielded the highest value of breaking strength when compared to C0 and C10 (though not significant to each other). C15 and C20 possessed five times more breaking strength than C0 and four times more than C10. This result suggests that sago starch has the ability to increase the breaking strength up to 15% concentration only. Addition of more sago above that did not display any significant difference on the breaking strength.

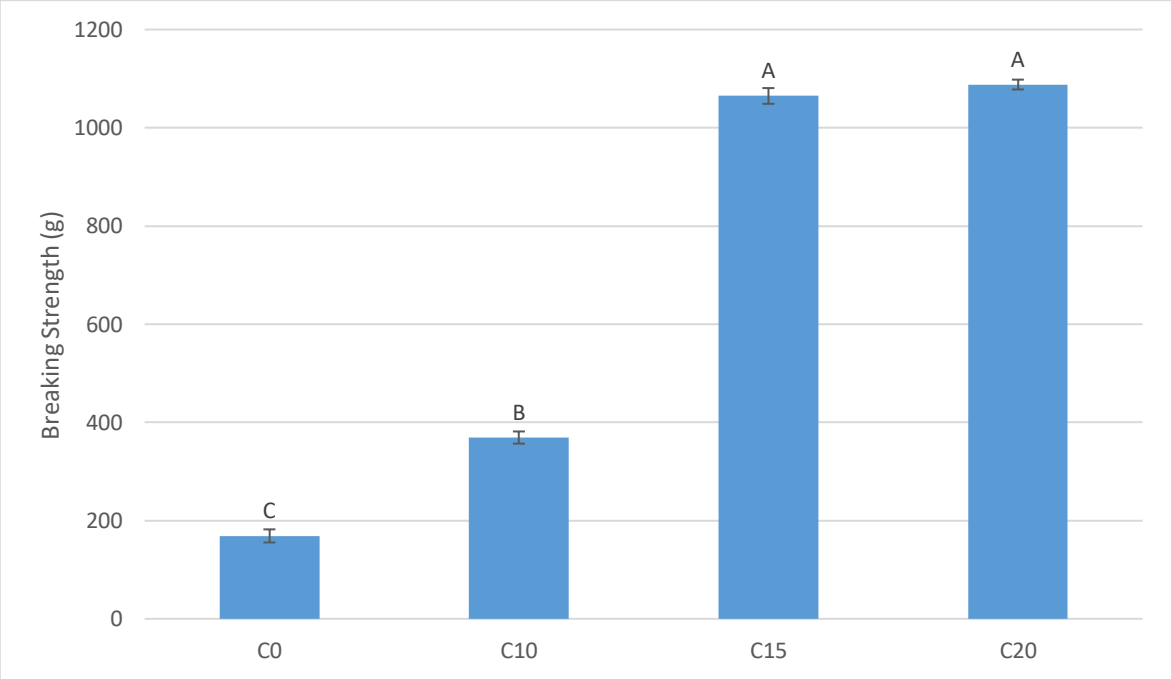


Figure 6.6: Breaking strength of surimi-starch gel at different sago concentrations following overnight frozen storage. *Data are means of triplicates (n = 3) with bars indicating standard deviation (SD). Different letters indicate significant difference (p ≤ 0.05).*

Figure 6.7 shows the breaking deformation values for surimi-starch gel samples added with sago starch at different concentrations following overnight frozen storage.

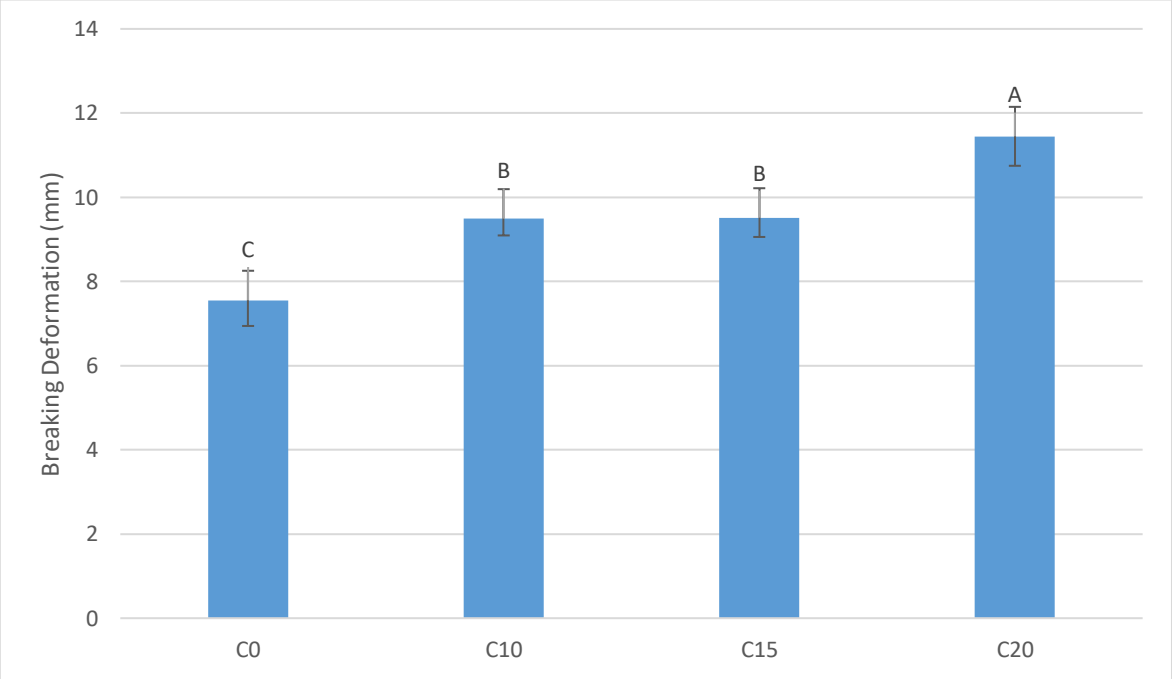


Figure 6.7: Breaking deformation of surimi-starch gel at different sago concentrations following overnight frozen storage. *Data are means of triplicates (n = 3) with bars indicating standard deviation (SD). Different letters indicate significant difference (p ≤ 0.05).*

From the Figure, C20 yielded the highest breaking deformation value. This has been predicted as C20 showed the highest strain max value (γ_{max}) as discussed in Table 6.5. C10 and C15 did not show any significant difference ($p > 0.05$) which indicates that they might possess similar cohesiveness. C0 showed the smallest breaking deformation value and is predicted to possess the lowest gel strength which is prone to deform at smaller stress and strain. C20 was found to yield 47.77% higher breaking deformation than C0 indicating that addition of sago starch has a significant effect on the breaking deformation.

A combination of breaking strength and breaking deformation will be translated into gel strength. Gel strength is one of the most important qualities of surimi. Surimi grades are determined by the gelling strength from low to high. Figure 6.8 displays the gel strength values for surimi-starch gel samples added with sago starch at different concentrations following overnight frozen storage.

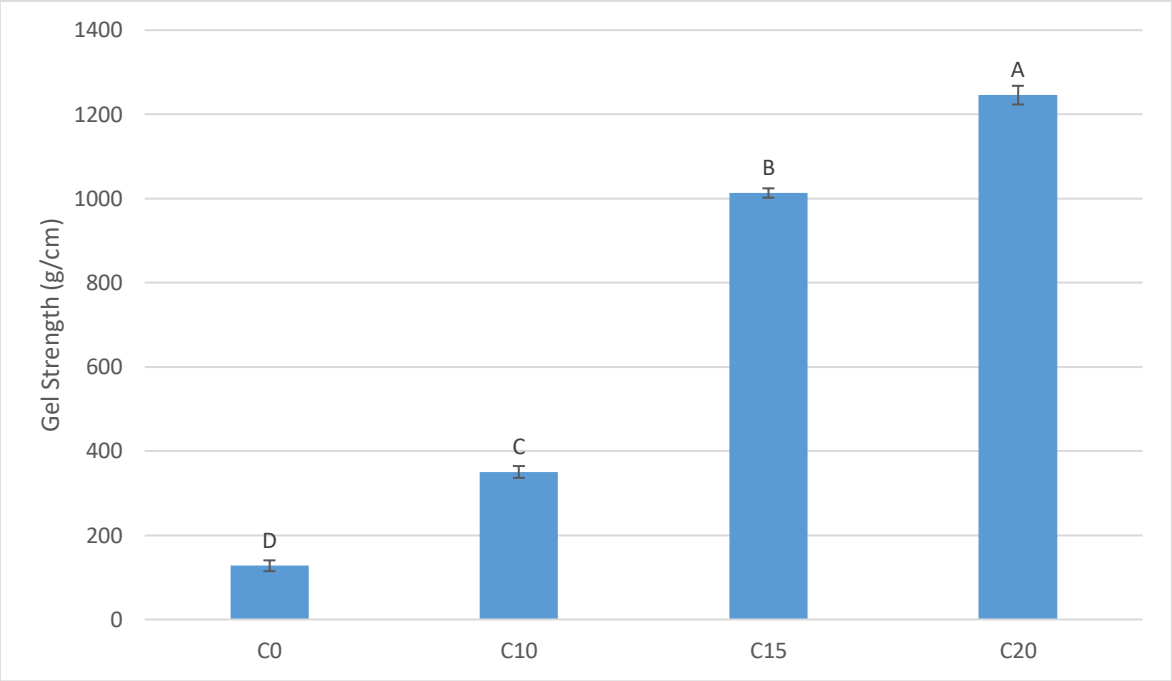


Figure 6.8: Gel strength of surimi-starch gel at different sago concentrations following overnight frozen storage. *Data are means of triplicates (n = 3) with bars indicating standard deviation (SD). Different letters indicate significant difference (p ≤ 0.05).*

From the Figure, C20 was found to display the highest gel strength value followed by C15, C10 and C0. C20 displayed the highest gel strength which was 83.33% higher than the control sample C0. Other than that, gel strength showed a significant increase with addition of 5% sago starch up to 20% ($p < 0.05$). This concludes that sago starch concentration has a significant effect on the gelling strength of surimi gel.

Initially, myofibrillar protein will form gel network and gel matrix which entraps water. Addition of starch increases the gelling strength due to the role of starch as filler (Park, 2006). Starch granules are dispersed within the protein gel matrix. As starch gelatinises, the granule expands and exerts pressure to the fish gel matrix which offers a much compact and tough structure (Kong *et al.,* 2016; Park, 2005; Yang 1998). The concentration of starch and ratio of amylose to amylopectin of starch will influence the texture characteristics of starch-surimi gel (Yang, 1998).

From the results, a correlation can be made between sago concentration and gel strength. Increased number of starch swelling produces much more compact structure due to stronger reinforcement of gel matrix, therefore, increasing gel strength (Kong *et al.,* 2016). As sago concentration increased, the amount of amylose and amylopectin present in surimi increased, thus, contributing to the strong gel characteristics. High amount of amylose content increased gel strength (Ahmad *et al.,* 1999) which was evident when C20 showed the highest gel strength. Amylose and amylopectin present in the surimi-starch gel are important. Amylose helps stabilise the starch granule swelling whereas amylopectin provides the swelling power (Yang, 1998). However, there are reports indicating that addition of starch above certain level could deteriorate the gel matrix (Campo-Deaño and Tovar, 2008). This could be caused by competition of water between myofibrillar protein and starch or the effect of starch retrogradation. Another factor that caused gel strength to deteriorate as starch increased is the high amount of amylose leeching out. Amylose that leeches out from the granule will form hydrogen bonds with water

molecules. These hydrogen bonds do not contribute to the swelling of starch granules and also decrease the amount of water needed by myofibrillar protein and starch granule to form gel network (Yang, 1998).

Figure 6.8 illustrates that C20 presented the highest significant value of gel strength ($p < 0.05$). This result is agreement with result presented by WHC previously (Table 6.4). C20 displayed the highest WHC value which is related to strong gel network (Nopianti *et al*., 2012; Chaijian *et al*., 2006). Table 6.5 shows that σ_{max} and γ_{max} increased significantly with the increase of sago starch concentration ($p < 0.05$). σ_{max} and γ_{max} of C20 recorded by the stress sweep test were the highest which indicates that C20 possessed the most rigid and firm structure (Campo-Deaño *et al.,* 2009). The gelation temperature profile also complements this result. C20 displayed the highest G' value throughout the heating process. Rheological test performed on C20 in paste form agrees with the result displayed by surimi in gel form which was high in rigidity and strong in gelation network. This suggests that rheological tests performed in paste form could provide crude information on surimi characteristics during gel form.

6.3.5 Effects of frozen storage on surimi starch-gel

One of the qualities of a functional surimi ingredient is the ability to increase shelf life during transportation and storage. However, starch has only been known as a gel enhancer. No literature has been found on the effects of starch in prolonging surimi quality in a frozen state. Park (2005) reported various kinds of starch and their effectiveness on the stability of surimi only up to ten days.

However, sago was not one of the starches reported in that finding. Thus, this experiment was done to determine the feasibility of sago starch in maintaining the surimi gel texture for a period of six months in frozen storage.

Figure 6.9 shows that as sago starch concentration increased, the breaking strength increased linearly. However, C20 was found to possess the highest significant ($p < 0.05$) breaking strength after one month in frozen storage. According to the test previously done on temperature sweep (Figure 6.4), myofibrillar protein gelation was overwhelmed by starch gelatinisation. A significant high value of breaking strength might be due to the presence of higher starch gelatinisation density and low protein matrix. Thus, minimum protein denaturation occurred which did not disrupt the gelling strength of C20. Other than that, the concentration of starch granules present might suggest that starch retrogradation occurred during frozen storage. Teng, Chin and Yusof (2013) reported that gel hardness was related to starch retrogradation. Fu *et al.* (2015) reported that starch retrogradation broke the linkages that form bonds with free water molecules. Short-term retrogradation has the ability to improve texture due to the formation of irreversible amylose-amylose interaction to form crystal nuclei. Furthermore, the slow crystalline formation of amylopectin which evolved around the amylose crystal nuclei increased crystal integrity and exhibited characteristics of a perfect crystal (Fu *et al.,* 2015).

Nevertheless, as frozen storage continued, breaking strength of all samples decreased. Long term retrogradation has been shown to shorten shelf life and decrease product quality (Fu *et al.,* 2015). Decreased breaking strength might

also be caused by water hydration and ice crystal formation (Carvajal, Lanier and MacDonald, 2005; Park, 2005; Badii and Howell, 2002). Ice crystal formation induces protein deformation and aggregation affecting the gel network formed.

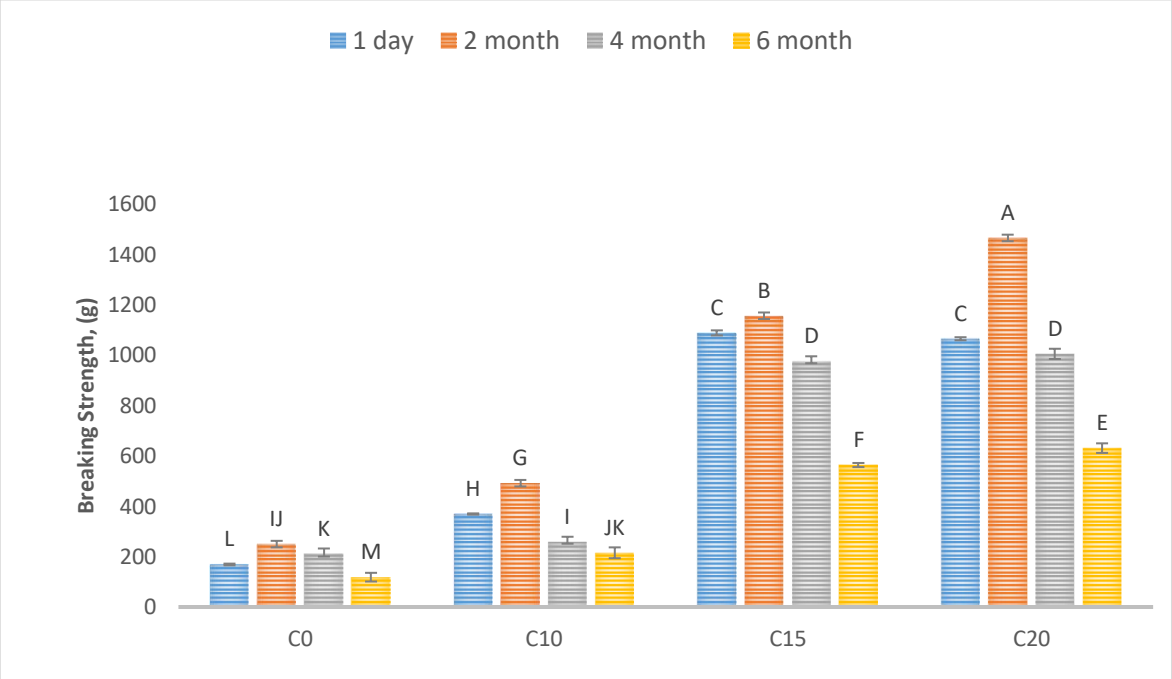


Figure 6.9: Effects of frozen storage on breaking strength of surimi-starch gel at different sago concentrations. *Data are means of triplicates (n = 3) with bars indicating standard deviation (SD). Different letters indicate significant difference ($p \leq 0.05$).*

Sago starch was found to be capable of maintaining breaking strength only up to two months. Breaking strength was found to significantly decrease after four months storage. After six months, breaking strength values were found to decrease 50% when compared to the first day. This result is constant for all sago concentrations which concludes that different sago concentrations did not have an effect on breaking strength stability. However, C0 was able to maintain the breaking strength of the sample up till four months of frozen storage and only showed a decrease in breaking strength after six months even though the

breaking strength was significantly low (p < 0.05) when compared to other samples. This might suggest that myofibrillar protein underwent slower denaturation process compared to a mix system of starch-surimi gel. Other than breaking strength, the breaking deformation of the samples was also analysed for a period of six months frozen storage (Figure 6.10).

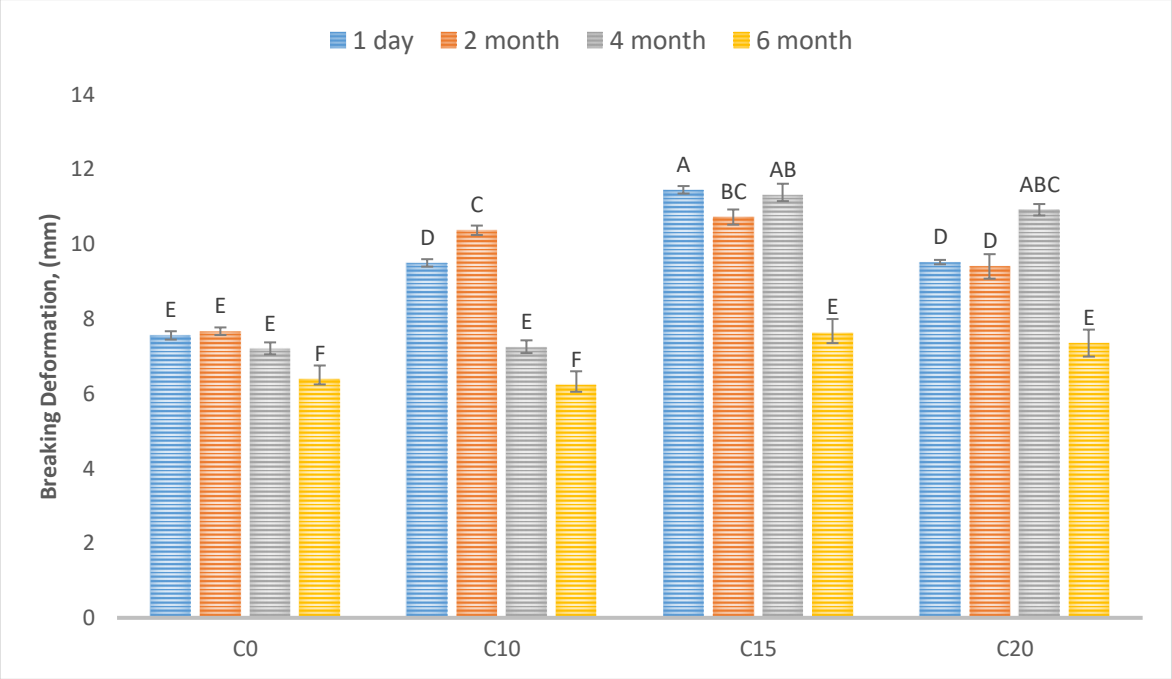


Figure 6.10: Effects of frozen storage on breaking deformation of surimi-starch gel at different sago concentrations. *Data are means of triplicates (n = 3) with bars indicating standard deviation (SD). Different letters indicate significant difference (p ≤ 0.05).*

It was found that the breaking deformation of samples without addition of sago starch (C0) showed no significant difference throughout the four months frozen storage (p > 0.05). This shows that some of the fish gel characteristics could be maintained for a long storage period under frozen temperature without any additives. Unfortunately, the breaking deformation value was significantly low (p < 0.05) when compared to other samples, even though breaking deformation was maintained. Additives are often used in the surimi industry to prevent

denaturation and increase the texture characteristics of surimi. Other than that, C15 and C20 showed positive results until four months of frozen storage. Both samples maintained a significantly high breaking deformation value ($p < 0.05$) up till the fourth month. However, similar result was obtained where all samples began to deteriorate after six months. This indicates that addition of sago starch did not have a significant effect on the shelf-life storage period of surimi breaking deformation. C15 displayed the biggest decrease which was about 60% of the value compared to overnight frozen storage.

Figure 6.11 illustrates that gel strength of all samples displayed an increase on the second month of frozen storage except for C15. The increase in gel strength could be caused by strengthening of protein gel network and starch gelatinisation during frozen storage. An increase in gel strength might also be due to the protein-protein interaction which caused product aggregation and produced stronger myofibrillar gel network at low temperature (Mahanawich *et al.,* 2010; Badii and Howell, 2002; Sych *et al.,* 1991A). This is further supported by result shown by C0. However, starch retrogradation also caused an increase in gel strength for a short period. Low temperature caused high amylose content of starch to crystallise. These crystals are further fortified with a coat of crystallise amylopectin on the outside (Fu *et al.,* 2015; Teng *et al*., 2011). The formation of crystal provided structure stability (Ahmad *et al.,* 1999).

After four months of frozen storage, gel strength seemed to decrease. C10 displayed a significant decrease ($p < 0.05$) which was about 60% as compared to the second month. After six months, all samples showed a significant

decrease which was lower than the value recorded on the first day of frozen storage ($p < 0.05$). C15 and C20 showed the most significant decrease when compared with the value recorded in the fourth month ($p < 0.05$). On the six months, C20 loss 60% gel strength and C15 loss 75% gel strength when compared to the first day.

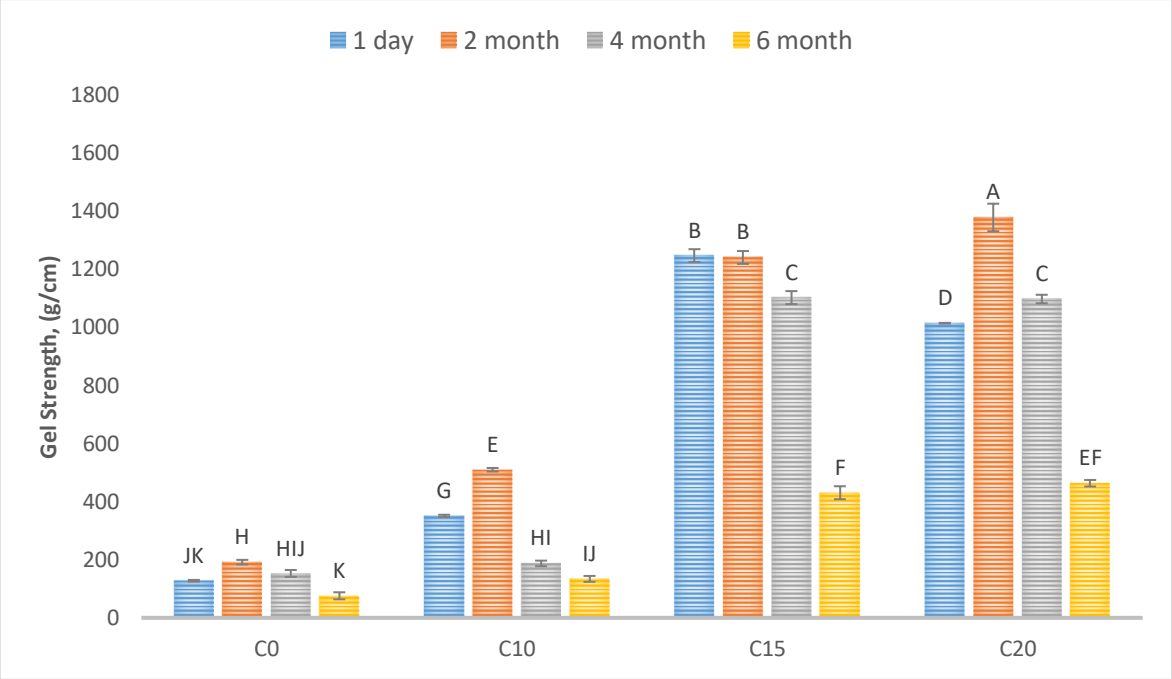


Figure 6.11: Effects of frozen storage on gel strength of surimi-starch gel at different sago concentrations. *Data are means of triplicates (n = 3) with bars indicating standard deviation (SD). Different letters indicate significant difference (p ≤ 0.05).*

The decrease in gel strength was associated to the denaturation of myosin (Dileep *et al.,* 2005). Even though starch has the ability to increase gel strength of surimi, prolonged frozen storage caused chemical changes due to the dissociation of amylose and amylopectin component (Teng *et al*., 2011). Breaking of linkages compromised structural integrity. As starch granules lost their swelling capability, fish gel matrix which was previously fortified became weak and lost its strength. In addition to that, the myofibrillar protein network

itself also undergoes changes and denaturation during pro-long frozen storage. This weakens the gel strength of the surimi-starch gel.

From the result, addition of sago starch did not have significant effect on the shelf life of surimi gel. However, it increased the gel strength initially and maintained the high gelling strength up to four months. Even though a decrease in gelling strength was found after six months, addition of sago starch at 15% and 20% (C15 and C20) was able to provide a higher value of gelling strength which was 87.5% more than surimi gel without sago starch. This provides options for food manufacturers either to produce a better gelling quality surimi with lower shelf life or a longer shelf life surimi with lower gel quality.

6.4 CONCLUSION

Several types of starch including potato starch and corn starch have been reported to increase the gel quality of surimi. However, the feasibility of using sago starch has not been researched. The results in the present Chapter have shown that sago starch exhibited a positive interaction with surimi and thereby increased the surimi gel quality. Addition of sago starch caused a decrease in moisture content due to its natural absorption behaviour. Although sago starch lowered the moisture content, the moisture content of surimi up to 20% sago starch was still in the range of acceptable moisture content which is 72%-77% (Park and Lin, 2005). WHC of surimi displayed a linear increase with increased amount of sago starch. Addition of 5% sago starch caused about 16.5% increase in WHC from 10% sago concentration to 15% and finally 20%, indicating that sago starch increased the WHC of surimi.

Rheological tests displayed an increasing trend with the increase of sago concentration. Viscoelastic moduli (G' and G'') were found to increase with increased amount of sago starch. Power law equation derived from the frequency sweep test also suggests that rigidity and stability increased as sago concentration increased. Rheological data also suggest that addition of 20% sago concentration yielded the highest structural rigidity. C20 was found to display the highest G' value during stress sweep, frequency sweep and temperature sweep tests. C20 also displayed a significant high value of for σ_{max}, γ_{max} and low δ_{max} which indicates a firm and stable structure.

The gelation profile of surimi-starch paste was found to be different than the normal surimi paste. Surimi starch showed a peak at temperature range of 60°C to 70°C. This peak has been identified as starch gelatinisation temperature. Starch gelatinisation process increased the gel strength of surimi by swelling and expanding its granule to increase the integrity of myofibrillar protein matrix. The number of starch granules present within the surimi-starch system also influenced the G' value during heat gelation. Higher volume of starch concentration displayed higher G' value.

Surimi starch gel formed was then tested for its textural properties. As predicted by early rheological tests on the surimi-starch paste form, increasing amount of sago starch increased the breaking strength, breaking deformation and gel strength. The gel strength was found to increase about 133% with 10% addition of sago starch. The gel strength showed a significant difference with addition of

5% sago starch up to 20%. C20 displayed the highest gel strength which was 83.33% higher than the control sample C0.

Other than that, sago starch was tested for its role as a preservative and C20 was found to be able to maintain gel strength up to four months. C10 and C15 showed gel strength deterioration after two months of frozen storage. C15 suffered the highest gel strength loss which was 57.14% in gel strength after four months of frozen storage.

Rheological tests used were found to complement the results displayed by textural analysis. This suggests that rheological test might be used as a crude prediction method in understanding the surimi-starch gelation behaviour and quality. Furthermore, sago starch was found to have the potential to act as a functional ingredient at par with other starches that have been commercially used by the surimi industry. Kong *et al*. (2016) reported that a commercial Alaska pollock surimi (grade AAA) has a gelling strength of 550 g/cm. In this research, C15 and C20 showed a significantly higher value of gel strength (1000 g/cm and 1200 g/cm) which could be maintained up to four months of frozen storage. Thus, research on sago starch and its potential as a functional ingredient in the surimi industry should be further investigated by food technologists and utilised by the seafood industry.

CHAPTER 7

Effects of Numbers of Washing Cycle and Concentrations of Nanobubble Water on the Textural Properties of Surimi Gel

7.1 INTRODUCTION

One of the most consumed fish protein products in the world is surimi. Surimi is produced from extracted and concentrated fish myofibrillar protein. Surimi provides alternative for consumers to consume fish protein which is low in fat, high in protein and good gelling texture in various product forms (Jin *et al.,* 2009). Surimi is obtained through a series of processes which include deboning and washing. Washing is a critical step as it determines the gel quality of surimi produced (Amiza and Ain, 2012).

Washing does not only clean the blood but it also removes undesirable components such as lipid, amines and water-soluble proteins which inhibit the formation of myofibrillar gel network (Karthikeyan *et al*., 2006). Sarcoplasmic protein has been reported as a water-soluble protein which inhibits gel formation. It is situated between the muscle fibres and produces various metabolic enzymes which disrupt the stability of functional protein (Lanier, Carvajal and Yongsawatdigul, 2005). Discarding all the unwanted components will produce high quality concentrated myofibrillar protein. The more concentrated the myofibrillar protein, the higher the gelling strength of surimi (Carvajal, Lanier and MacDonald, 2005; Park and Lin, 2005).

It is estimated that to wash 1 kg of surimi requires about 15 kg of water (Priyadarshini *et al.,* 2017). This extensive use of water causes too many water wastage and harmful effects on the environment. Several washing media alternatives have been tested to decrease the utilisation of water during washing whilst maintaining the surimi quality. Salt solution has been researched

to determine its feasibility as an alternative. Park (2005) reported that salt water used below 0.25% concentration was able to reduce washing cycle and maintain high gelling strength when compared to higher salt solution concentration. However, using salt water still causes detrimental effects on the environment if the solution is not discarded properly after washing. The same condition occurs when various washing media such as alkaline saline, calcium chloride and magnesium chloride are used (Priyadarshini *et al*., 2017; Zhang *et al.,* 2018). The gel strength of surimi increases with the usage of these washing media. However, these solution waste causes harm to the environment if it is not properly handled and disposed after washing.

There are two factors that need to be considered when washing. One is the meat:water ratio and the other is the number of cycles. This is presented in Figure 7.1 where different meat:water ratios and washing cycles have an effect on the total extractable proteins. Total extractable protein represents the amount of protein left after washing. Figure 7.1 illustrates that increasing washing cycle (WS) increases the total extractable proteins which will produce better gelling characteristics with increasing storage period in months (m). Currently, surimi manufacturers adopt the 3:1 water (W): meat (M) ratio with 3-cycle washing step (Karthikeyan *et al*., 2006). Surimi is washed three times to ensure that all the unwanted components especially sarcoplasmic proteins are discarded thoroughly (Amiza and Ain, 2012). Lipids are also another concern in surimi processing. Lipids that are not properly discarded will cause lipid oxidation during surimi processing and prolonged storage.

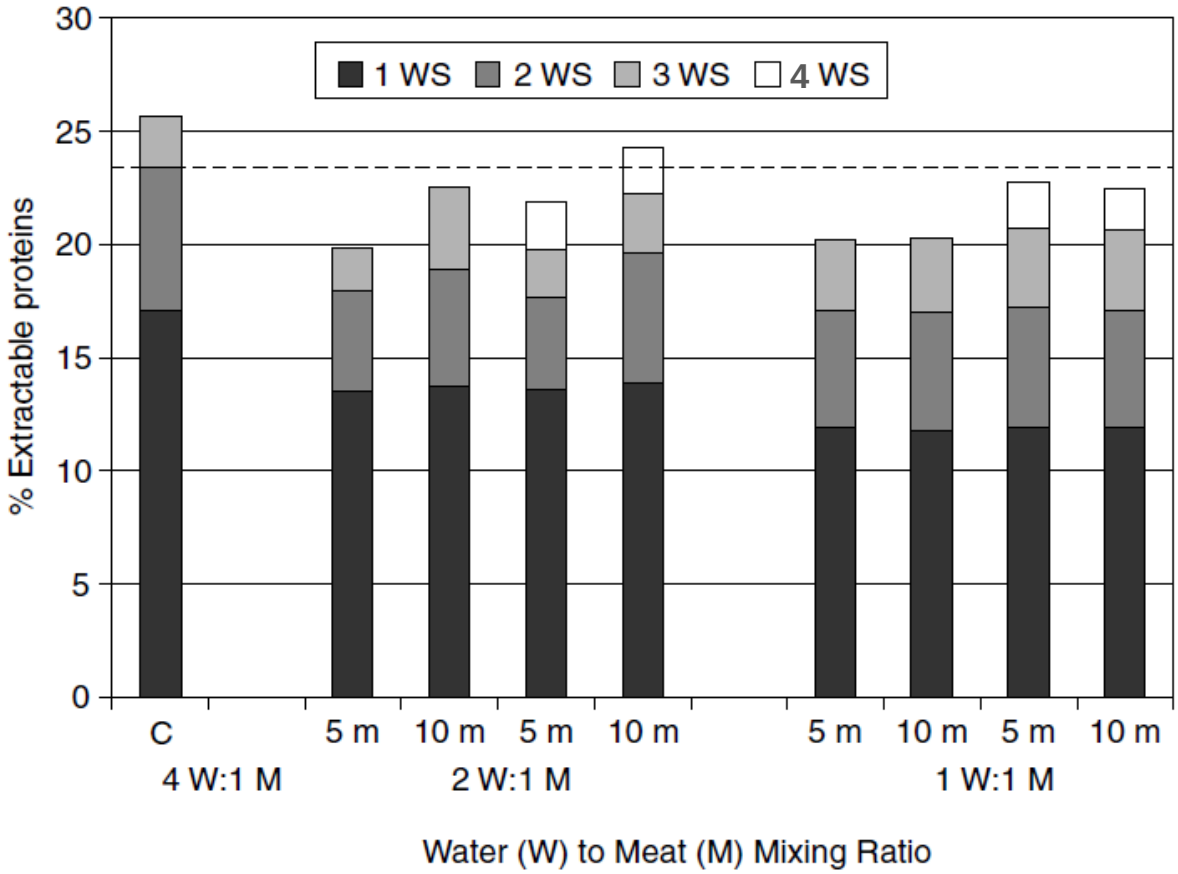


Figure 7.1: Effects of various washing conditions on total extractable proteins (Park and Lin, 2005).

Nanobubble technologies are currently getting attention in various fields of research and processing (Ushikubo *et al.,* 2010). Nanobubble has been applied in water-treatment technologies and was deemed to possess great potential (Temesgen *et al.,* 2017) Nanobubble water is water with nano-scale air bubbles. Industry currently incorporates nanobubbles in washing and defouling processes and water treatment due to their ability to efficiently discard unwanted components (Temesgen *et al.,* 2017). It has been reported that nanobubbles had the ability to improve organic degradation due to the presence of OH^- radical (Ghadimkani *et al*., 2016). Their ability to attach to oil droplets, metal ions, proteins and several other components widens their application in various areas (Zimmerman *et al*., 2011). Food industry utilises

nanobubbles in producing food products such as carbonated drinks, confectionary with foamy texture and food supplements (Temesgen *et al.,* 2017; Ushikubo *et al.,* 2010). Therefore, utilising nanobubble water during the washing step in surimi processing might have the potential to produce better quality surimi.

Environmental and biological safety awareness is currently increasing worldwide. Various steps and researches have been done to decrease the amount of water used during washing and hence decrease water waste. The objectives of this study were therefore (i) to determine the effect of using nanobubbles as a washing medium as compared to the normal practice which uses distilled water, and (ii) to investigate the efficiency of different nanobubble concentrations on surimi washing. The efficiency of nanobubble concentrations were measured by the resulting gel strength quality. Furthermore, different numbers of washing cycles were also investigated to determine the feasibility of reducing the number of washing cycle to support the environmental preservation.

7.2 MATERIALS AND METHODS

7.2.1 Nanobubble water preparation

Distilled water was used as a medium to generate nanobubble water. Nanobubble water was generated using the scheme presented in Figure 7.2. Nanobubble water obtained was then diluted to achieve three different concentrations which were characterised as low nanobubbles concentration (LN), medium nanobubbles concentration (MN) and high nanobubbles (HN)

concentrations. The amounts of nanobubble concentration and droplet size distribution were measured using Zetasizer Nano-ZSP Zen 5600 (Malvern Instruments Ltd., UK). Table 7.1 depicts the results of nanobubble water prepared with three different concentrations.

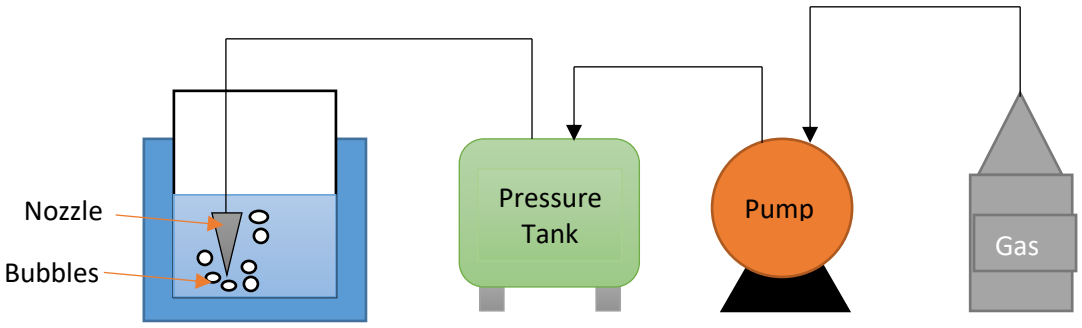


Figure 7.2: Nanobubble water generator model

Table 7.1: Amounts of nanobubbles present in washing media and their average size.

SAMPLE	AMOUNT OF NANOBUBBLES	AVERAGE SIZE OF NANOBUBBLES
Low concentration (LN)	0.75×10^8 bubbles/mL	149 nm
Medium concentration (MN)	11.15×10^8 bubbles/mL	141 nm
High concentration (HN)	22.35×10^8 bubbles/mL	133 nm

7.2.2 Sample preparation

Fish meat underwent mincing by using a food processor (Philips Jamie Oliver Model HR7782/00; the Netherlands) to attain uniformity and homogeneity. The fish mince was then weighed prior to washing step. Washing was done using different concentrations of chilled nanobubble water as presented in Table 7.1

with 3:1 (w/w) water to mince ratio. The fish meat slurry was then stirred gently for 5 min and filtered using cheesecloth. The fish paste collected was then divided into four samples which were DW (distilled water; control), LN (low nano), MN (medium nano) and HN (high nano).

Samples were then extruded into polyvinilydine casings (25 mm Ø). Both ends of the casings were tightly sealed. The samples were then boiled in water at 40°C for 30 min and 90°C for 20 min (two-step heating as described by Benjakul *et al*., 2002). The samples were then stored inside a freezer for 24 h.

7.2.3 Effects of number of washing cycles

Preparation of sample was done as mentioned in section 7.2.2 with additional washing step. Following homogenisation with a food processor, the sample was washed with four different types of chilled water (4°C) which were DW (distilled water), LN (low nanobubbles concentration water), MN (medium nanobubbles concentration water) and HN (high nanobubbles concentration water). Washing was done by stirring the fish mince with chilled water for 5 minutes. The sample was then filtered using cheesecloth to obtain the concentrated fish myofibrillar. Washing was then repeated again and filtered to obtain the 2-cycles washing samples. For the next sample, the fish mince was then washed and filtered again to obtain 3-cycles washing sample. The washing and filtration process was repeated till 3 washing cycles to obtain all the samples. The washing and sample collecting process is shown in Figure 7.3.

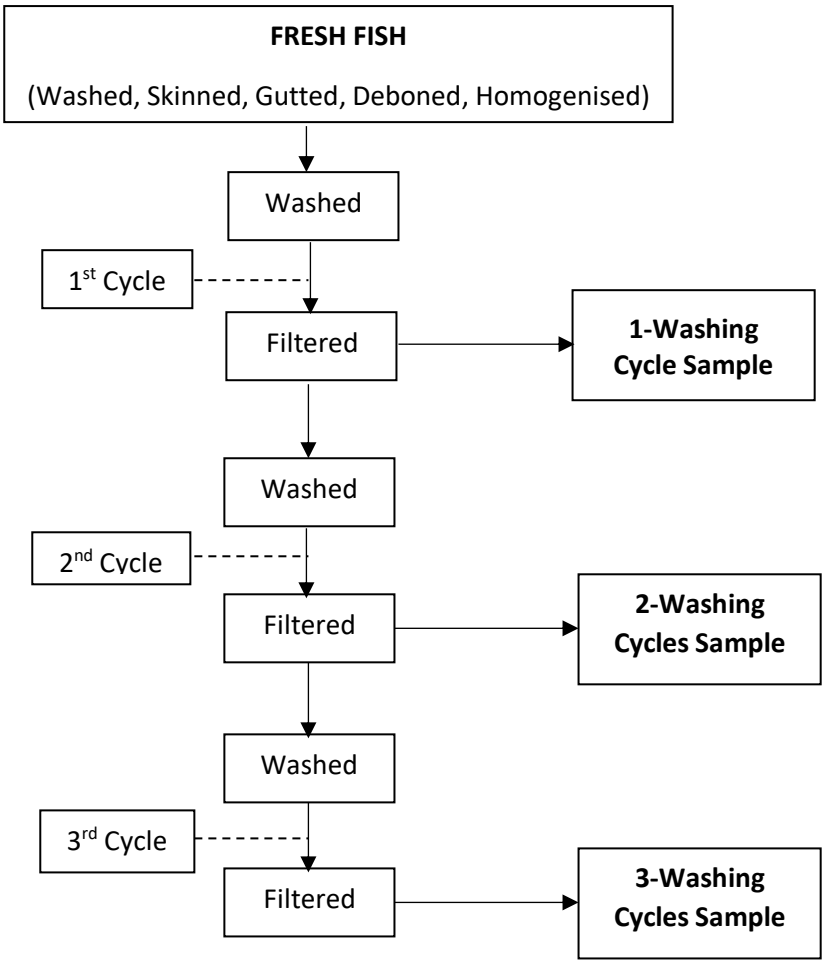


Figure 7.3: The washing and sample collecting process for surimi washed with nanobubble water.

At each cycle washing, sample was collected and coded as presented in Table 7.2. Samples were then extruded into polyvinilydine casings (25 mm Ø). Both ends of the casings were tightly sealed. The samples were then boiled in water at 40°C for 30 min and 90°C for 20 min (two-step heating as described by Benjakul *et al*., 2002). The samples were then stored inside a freezer for 24 h. The samples then undergo rheological testing, textural analysis and shelf life frozen stability analysis.

Table 7.2: Sample identification codes for different washing treatments.

TYPE OF WATER	NUMBER OF WASHING	SAMPLE CODE
Distilled (control)	1 Cycle	DW1
	2 Cycle	DW2
	3 Cycle	DW3
Low Nano	1 Cycle	LN1
	2 Cycle	LN2
	3 Cycle	LN3
Medium Nano	1 Cycle	MN1
	2 Cycle	MN2
	3 Cycle	MN3
High Nano	1 Cycle	HN1
	2 Cycle	HN2
	3 Cycle	HN3

7.3 RESULTS AND DISCUSSION

7.3.1 Moisture content

Moisture content is defined as free water which is present within the food system. Moisture content can influence the gel strength and viscosity of surimi (Kim *et al*., 2005). Table 7.3 records the moisture content of samples after overnight storage at -18°C. Based on the Table, the moisture content of LN was higher when compared to the ideal commercial grade surimi which is within the range of 72%-77% (Park and Lin, 2005).

Nanobubble present in the water might have caused interaction between protein and water which in turn retained more moisture. However, as the amount of nanobubbles present in the water increased, the moisture content continued to decrease. Nanobubble is known to possess OH^- radical and strong

hydrogen bond at its surface (Zimmerman *et al.,* 2011). Thus, the repulsive force due to these two factors causes water to separate from the nanobubble; which might have caused the moisture to decrease within the surimi sample as the nanobubble concentrations increased. At low nanobubble concentrations, the amount of nanobubble introduced to surimi might be enough to just transport small amounts of unwanted components without actually affecting the moisture content of the surimi sample.

It is also apparent from the Table that LN yielded the highest moisture content followed by MN, HN and DW after overnight storage. Even though LN recorded the highest moisture content, it did not display any significant difference with sample washed with MN ($p > 0.05$). MN and HN also did not show any significant difference with DW ($p > 0.05$) indicating that washing with either these three media will produce similar moisture content. This suggests that washing using nanobubble water does not have a significant effect on the moisture content of surimi samples except at low concentration. Thus, using distilled water might be better as it is simpler to procure rather than using nanobubbles water. Higher nanobubble concentration water used might lead to nanobubble residue in the surimi sample. Traces of nanobubble present in the surimi sample might have filled the cavity which was supposedly occupied by water, thus, decreasing the moisture of the surimi.

Table 7.3: Effects of different washing media on moisture content of surimi samples following overnight frozen storage (-18°C). *Data are means of triplicates (n = 3). Different letters represent significant difference (p < 0.05)*.

SAMPLE	MOISTURE CONTENT (%)
DW	$76.81^{b} + 0.13$
LN	$79.54^{a} + 1.41$
MN	$77.46^{ab} + 0.67$
HN	$75.77^{b} + 1.38$

7.3.2 Expressible moisture content and water holding capacity

Expressible moisture content (EMC) and water holding capacity (WHC) play an important role in determining the gel strength of a surimi gel. Fish gel which possesses lower EMC will display a much rigid structure (Yoon and Lee, 1990). Low WHC and high EMC have been found to cause a decrease in gelling ability of surimi (Nopianti *et al*., 2012; Benjakul *et al*., 1997). Thus, WHC can be used as an indicator of surimi quality.

Table 7.4 records the EMC and WHC of surimi samples washed with different types of water. HN displayed the highest EMC whilst LN the lowest. Only LN and HN displayed a significant difference in EMC when compared ($p < 0.05$). DW and MN did not display any significant difference between them ($p > 0.05$) and were found to be in between the lowest and highest EMC value.

As for WHC, LN significantly ($p < 0.05$) recorded the highest value when compared to DW, MN and HN. This might suggest that washing using LN water increased the WHC of surimi gel. Washing using LN was found to be able to increase about 8.47% WHC compared to using DW. Low nanobubble

concentration water might be able to remove some components which affect the WHC of the fish protein. This is due to the fact that nanobubble present in water is known to attach itself to other particles and is often washed away as flotation during cleaning (Che and Theodorakis, 2017). Other than that, nanobubble is also known to attract protein with different affinity (Temesgen *et al*., 2017). Therefore, it might successfully remove the water-soluble sarcoplasmic protein which disrupts WHC and gel forming ability. Data obtained suggest that washing at higher concentration of nanobubble did not have a significant effect on the WHC of fish gel. Higher concentration of nanobubble water might leave some traces of nanobubbles within the protein matrix which disrupt the formation of protein networks to hold water. This might be true as nanobubble possesses positive ions in normal water (Temesgen *et al.,* 2017; Zimmerman *et al*., 2011). Due to this, unwanted interaction such as ionic bonds with proteins could disrupt the gelation process.

Table 7.4: Effects of different washing media on the expressible moisture content and water holding capacity of surimi samples following overnight storage at -18°C. *Data are means of triplicates (n = 3). Different letters represent significant difference (p < 0.05)*.

SAMPLE	EXPRESSIBLE MOISTURE CAPACITY (%)	WATER HOLDING CAPACITY (%)
DW	$39.59^{ab} \pm 1.47$	$53.71^{b} \pm 1.70$
LN	$37.36^{b} \pm 1.01$	$58.26^{a} \pm 1.70$
MN	$39.94^{ab} \pm 1.15$	$54.34^{b} \pm 1.02$
HN	$41.89^{a} \pm 1.60$	$51.16^{b} \pm 1.70$

7.3.3 Texture analysis

Breaking force, breaking deformation and gel strength were determined and calculated for each sample to understand the effect of washing with different nanobubble concentrations on the texture of fish gel samples. These textural analyses will provide information on the elasticity, rigidity and other texture characteristics of fish gel. Figure 7.4 depicts the effect of different washing media on the breaking strength of fish gel. Fish gel washed using LN showed the highest value of breaking strength followed by MN, DW and HN.

In addition, as nanobubble concentrations increased, it was found that the breaking strength of the sample decreased which might suggest that an increase in the number of nanobubbles present during washing might have a detrimental and negative effect on breaking strength rather than improving it. Other than that, all samples showed a significant difference in breaking strength ($p < 0.05$) which suggests that different types of water used for washing had a significant effect on the breaking strength.

Figure 7.4 also shows that fish gel washed using LN has 25% more breaking strength when compared to DW. In contrast, fish gel washed using HN yielded 12% less breaking strength when compared to DW. This is a clear indication that washing using nanobubbles only at a certain concentration improves the breaking strength of surimi gel. Higher concentration of nanobubbles causes deteriorating effect on the breaking strength of the surimi gel.

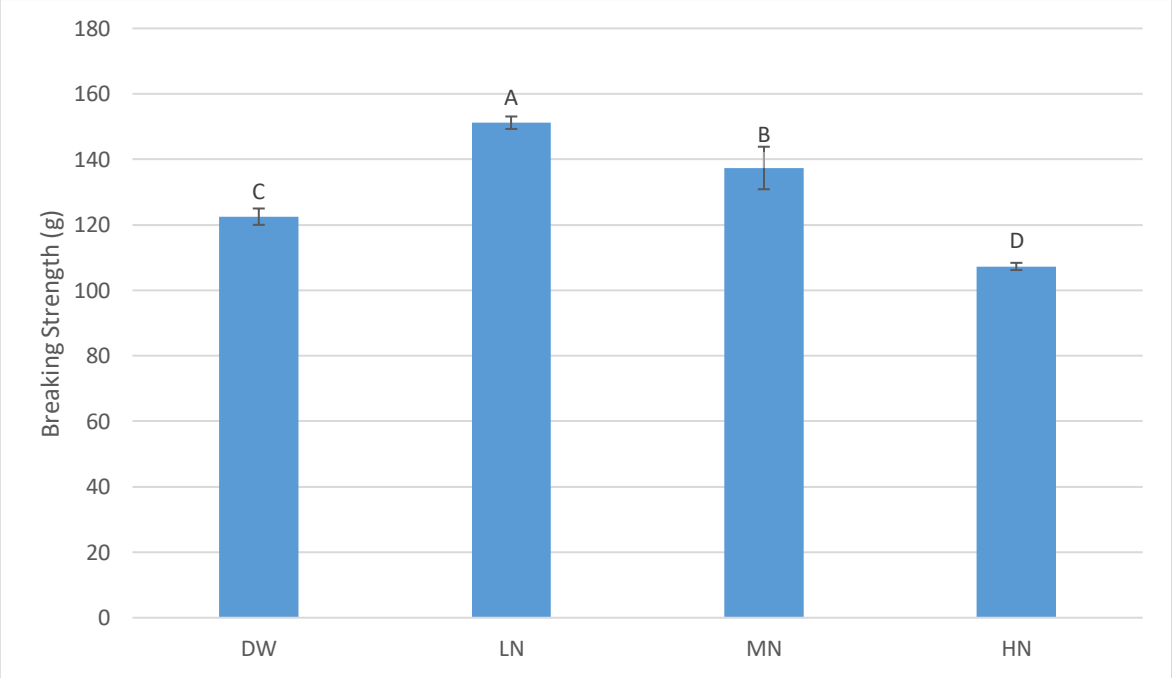


Figure 7.4: Breaking strength of surimi gel samples washed with different types of washing water. *Data are means of triplicates (n = 3) with bars indicating standard deviation (SD). Different letters indicate significant difference (p < 0.05).*

In contrast to that, Figure 7.5 shows an increase in breaking deformation as nanobubble water concentrations increased. From the Figure, DW recorded the highest breaking deformation followed by HN, MN and LN. LN showed a significant difference in breaking deformation when compared to all samples ($p < 0.05$). Meanwhile, MN and HN did not show any significant difference in breaking deformation between them ($p > 0.05$). However, no significant difference was found between samples washed with DW and HN. This indicates that washing using DW is preferable in order to maintain a high breaking deformation of fish gel compared to using HN which is more troublesome to procure.

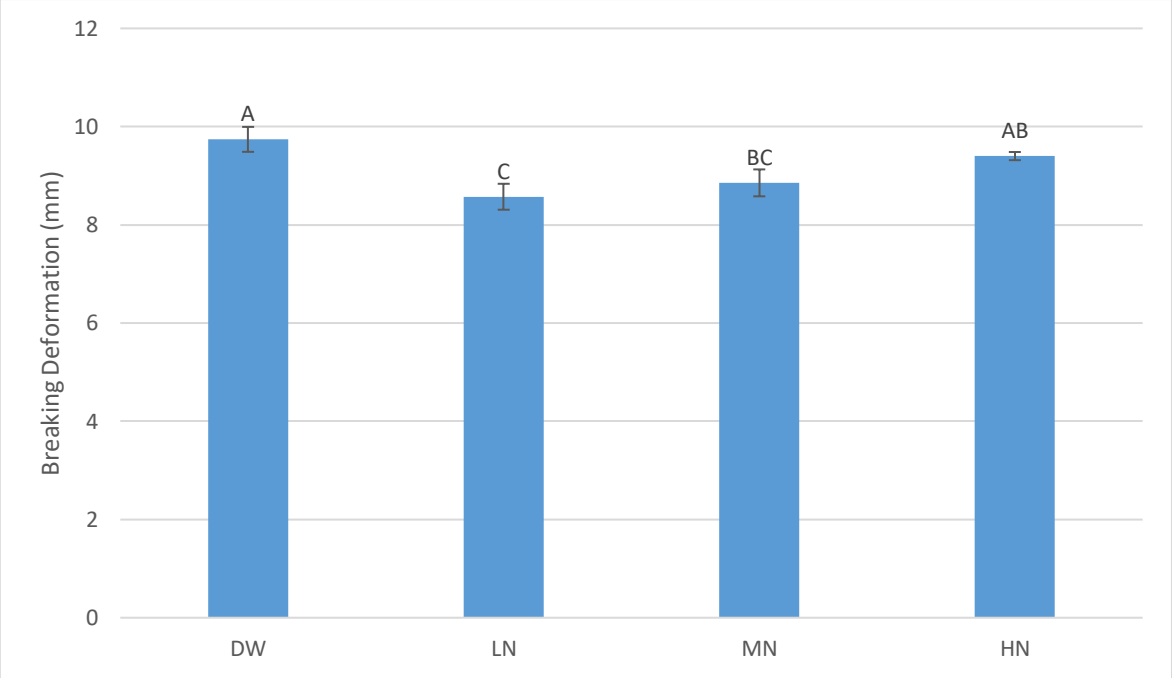


Figure 7.5: Breaking deformation of surimi gel samples washed with different types of washing water. *Data are means of triplicates (n = 3) with bars indicating standard deviation (SD). Different letters indicate significant difference (p < 0.05).*

Figure 7.6 shows the gel strength of surimi samples washed with different types of washing media. LN displayed the highest value of gel strength. This indicates that washing using LN might be more efficient in removing water soluble protein which inhibits gel formation. This result also supports earlier result where LN recorded the highest WHC. Higher gel strength is associated with good WHC. LN displayed a high gel strength which might suggest that the nanobubbles present in smaller amount attached itself to particles and these particles were successfully extruded out without leaving any nanobubble residue. Nanobubbles are well known for its high mass transfer efficiency and have been widely use in water treatments (Zimmerman *et al.,* 2011). Nanobubbles have been reported to remove up to 95% turbidity, total solids and silica in wastewater (Temesgen *et al.,* 2017) which could explain the efficiency in surimi washing.

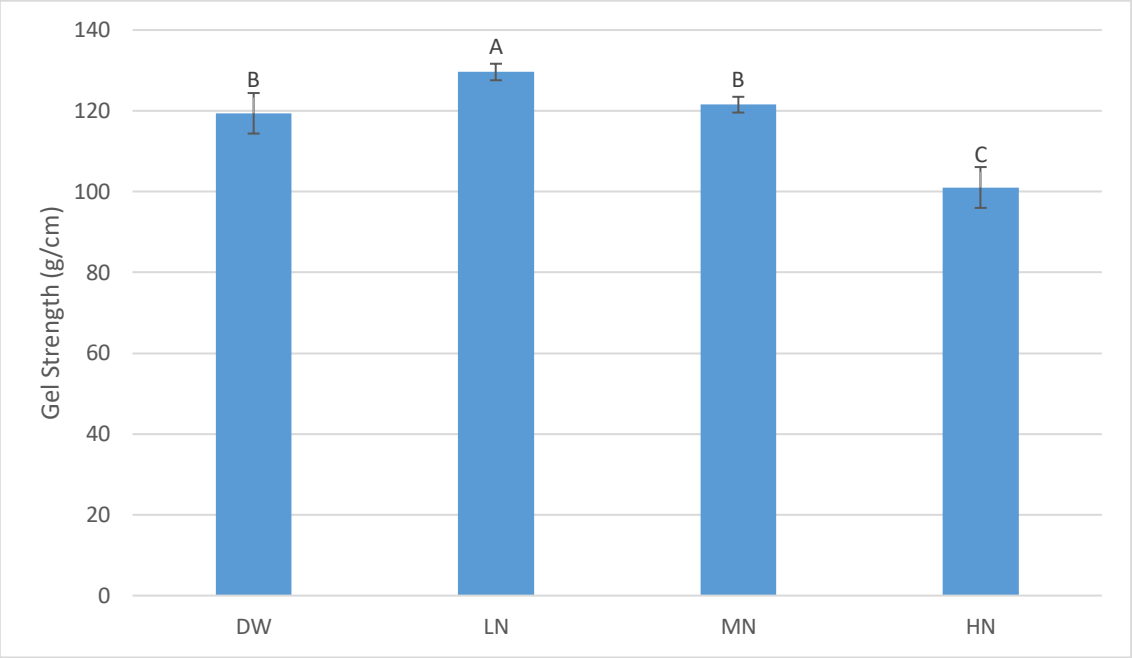


Figure 7.6: Gel strength of surimi gel samples washed with different types of washing water. *Data are means of triplicates (n = 3) with bars indicating standard deviation (SD). Different letters indicate significant difference (p < 0.05).*

Surimi washed using HN displayed the lowest gel strength when compared to other samples ($p < 0.05$). This suggests that higher concentration of nanobubbles might have washed some functional proteins which affected the gel strength of the surimi. High specific area (surface area per volume) increased with increased concentration of nanobubbles (Ushikubo *et al.,* 2010). The increased number of OH^- radical present might attract and clean other components which is desired to form gel network. Radical OH^- is known to remove organic matter efficiently (Ghadimkani *et al*., 2016). Positively charged nanobubbles also might attract some negatively charged components which is essential to gelation process. Low gel strength might also be caused by presence of nanobubble in the structure.

Another inference that can be made is that, a high amount of nanobubble might leave residues within the surimi which cause physical interactions and changes. Low gel strength might also be caused by presence of nanobubble in the structure that changes the physical properties of the gel such as density, porosity and rigidity. Nanobubbles are known to possess charges which could interact with hydrophobic and ionic bonds (Che and Theodorakis, 2017; Agarwal *et al*., 2011). It also contains strong hydrogen bonds on the interfacial surface (Temesgen *et al.,* 2017). These might induce chemical interaction between nanobubbles with water and protein. The chemical interaction induced by nanobubble with hydrophobic and ionic bonds might disrupt the formation of gel protein matrix. Other than that, due to the present of nanobubble, undesired nanobubble-protein interaction might occur rather than the desired protein-protein interaction.

DW and MN did not show any significant difference between them ($p > 0.05$). There might be different factors which affect the gel strength of these two samples. Result displayed from HN indicates that higher concentration might wash away not only undesirable component which inhibits gel formation but also other important component which contributes to the gel strength (over efficient). On the other hand, the lower gel strength value recorded by DW might be caused by washing inefficiency. Some lipids and water-soluble protein such as sarcoplasmic protein might still be present in the fish protein system which inhibited gel network formation. Even though there was no significant difference between these two samples, the factor of its reduced gel strength might not be the same.

7.3.4 Effect of different washing media and numbers of washing cycle

7.3.4.1 Moisture content

Table 7.5 records the moisture content of each sample following overnight frozen storage (-18°C). It is apparent that more washing cycles increased the moisture content of the sampled. Karthikeyan *et al*. (2006) also reported similar result and reported an increase in moisture content as washing cycles increased. Repeated washing increased hydration of myofibrillar which changed the moisture content of surimi (Park and Lin, 2005).

LN was found to display the highest moisture content at all washing cycle and recorded the highest moisture content on the third washing cycle (LN3). HN however, displayed a contrast result with low percentage of moisture content throughout all washing cycles (HN1, HN2 and HN3) when compared to other samples.

As discussed earlier, higher amount of nanobubble concentration present in the water might attach itself to the myofibrillar concentrate and was not discarded out fully during filtration. However, it seems that as washing cycle increased, the moisture content of all samples increased as well. This suggests that hydration of myofibrillar protein supersedes the effect of nanobubble concentration present within the system.

Another inference that can be made is that the nanobubble which was attached to myofibrillar protein might already cover all the cavities, gaps and holes within the myofibrillar network making it compact. Thus, increasing amount of

nanobubble added might not have an effect on the moisture content due to the absence of space within the surimi. Both these inferences could be further studied to determine the actual reaction and interaction within the surimi.

Table 7.5: Effects of different washing media and numbers of washing cycle on moisture content of surimi samples following overnight frozen storage (-18°C). *Data are means of triplicates (n = 3). Different letters indicate significant difference (p < 0.05).*

SAMPLE	MOISTURE CONTENT (%)
DW1	$76.81^{C} \pm 0.13$
DW2	$76.12^{C} \pm 0.42$
DW3	$79.25^{AB} \pm 0.27$
LN1	$79.54^{A} + 1.41$
LN2	$79.00^{AB} \pm 0.69$
LN3	$77.35^{ABC} \pm 0.44$
MN1	$77.46^{ABC} \pm 0.67$
MN2	$77.65^{ABC} \pm 0.48$
MN3	$79.19^{AB} \pm 0.42$
HN1	$75.77^{C} \pm 1.38$
HN2	$75.81^{C} \pm 1.00$
HN3	$77.04^{BC} \pm 0.44$

Furthermore, increased washing cycle only increased the hydration of the myofibrillar protein which are in agreement with other findings (Amiza and Ain, 2012; Karthikeyan *et al*., 2006; Park and Lin, 2005). This result could be supported by the moisture content shown by samples washing with 1- and 2-

washing cycle. Some samples did not even show any significant difference between the washing cycles ($p > 0.05$).

7.3.4.2 Expressible moisture content and water holding capacity

Expressible moisture content (EMC) and water holding capacity (WHC) of each sample were determined to understand the gelling structure and protein network ability of the surimi samples. Figure 7.7 displays the EMC and WHC of all samples following overnight frozen storage at -18°C. It was found that EMC decreased with increasing number of washing cycles and *vice versa* for WHC. DW3, LN3, MN3 and HN3 recorded the highest WHC when compared to the samples washed with 1- and 2-cycles. Increasing WHC indicates that increased protein network was formed which could retain more water within the system. This in turn indicates that by increasing the number of washing cycle, the WHC of fish gel also improves regardless of the type of washing media used. As number of washing increased, the myofibrillar protein became more concentrated thus improving WHC and EMC of the surimi (Dileep and Shamasundar, 2006). Nevertheless, type of washing media used did affect the strength of fish gel WHC. LN3 displayed the highest WHC followed by DW3, MN3 and HN3, thus, indicating that LN3 might yield the best gelling strength according to the WHC value recorded.

The most significant difference in WHC can be observed in DW and HN. There was an increase up to 10% in WHC from the washing with 2-cycles to washing with 3-cycles. Dileep and Shamasundar (2006) reported the same findings using normal water. However, using higher concentration of nanobubble water

might also create an internal reaction or chemical interaction which strengthens the WHC. These interactions might be a side-effect of the increased myofibrillar concentration and high amount of nanobubbles present with each washing cycles. However, further investigation on the microstructure of the samples is required to validate these claims.

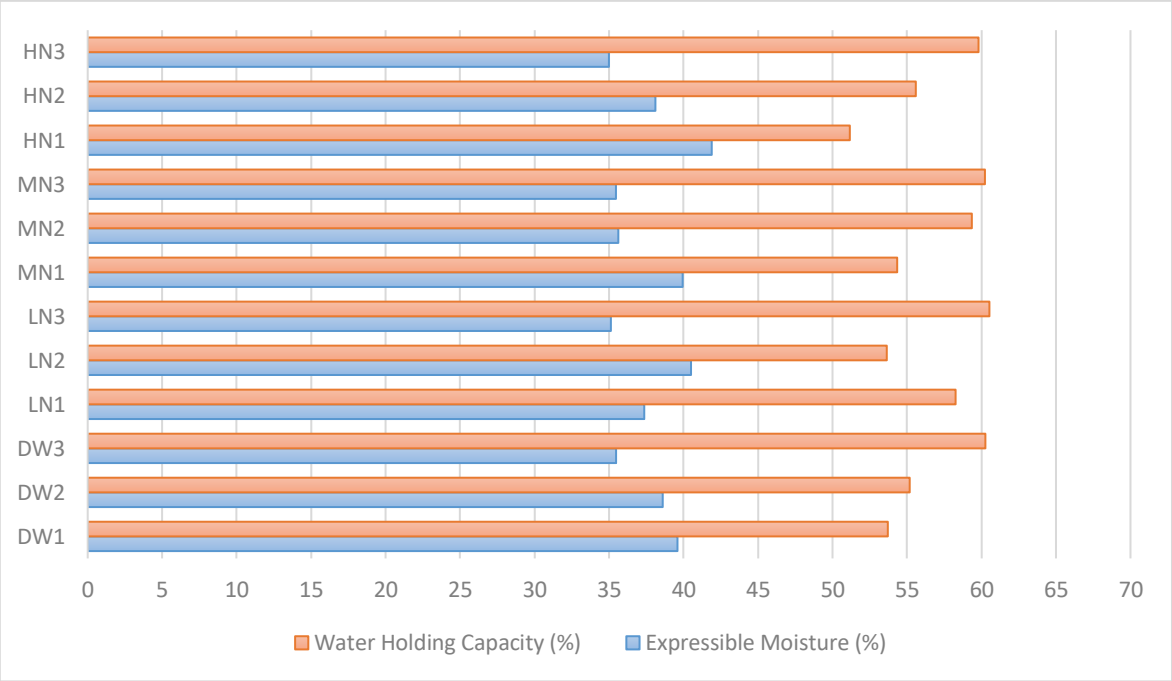


Figure 7.7: Effects of different washing media and numbers of washing cycle on the expressible moisture content and water holding capacity of surimi samples following overnight frozen storage at -18°C.

7.3.4.3 Texture analysis

Surimi samples washed with different types of washing media were subjected to three different washing cycles. These samples were then made into surimi gel and their textural properties were analysed. The textural properties will indicate the quality of surimi and determine if different washing cycles have a significant effect on the quality of surimi. Breaking strength, breaking deformation and gel strength were used as a quality indicator of texture.

Figure 7.8 shows the effects of different washing media and numbers of washing cycle on breaking strength of surimi samples following overnight frozen storage at -18°C. From the Figure, it can be seen that DW showed increasing amount of breaking strength as washing cycle increased. The breaking strength of DW was found to increase to 8.3% on the second washing cycle and 25% on the third washing cycle. Sample washed with HN also displayed similar trend to DW. This indicates that washing cycle has a significant effect on the breaking strength of samples washed using DW and HN. MN did not show any significant difference with the increasing number of washing cycle ($p < 0.05$) whilst LN showed instability of breaking force with no trend.

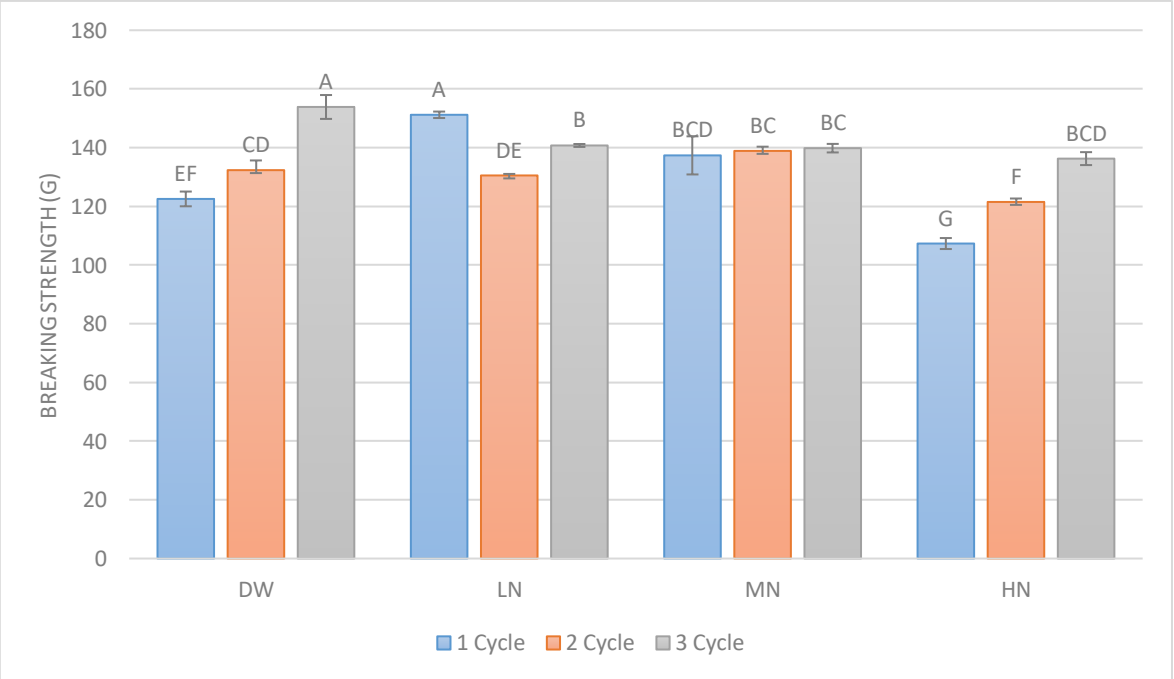


Figure 7.8: Effects of different washing media and numbers of washing cycle on breaking strength of surimi samples following overnight frozen storage at -18°C. *Data are means of triplicates (n = 3) with bars indicating standard deviation (SD). Different letters indicate significant difference (p < 0.05).*

Sample which was prepared with 3-washing cycle with DW and 1-washing cycle with LN showed similar result and recorded the highest significant breaking strength ($p < 0.05$). This suggests that by using LN instead of DW, the amount of water used for surimi preparation could be reduced whilst still maintaining a high breaking strength. HN showed the lowest breaking strength at 1- and 2-cycle washing. It has been discussed earlier that high concentration of nanobubbles present during washing might remove important components which contribute to protein functionality. However, after 3-cycle washing, HN displayed similar result to MN.

Figure 7.9 shows the effects of different washing media and numbers of washing cycle on breaking deformation of surimi samples following overnight frozen storage at -18°C. From the Figure, it is apparent that breaking deformation increased as the number of washing cycle increased. This indicates that washing cycle has a significant effect on the breaking deformation. LN3, MN3 and HN3 recorded the highest breaking deformation which further supports the significant effect of washing cycle on breaking deformation. Although these three samples showed the highest breaking deformation; LN2, MN2 and HN2 did not show any significant difference when compared between each other ($p < 0.05$). An inference can be made that 2-cycle washing might produce similar result to 3-cycle washing on breaking deformation. Thus, lower number of washing cycle might be preferred and recommended to the industry to avoid water waste.

Figure 7.9 also shows that the addition of nanobubbles in washing media for 2- and 3-cycles washing improved the breaking deformation significantly. Breaking

deformation was found to increase up to 44% when compared to using nanobubbles water for 1-cycle washing. Washing using nanobubble water also increased breaking deformation at least 10% when compared to using DW.

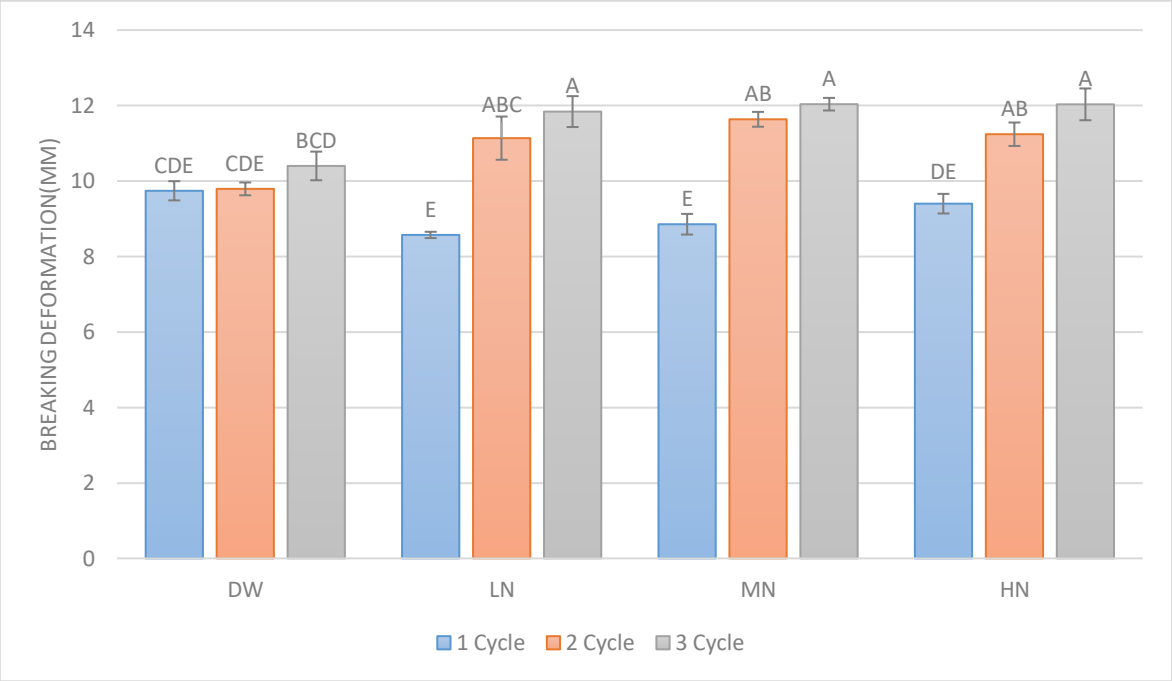


Figure 7.9: Effects of different washing media and numbers of washing cycle on breaking deformation of surimi samples following overnight frozen storage at -18°C. *Data are means of triplicates (n = 3) with bars indicating standard deviation (SD). Different letters indicate significant difference ($p < 0.05$).*

Figure 7.10 shows the effects of different washing media and numbers of washing cycle on gel strength of surimi samples following overnight frozen storage at -18°C. The obtained data are in agreement with several researchers which reported that gel strength of surimi increased as the number of washing cycle increased (Amiza and Ain, 2012; Karthikeyan *et al*., 2006; Park and Lin, 2005). The obtained data are also in agreement with this result in Figure 7.7 (WHC) where washing using 3-cycles exhibited the highest WHC. It was also found that the gel strength of all samples using 3-cycles washing displayed no

significant difference between them (p < 0.05). This suggests that 3-cycles washing might be the optimum washing cycle for all samples. By the third washing cycle, all unwanted components which disrupted gel formation had been efficiently removed. Protein functionality might be well preserved during overnight storage after washing with 3-cycles.

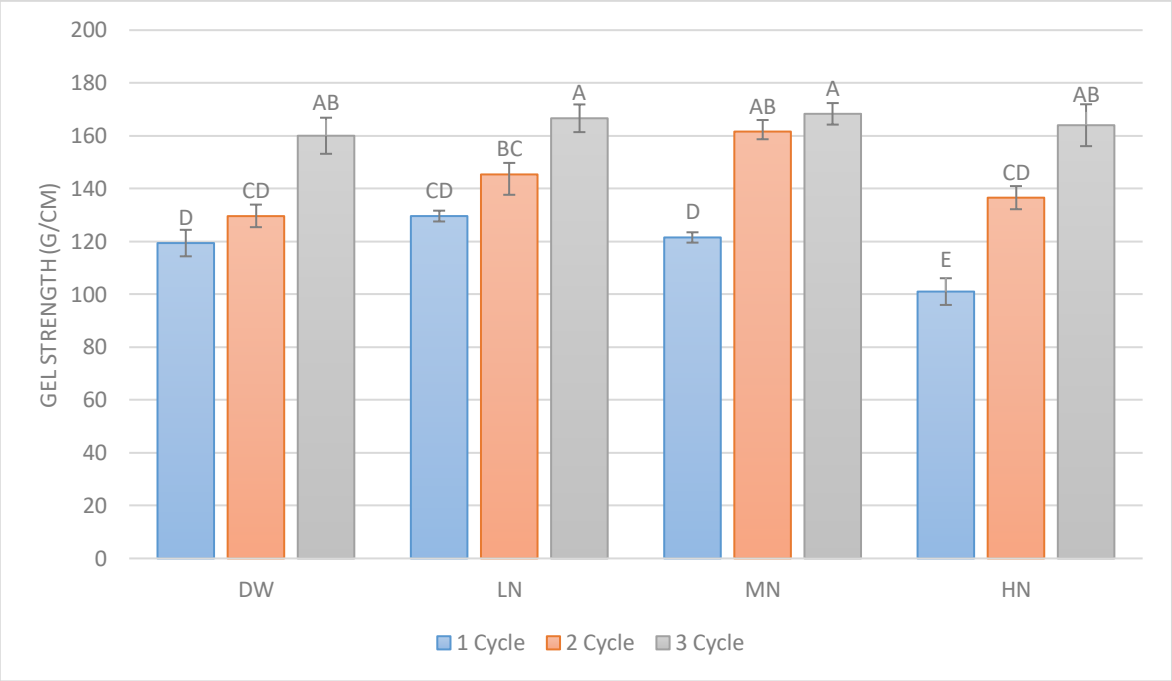


Figure 7.10: Effects of different washing media and numbers of washing cycle on gel strength of surimi samples following overnight frozen storage at -18°C. *Data are means of triplicates (n = 3) with bars indicating standard deviation (SD). Different letters indicate significant difference (p < 0.05).*

Although there was no significant difference between all samples at 3-cycle washing, the average gel strength for DW3 was still lower when compared to LN3, MN3 and HN3. This suggests that nanobubble water used for washing still provides positive outcome when compared to washing using the conventional water. There are two factors that might explain why washing using nanobubble water yields higher gel strength. One is the efficiency of nanobubble to attract

unwanted component and float it out during washing and filtration. Nanobubbles has the ability to attract organic matters, metal ions and oils due to the attraction of opposites charge between them (Temesgen *et al.,* 2017). Another factor is the nanobubble residue actually strengthens the gel network by acting as filler and producing ionic bonds with exposed protein network during gelation. This might be the case as Zimmerman *et al*. (2011) reported that nanobubbles possessed positive charges in normal water. However, further investigation of nanobubble used as a filler should be conducted to support this inference. Microstructure study could very well validate these inferences.

Washing using MN displayed no significant difference in gel strength between 2- and 3-cycle washing ($p > 0.05$). This indicates that lesser amount of water could be used to achieve higher gelling strength by incorporating nanobubble. By using only 2-cycle washing, about 3 kg of water could be saved in the processing of 1 kg surimi. A positive outcome could be achieved by using nanobubble water which could reduce water waste while maintaining the surimi gel quality.

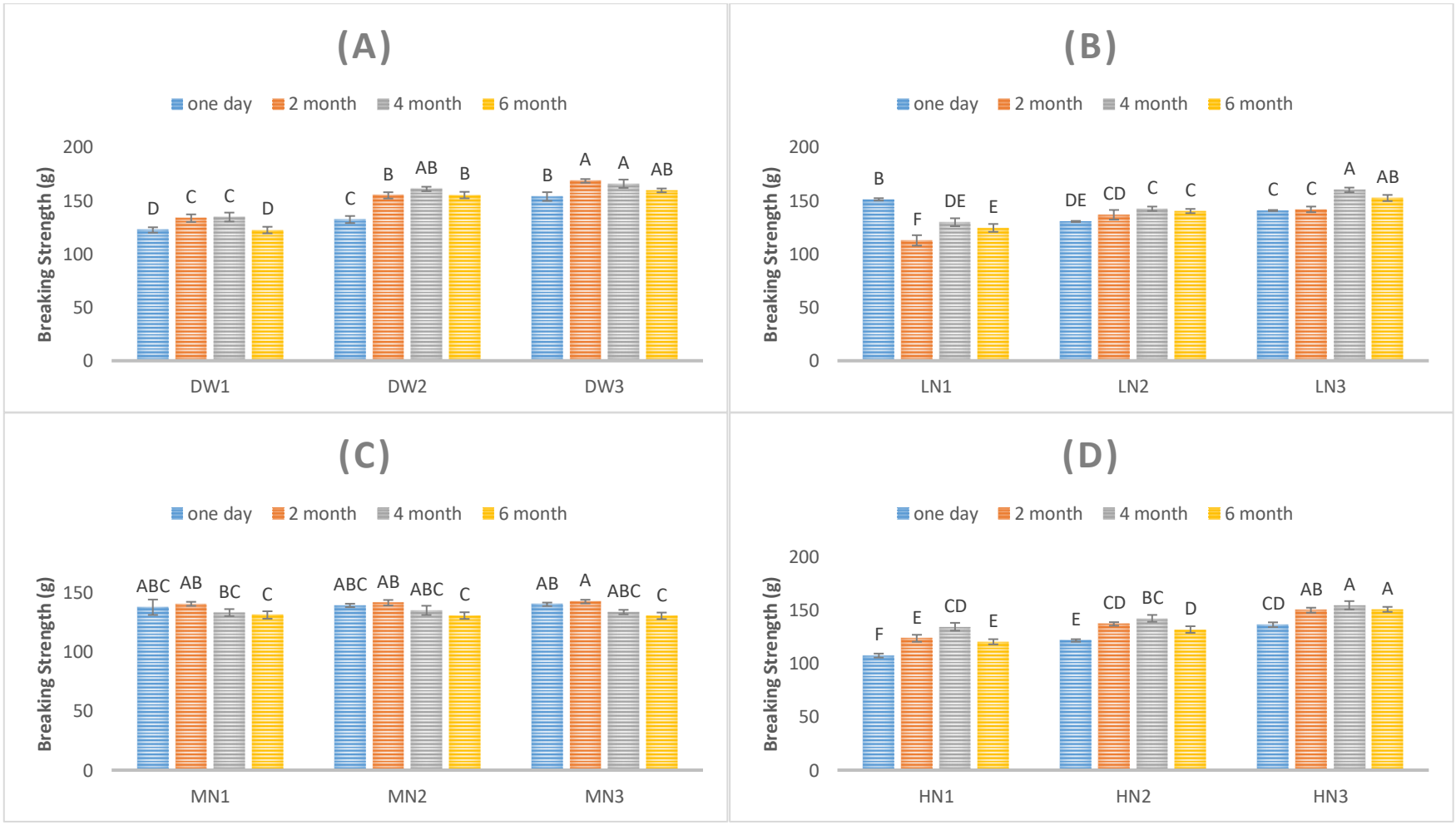
7.3.4.4 Effects of frozen storage on texture of surimi gel

Frozen storage is the most common means of food preservation used by fishermen and surimi manufacturers. However, frozen storage causes deterioration and denaturation of myofibrillar protein. Therefore, surimi needs to be properly washed before it is frozen. Components such as lipid, blood and sarcoplasmic protein cause protein denaturation during frozen storage. Lipid causes oxidation and release of free radical during frozen storage. Figures

7.11a-d show the effects of frozen storage (one day, two months, four months, six months) on breaking strength of surimi samples washed with different washing media and at different washing cycles.

Figure 7.11(a) shows that DW1 yielded the lowest breaking strength when compared to other samples. DW2 and DW3 showed good frozen stability up to six month. This is indicated when the value of breaking strength did not display any significant difference up till six month of frozen storage ($p > 0.05$). This indicates that washing using 2- or 3-cycles of DW did not have a significant effect on the breaking strength stability of sample. Thus, 2-cycles washing using DW could be adapted by the industry to reduce water waste while preserving the breaking strength of surimi.

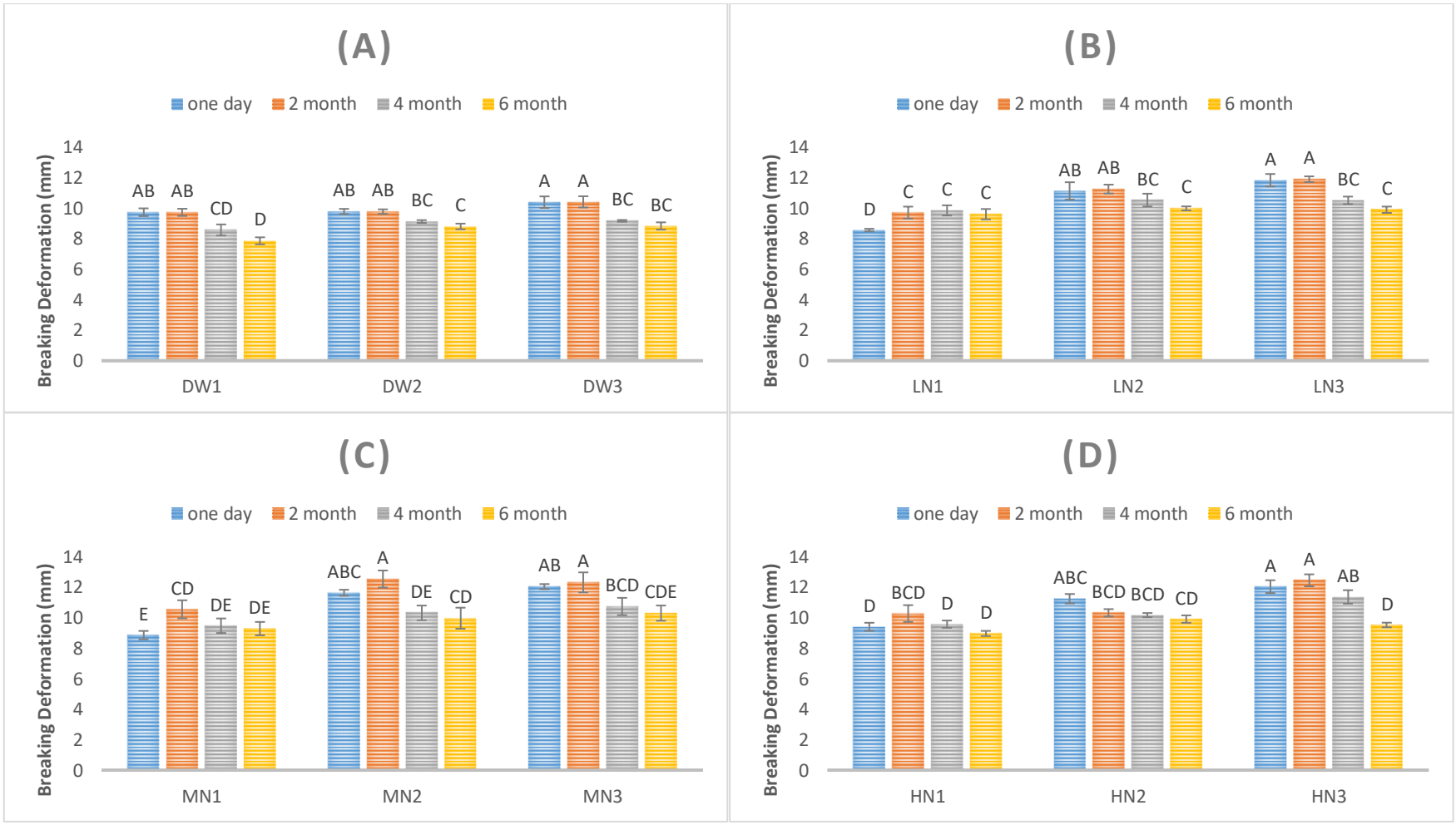
Figure 7.11(b) shows that the breaking strength increased with increasing washing cycles and frozen storage. LN3 at four month frozen storage displayed the highest breaking strength when compared to LN1 and LN2. This inidicates that washing using LN improved the breaking strength of surimi gel with increasing washing cycle. However, LN2 showed promising result on maintaining the breaking strength stability thoroughout six month frozen storage. Although the breaking strength was 12.5% less than LN3, the breaking strength showed no significant difference up to 6 month frozen storage ($p > 0.05$).



Figures 7.11: Effects of frozen storage (one day, two months, four months, six months) on breaking strength of surimi samples washed with (a) DW, (b) LN, (c) MN and (d) HN at different washing cycles. *Data are means of triplicates (n = 3) with bars indicating standard deviation (SD). Different letters indicate significant difference ($p < 0.05$).*

Figure 7.11(c) shows that MN1, MN2 and MN3 display similar result throughout the six month frozen storage. This indicates that increasing washing cycle did not affect the breaking strength of surimi samples and their frozen stability. Figure 7.11(d) suggests that breaking strength increased with increasing washing cycle and also frozen storage time. This is represented by result displayed by HN3 at four and six month which recorded the highest breaking strength when compared to HN1 and HN2.

Figures 7.12a-d show the effects of frozen storage (one day, two months, four months, six months) on breaking deformation of surimi samples washed with different washing media and at different washing cycles. Figure 7.12(c) shows that washing using MN for 2-cycle compromised the breaking deformation. MN2 displayed a significant reduction in breaking deformation at four months frozen storage. ($p < 0.05$). MN1 maintained breaking deformation up to six months without any significant difference ($p < 0.05$). In addition, HN1 and HN2 displayed similar result which is displayed in Figure 7.12(d). Only HN3 was found to display a significant reduction in breaking deformation after six months frozen storage ($p < 0.05$). An inference can be made that increased washing cycle caused a significant decrease in breaking deformation frozen stability. One cycle washing was deemed to display the best breaking deformation frozen stability for all samples regardless of the different washing media used.

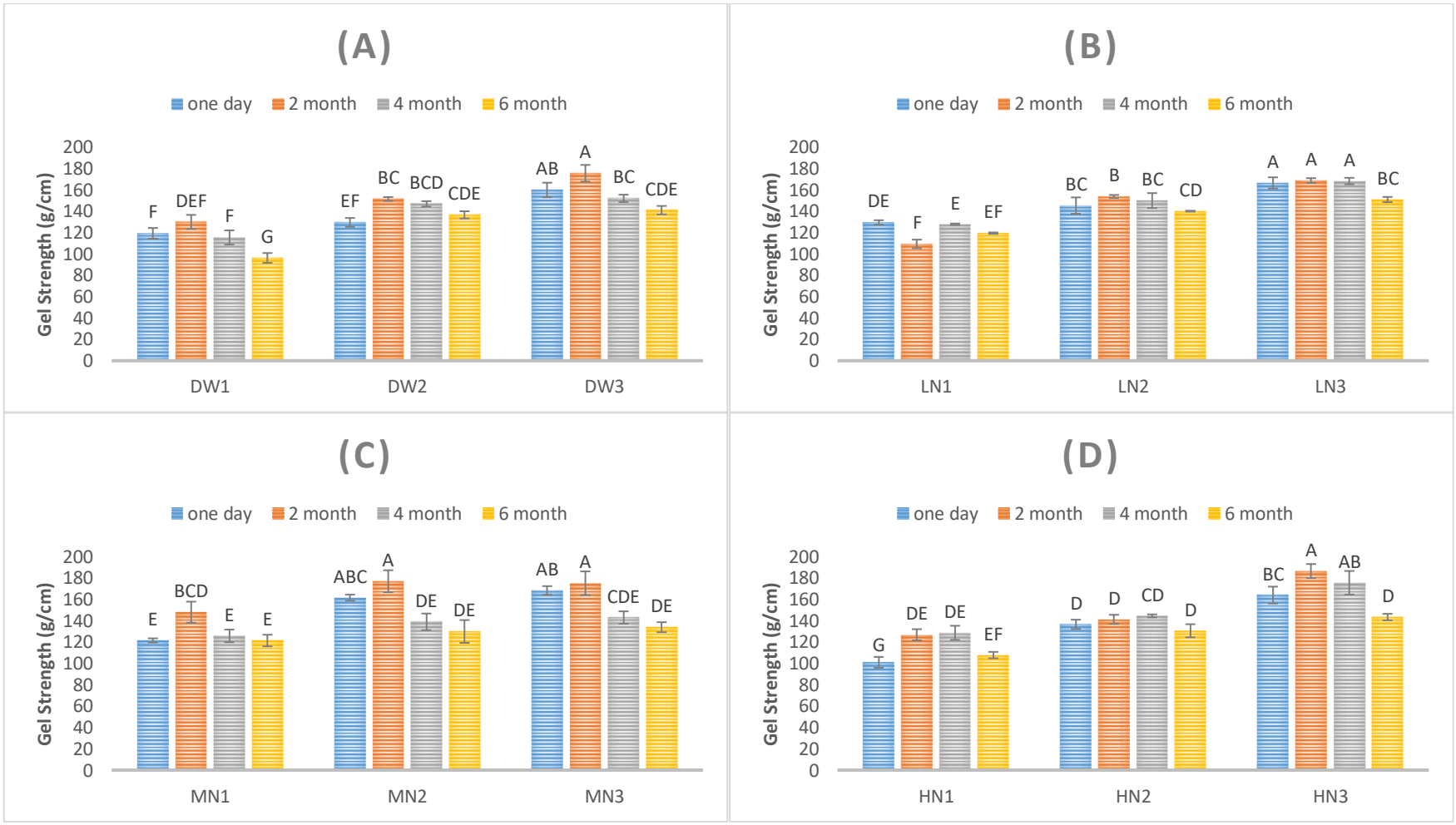


Figures 7.12: Effects of frozen storage (one day, two months, four months, six months) on breaking deformation of surimi samples washed with (a) DW, (b) LN, (c) MN and (d) HN at different washing cycles. *Data are means of triplicates (n = 3) with bars indicating standard deviation (SD). Different letters indicate significant difference ($p < 0.05$).*

Figure 7.13 shows the gel strength of all samples for a period of six months frozen storage. Samples which were washed for only one washing cycle maintained stable gel strength without any significant change up to six months except for DW1. This indicates that the presence of nanobubbles during one washing cycle had a significant effect on gel strength stability of fish gel during frozen storage.

DW1 showed the lowest stability after six months frozen storage as it recorded the lowest gel strength after six months when compared to all samples. DW2 on the other hand displayed the best gel strength frozen stability by maintaining the gel strength value without any significant change up to six months. After six months frozen storage, the gel strength of DW2 and DW3 displayed similar results which suggest that by increasing the washing cycle from 2 to 3 using DW did not significantly increase the gel strength frozen stability.

Figure 7.13(b) shows that LN had the best stability during frozen storage. This is presented by the result shown by LN1 and LN2 which were found to be able to maintain gel strength up to six months without any significant changes in its value ($p > 0.05$). Meanwhile, LN3 showed the highest gel strength and did not display any significant difference up to four months of frozen storage. HN1 and HN2 (Figure 7.13d) showed similar result as LN1 and LN2 which maintained the gel strength in frozen storage up to six months. HN3 only recorded gel strength stability up to four months. Even though the gel strength of HN3 was found to decrease at six months, HN3 still recorded the highest gel strength compared to all samples after six months frozen storage.



Figures 7.13: Effects of frozen storage (one day, two months, four months, six months) on gel strength of surimi samples washed with (a) DW, (b) LN, (c) MN and (d) HN at different washing cycles. *Data are means of triplicates (n = 3) with bars indicating standard deviation (SD). Different letters indicate significant difference (p < 0.05).*

Figure 7.13(c) shows that MN had the lowest frozen stability due to the result shown by MN2 and MN3. These two samples began to display a significant reduction in gel strength on the fourth month of frozen storage ($p < 0.05$). Even though MN2 and MN3 recorded high values of gel strength, these values could not be maintained and were found to deteriorate earlier than other samples. Result presented in Figure 7.13 also suggests that increased washing cycle reduced the gel strength of surimi gel during prolonged frozen storage thus suggesting that increased number of washing cycle increased the gel strength of fish gel but compromised its frozen stability.

Another reason for decreasing gel strength is protein denaturation. Protein denaturation could be caused by several factors such as changes in ionic strength, physical damage by ice crystals, dehydration and changes in surface tension (Mosavi-Nasab, 2003). Frozen storage also causes myosin to aggregate thus altering the structure of fish gel (Mosavi-Nasab, 2003). However, nanobubble was found to be very stable even in cold temperatures (Che and Theodorakis, 2017). By washing surimi with nanobubble water, inadvertently some nanobubbles attach to the myofibrillar protein and increase the gel strength and also improve the frozen stability of surimi gel. With increased washing cycles, more and more nanobubbles will attach to fish myofibrillar protein and subsequently increase the frozen stability. In general, washing using nanobubble water does not increase the shelf-life stability of surimi.

7.4 CONCLUSION

Washing is an important step in surimi processing. By washing adequately and efficiently, surimi quality and shelf life could be increased. However, the surimi industry currently wastes too much water to wash surimi. In order to produce 1 kg of surimi, 3 kg of water is needed for washing. Thus, an alternative for washing could remediate this and help protect the environment.

From the work carried out in the present Chapter, low concentration of nanobubbles in water (LN) was found to yield the best gel strength compared to distilled water. Another observation found is that as concentration of nanobubble used in the washing water increased, the gel strength of fish gel decreased after an overnight frozen storage. This suggests that higher concentration of nanobubble might wash away some functional protein which is critical to gel network formation. Fish gel washed with low nanobubble concentration was found to possess 8.3% higher gel strength than washed with distilled water. This information might suggest that using low nanobubble concentration water might produce a better-quality surimi.

In addition to that, the effect of different washing cycles using nanobubble water on the fish gel quality was also investigated. It was found that, as the number of washing cycles increased, the gel strength of all the samples also increased regardless of the type of washing media used. Initially, there was a significant difference in gel strength at 1-cycle and 2-cycle washing depending on type of washing media used. However, when 3-cycles washing was used, all samples displayed no significant difference in gel strength. Nevertheless, washing using

medium nanobubble concentration (MN) was found to yield similar gel strength at 2- and 3-cycle washing. Thus, the surimi industry could research the potential of using medium nanobubble concentration water as an alternative to reduce water waste and number of washing cycle to process surimi. The number of washing cycle using MN could be reduced to 2-cycle rather the commercial practice, 3-cycle. However, this sample is not stable for longer storage period. Manufacturers could adopt this technique if surimi manufacturing is done immediately or less than 2 to 4 months.

To further complement the effects of using nanobubble water during washing, the frozen stability of fish gel was also investigated. Sample which was washed with nanobubble water for 1-cycle was found to be the most stable. LN1, MN1 and HN1 were able to maintain gel strength up to six months in contrast to DW (four months). Although these samples were stable, they still yielded the lowest gel strength when compared to 2- and 3-cycle washing. LN3 yielded the highest and stable gel strength without any significant difference ($p > 0.05$) throughout frozen storage up to four months.

To summarise, low nanobubble concentration water yielded the highest gel strength at 1-cycle washing after overnight frozen storage. If a product is processed overnight, then washing using LN for only 1-cycle would be the best option. By using medium nanobubble concentration water, washing cycle of surimi can be reduced to 2-cycle washing. This means that washing using MN could reduce the amount of water waste whilst maintaining high gelling strength compared to other washing media. Although 1-cycle washing displayed the best

frozen shelf life stability, MN3 however yielded the highest gel strength and consistency. By reducing the washing cycle to 1-cycle, the samples were stable for up to six months but with lower gel strength compared to higher number of washing cycles used.

This preliminary research on nanobubble water used as a washing medium could be beneficial to the industry if a much thorough and further investigation could be done. The microstructure study of fish gel washed with nanobubbles water would further validate the variation of gel strength with different washing media. Other than that, the analysis of washed water could help identify the particles which are efficiently washed and extracted by nanobubble water. Water waste during surimi processing could be reduced in addition to increasing gel strength and frozen shelf life stability. The potential of using nanobubbles water might improve the surimi industry and further sustain the biological environment.

CHAPTER 8

General Conclusions and Recommendations for Future Work

8.1 GENERAL CONCLUSIONS

Various researches have been done on surimi due to new emerging theories and technologies. However, the basic and fundamental chemistry of surimi needs to be understood and studied again due to the changes in environment and technology. With advanced and more efficient technology, surimi industry should consider producing surimi with minimal processing and additives to produce a much healthier and high-quality surimi. At present, as consumers' awareness increases, they prefer fresh and more nutritious foods as compared to processed foods laden with additives and preservatives. Data obtained in the present work therefore can help the surimi industry towards achieving a healthier, more economical and more environmental friendly surimi product.

In Chapter 3, mannitol (a sugar with lower calorie and less sweetness) has been demonstrated as having promising results and could be considered as an alternative cryoprotectant over sucrose and sorbitol which are commercially used in the surimi industry. Rheological and textural analyses performed showed that mannitol yielded the highest gel strength which indicates a high-quality surimi product. The efficiency of rheological testing to predict the gel properties of surimi has also been shown. The effects of sugar concentrations on surimi gelling strength showed significant difference from 2% (w/w) to 4% (w/w) but not from 6% to 8%, suggesting that using 6% sugar concentration would be better in achieving a healthier low-calorie surimi as compared to the current commercial practice of 8%. The surimi industry could therefore consider applying these findings in the industry to produce healthier and more economical products.

In Chapter 4, different concentrations of salt were tested to understand their effect on the rheological and textural properties of surimi paste and gel. It was shown that as salt concentrations increased, so did the gel strength of the surimi samples. Additionally, it was also found that, salt alone without any addition of cryoprotectant could produce similar texture with surimi added with sugar which indicates that sugar could be eliminated if salt is added to surimi, hence, reducing the calorie and achieving a healthier surimi. However, although the highest salt concentration tested displayed the highest value of gel quality, shelf life study showed that the gel and protein networking was the least stable over six-month frozen storage when compared to other salt concentrations. The lowest salt concentration was found to yield the most stable surimi samples which suggest that salt does not act as a stabilizer, rather it alters the chemical structure of protein to produce better gelation. The obtained data could prompt the surimi industry to select the best salt-sugar formulation to produce healthier surimi products with improved quality.

In Chapter 5, the feasibility of using sago starch to increase the gel quality of surimi has been demonstrated. It was shown that sago starch exhibited a positive interaction with surimi and thereby increased its gel quality (structural rigidity). Increasing amount of sago starch also increased the breaking strength, breaking deformation and gel strength of the surimi sample. The gel strength was found to increase about 133% with 10% addition of sago starch. Sago starch was also tested for its role as a preservative and was found to be able to maintain gel strength up to four months of frozen storage. The obtained findings provide the surimi industry with yet another alternative which has never been

used before. It was also shown that sago starch had similar measurable quality to other starches (waxy-maize starch, wheat starch) commercially used in the surimi industry. Combination of additives also plays a role in producing high quality surimi as each additive interacts differently with myofibrillar protein. For example, sugar acts as a cryoprotectant that prevents protein denaturation without compromising the protein structure. Salt causes protein to solubilize thus forming protein gel network when protein is heated. Starch increases the gel strength of myofibrillar fish by acting as filler in a molecular level. By understanding these characteristics and interaction, combination of additives might produce a healthier, functional and quality surimi.

Finally, in Chapter 6, an alternative for washing surimi with nanobubble water was researched as compared to the current practice of using too much water which leads to high water wastage. It was found that the low concentration of nanobubbles in water yielded the best surimi gel strength as compared to distilled water. It was also found that as concentrations of nanobubble used in the washing media increased, the gel strength of surimi sample decreased after an overnight frozen storage. In addition to that, the effect of different washing cycles using nanobubble water on the fish gel quality was also investigated. It was found that, as the number of washing cycles increased from 1- to 2-cycle, the gel strength of all the samples also increased regardless of the type of washing media used, but not from 2- to 3-cycle. So, the number of washing cycle could be reduced to just 2-cycle rather than the commercial practice of 3-cycle. To further complement the effects of using nanobubble water during washing, the frozen stability of surimi samples was also investigated. Sample

which was washed with nanobubble water for 1-cycle was found to be the most stable up to six months of frozen storage.

Summarised below are the conclusions derived from the present work:

1. Mannitol can be an alternative to sucrose and sorbitol as surimi cryoprotectant.
2. Lesser sugar concentration could also produce similar gel strength.
3. Surimi added with 1% salt concentration (w/w) showed the best frozen shelf-life stability.
4. Sago starch produced positive result which could be used as a filler to increase gel strength equivalent to other starch sources used in current practice.
5. Surimi added with starch could only maintain high gel strength up to four months.
6. Incorporating nanobubble in the washing water was found to increase gel strength when compared to distilled water.
7. Washing cycle could be reduced to a minimum of two washing cycles with the use of medium concentration nanobubble water (11.15×10^8 bubbles/mL).
8. One washing cycle showed the best frozen shelf-life stability.
9. Rheological tests could be used to predict quality and textural properties.

8.2 RECOMMENDATIONS FOR FUTURE WORK

8.2.1 Rheology

Rheological tests done were able to determine the structure and characteristics of various samples in this study. However, several other rheological tests could also be employed to further understand the properties of samples with different additives. Recovery test could further determine the stability of sample during processing. Other than that, studying the yield stress of samples could add to the characterisation of each sample. By comparing the result of several geometries, consistent and accurate data could be extracted. A more elaborate analysis of texture by using the texture profile analysis graph would also help in further characterising the texture and sensory attributes of surimi such as chewiness, springiness, and cohesiveness. A further improvement could be achieved if a mathematical model could be designed to relate the behaviour of surimi in paste form and the texture during the gel form.

8.2.2 Nanobubble water and washing

In order to complement this research better, a study on the properties of nanobubble used is important. The density, surface tension and contact angle of nanobubbles might provide information on the effectiveness of the washing medium to discard all unwanted components. Although nanobubble water was found to produce beneficial result at certain concentration, the chemistry of interaction between nanobubble and myofibrillar protein is yet to be understood. The study of nanobubble behaviour and its interaction with solid particles will also be useful and informative. The charge possessed by nanobubble could cause several outcomes which lead to increased texture quality. Zeta-potential

which is an analysis of energy and charges is an option to be considered in expanding this research. The zeta-potential of nanobubble and myofibrillar protein interaction study could benefit this study further. This study could identify the best nanobubble size and nanobubble distribution to further optimize washing. Other than that, an understanding of the forces and energy distribution between nanobubble and water molecule will help increase the potential of using nanobubble as a washing alternative than normal water. Additionally, an analysis of the washed water also could help identify which component that was washed out. FTIR or LC/MS could be used to identify these components. A comparison between component that was washed out using distilled water and nanobubble water could determine the effectiveness of using nanobubble water. Also, a series of rheological tests could be done to understand the effects of nanobubble water washing on surimi.

8.2.3 Microstructure study

A microstructure study would further complement the outcome of the present work as it would validate some claims and theories on the changes of microstructure during processing and during frozen storage. Microstructure study would further increase the understanding on the changes in texture with addition of additive, washing and also heating. SEM or TEM could provide the best image of microstructure.

8.2.4 Frozen shelf-life study

Data on the effects of different ranges of cold storage temperature and cooling rate should be further investigated. By changing the cooling rate (*e.g.*, slow

cooling, rapid cooling), the stability of surimi could be studied. This could also help to understand further the frozen stability of surimi and role of additives in prolonged frozen storage. Other than that, the best combination of additives and its preservation characteristics during frozen storage could help surimi manufacturers to produce the best quality surimi. A longer period of frozen storage could further benefit this study.

8.2.5 Chemical analysis

SDS-PAGE would help determine the protein band present in the surimi. This will further validate the protein interaction and presence in the surimi. In addition to that, FTIR could be used to determine the group of amines that react with additives. This will increase the understanding of chemical interaction happening within the surimi. Other than that, DSC could be used to validate the rheological data on the gelation profile and further understand the changes in heat and energy caused by the protein during transition (gelation).

8.2.6 Sensory application

Conducting a series of sensory tests with trained panellists will further help characterize the texture and taste of the sample. This will be beneficial as it helps understand the consumer perception and acceptance of the samples. Other than that, it can work as an initial market survey for the feasibility of future products with the additives added. For example, the acceptance of taste for surimi added with mannitol compared to the conventional sucrose and sorbitol.

CHAPTER 9

Reference

9.0 REFERENCES

Alakhrash, F., Anyanwu, U., & Tahergorabi, R. (2016). Physicochemical properties of Alaska Pollock (*Theragra chalcograma*) surimi gels with oat bran. *LWT – Food Science and Technology, 66,* 41-47.

Agarwal, A., Ng, W.J., & Liu, Y. (2011). Principle and applications of microbubble and nanobubble technology for water treatment. *Chemosphere, 84*, 1175-1180.

Ahmad, F. B., Williams, P. A., Doublier, J. L., Durand, S., & Buleon, A. (1999). Physico-chemical characterisation of sago starch. *Carbohydrate Polymers*, *38*(4), 361-370.

Amiza, M.A., & Ain, K.N. (2012). Effect of washing cycle and salt addition on the properties of gel from Silver Catfish (*Pangasius sp.*) surimi. *UMT 11th International Annual Symposium on Sustainability Science and Management* 9th-11th July 2012, 485-491.

An, H., Peters, M.Y., & Seymour, T.A. (1996). Roles of endogenous enzymes in surimi gelation. *Trends in Food Science and Technology, 7,* 321-327.

Auh, J.H., Lee, H.G., Kim, J.W., Kim, J.C., Yoon, H.S., & Park, K.H. (1999). Highly concentrated branched oligosaccharides as cryoprotectant for surimi. *Journal of Food Science, 64*(3), 418-422.

Badii, F., & Howell, N.K. (2002). Changes in texture and structure of cod and haddock fillets during frozen storage. *Food Hydrocolloids, 16*, 313-319.

Barroso, M., Careche, M., & Borderías, A.J. (1998). Quality control of frozen fish using rheological techniques*. Trends in Food Science and Technology, 9*, 220-239.

Belibagli, K.B., Speers, R.A., & Paulson, A.T. (2003). Thermophysical properties of silver hake and mackerel surimi at cooking temperatures. *Journal of Food Engineering, 60*, 439-448.

Benjakul, S., Chantarasuwan, C., & Visessanguan, W. (2003). Effect of medium temperature setting on gelling characteristics of surimi from some tropical fish. *Food Chemistry*, *82*, 567-574.

Benjakul, S., Visessanguan, W., Riebroy, S, Ishizaki, S., & Tanaka, M. (2002). Gel-forming properties of surimi produced from bigeye snapper, *Pricanthus tayenus* and *P macracanthu,* stored in ice. *Journal of the Science of Food and Agriculture, 82*, 1442-1451.

Benjakul, S., Visessanguan, W., Thongkaew, C., & Tanaka, M. (2005). Effect of frozen storage on chemical and gel-forming properties of fish commonly used for surimi production in Thailand. *Food Hydrocolloids, 19*, 197-207.

Bentis, C.A., Zotos, A., & Petridis, D. (2005). Production of fish-protein products (surimi) from small pelagic fish (*Sardinops pilchardusts*), underutilized by industry. *Journal of Food Engineering, 68*, 303-308.

Binsi, P.K., Shamasundar, B.A., Dileep, A.O., Badii, F., & Howell, N.K. (2009). Rheological and functional properties of gelatine from the skin of Bigeye snapper (*Priacanthus hamrur*) fish: Influence of gelatine on the gel-forming ability of mince fish. *Food Hydrocolloids, 23,* 132-145.

Borderías, A.J., Jiménez-Colmenero, F., & Tejada, M. (1985). Parameters affecting viscosity as a quality control for frozen fish. *Marine Fish Review, 47*, 43-45.

Bouraoui, M.M., Fichtali, J., Pinder, K.L., Nakai, S., & Bowen, B.D. (1997). Viscous properties of salmon surimi paste. *Journal of food Engineering, 34*, 441-452.

Colmenero, F.J. (2014). Chemistry and physics of comminuted products: nonmeat proteins. Reference Module in Food Science, Encyclopedia of Meat Sciences (2nd ed.), Elsevier.

Campo, L., & Tovar, C. (2008). Influence of starch content in the viscoelastic properties of surimi gels. *Journal of Food Engineering, 84*, 140-147.

Campo-Deaño, L., & Tovar, C. (2009A). The effect of egg albumen on the viscoelasticity of crab sticks made from Alaska Pollock and Pacific Whiting surimi. *Food Hydrocolloids, 23*, 1641-1646.

Campo-Deaño, L., Tovar, C.A., Pombo, M.J., Solas, M.T., & Borderías, A.J. (2009B). Rheological study of giant squid surimi (*Dosidicus gigas*) made by two methods with different cryoprotectants added. *Journal of Food Engineering, 94*, 26-33.

Campo-Deaño, L., Tovar, C.A., & Borderías, A.J. (2010). Effect of several cryoprotectants on the physicochemical and rheological properties of suwari gels from frozen squid surimi made by two methods. *Journal of Food Engineering, 97*, 457- 464.

Cando, D., Herranz, B., Borderías, A.J, & Moreno, H.M. (2015). Effect of high pressure on reduced sodium chloride surimi gels. *Food Hydrocolloids, 51*, 176-187.

Cando, D., Herranz, B., Borderías, A.J, & Moreno, H.M. (2016). Different additives to enhance the gelation of surimi gel with reduces sodium content. *Food Chemistry,* 196, 791-799.

Carvajal, P.A., Lanier, T.C., & Macdonald, G.A. (2005). Stabilization of proteins in surimi in J.W. Park (2nd Edition), 493-576 pp. CRC Press, Boca Raton.

Carvajal, P.A., MacDonald, G., & Lanier, T.C. (1999). Cryostabilization mechanism of fish muscle proteins by maltodextrins. *Cryobiology, 38*,(1), 16-26.

Chaijan, M., Benjakul, S., Visessanguan, W., & Faustman, C. (2004). Characteristics and gel properties of muscles from sardine (*Sardinella gibbosa*) and mackerel (*Rastrelliger kanagurta*) caught in Thailand. *Food Research International, 37*, 1021-1030.

Chaijan, M., Klomklao, S., & Benjakul, S. (2013). Characterisation of muscle from Frigate mackerel (*Auxis thazard)* and catfish (*Clarias macrocephalus*). *Food Chemistry, 139*, 414-419.

Chan, E.L., Nakai, S., & Wood, D.F. (1987). Muscle protein structure-function relationships and discrimination of functionality by multivariate analysis. *Journal of Food Science, 52*(1), 31-41.

Che, Z., & Theodorakis, P.E. (2017). Formation, dissolution and properties of surface nanobubbles. *Journal of Colloid and Interface Science, 487*, 123-129.

Chen, H-H., & Huang, Y.C. (2008). Rheological properties of HPMC enhanced surimi analysed by small- and large-strain tests – II: Effects of water contents and ingredients. *Food Hydrocolloids*, *22*, 313-322.

Chen, W.S., Liu, D.C., & Chen, M.T. (2002). Effects of high level of sucrose on the moisture content, water activity, protein denaturation and sensory properties in Chinese-style pork jerky. *Asian-Australasian Journal of Animal Science, 15*(4), 585-590.

Choi, Y., Lin, T., Tomlinson, K., & Park, J.W. (2008). Effect of salt concentration and temperature of storage water on the physicochemical properties of fish proteins. *LWT,* 41, 460-468.

Choi, S.S. & Regenstein, J.M. (2000). Physicochemical and sensory characteristics of fish gelatin. *Journal of Food Science, 65*, 194-199.

Damodaran, S. (1997). Food proteins and their applications (Vol. 80). CRC Press.

Debusca, A., Tahergorabi, R., Beamer, S.K., Matak, K.E., & Jaczynski, J. (2014). Physicochemical properties of surimi gels fortified with dietary fibers. *Food Chemistry, 148*, 70-76.

Dileep, A.O., Shamasundar, B.A., Binsi, P.K., Badii, F., & Howell, N.K. (2005). Effect of ice storage on the physicochemical and dynamic viscoelastic properties of ribbonfish (*Trichiurus spp*) meat. *Journal of Food Science: Food Engineering and Physical Properties*, *70*(9), 537-545.

Dileep, A.O., Shamasundar, B.A., Binsi, P.K., Badii, F., & Howell, N.K. (2011). Composition, physicochemical and rheological properties of fresh bigeye snapper fish (*Pricanthus hamrur*) mince. *Journal of Food Biochemistry,* ISSN 1745-4514.

Elgadir, M.A., Akanda, M.J.H., Ferdosh, S., Mehrnoush, A., Karim, A.A., Noda, T., & Sarker, M.Z.I. (2012). Mixed biopolymer system based on starch. *Molecules*, *17*, 584-597.

Esturk, O. (2003). Characterization of rheological properties and thermal stability of fish myofibrillar protein. PhD Thesis. Oregon State University.

Ferry, J.D. (1980). Viscoelastic properties of polymers (3rd ed.) New York: John Wiley and Sons, Inc.

Fischer, P., & Windhab, E.J. (2011). Rheology of food materials. *Current Opinion in Colloid and Interface Science*, *16*, 36-40.

Feriyanto, N., Saleh, C., Ulandari, S. & Md Deros, B. (2016). Supply chain framework for selling halal meat in retail business: a case study. *International Business Management 10*(19), 4684-4689.

Fu, X., Hayat, K., & Li, Z. (2012). Effect of microwave heating on the low-salt gel from silver carp (Hypophthalmichthys molitrix) surimi. *Food Hydrocolloids, 27*, 301-308.

Fu, Z., Chen, J., Luo, S-J., Liu, C-M, & Liu, W. (2015). Effect of food additives on starch retrogradation: A review. *Starch*, *67*, 69-78.

Fukushima,H., Okazaki, E., Fukuda, Y., & Watabe, S. (2007). Rheological properties of selected fish paste at selected temperature pertaining to shaping of surimi-based products. *Journal of Food Engineering, 81*, 492-499.

Geirsdottir, M., Hlynsdottir, H., Thorkelsson, G., & Sigurgisladottir, S. (2007). Solubility and viscosity of Herring (*Clupea harengus*) proteins as affected by freezing and frozen storage. *Journal of Food Science*, *72*, 376-380.

Ghadimkani, A., Zhang, W., & Marhaba, T. (2016). Ceramic membrane defouling (cleaning) by air Nano bubbles. *Chemosphere*, *146*, 379-384.

Greiff, K., Aursand, I.G., Erikson, U., Josefsen, K.D., & Rustad, T. (2015). Effects of type and concentration of salts on physicochemical properties in fish mince. *LWT – Food Science and Technology, 64,* 220-226.

Guenneugues, P & Lanelli, J. (2013). Surimi resources and market in J.W. Park (3rd Edition), 26-52, CRC Press, Boca Raton.

Guenneugues, P., & Morissey, M.T. (2005). Surimi resources In J.W. Park (2nd Edition), 4-29, CRC Press, Boca Raton.

Gunasekaran, S., & Ak, M.M. (2000). Dynamic oscillatory shear testing for foods – selected applications. *Trends in Food Science and Technology, 11*(3), 115-127.

Haug, I.J., Draget, K.I., & Smidsrød, O. (2004). Physical and rheological properties of fish gelatin compared to mammalian gelatin. *Food Hydrocolloids, 18,* 203-213.

Herrera, J.R., & Mackie, I.M. (2004). Cryoprotection of frozen-storage actomyosin of farmed rainbow trout (*Oncorhynchus mykiss*) by some sugars and polyols. *Food Chemistry, 84,* 91-97.

Huda, N., Leng, O.H., & Nopianti, R. (2011). Cryoprotective effects of different levels of polydextrose in Threadfin Bream surimi during frozen storage. *Journal of Fisheries and Aquatic Sciences, 6*(4), 404-416.

Huda, N., Fazilah, A., Ismail, N., & Easa, A.M. (2012). Effect of different types of low sweetness sugar on physicochemical properties of threadfin bream surimi (*Nemipterus* spp.) during frozen storage. *International Food Research Journal*,*19*(3), 1011-1021.

Hudson, H.M., Daubert, C.R., & Foegeding, E.A. (2000). Rheological and physical properties of derivitized whey protein isolate powders. *Journal of Agricultural and Food Chemistry, 48*, 3112-3119.

Hultin, H.O., & Kelleher, S.D. (2000) Surimi processing from dark muscle fish. In J.W. Park (Ed.), 59-77 pp, Marcel Dekker, New York.

Hyseni, L., Elliot-Green, A., Lloyd-Williams, F., Kypridemos, C., O'Flaherty, M., McGill, R., Orton, L., Bromley, H., Cappuccio, F.P. & Capewell, S. (2017). Systematic review of dietary salt reduction policies: Evidence for an effectiveness hierarchy? PLoS ONE (12) 5, e0177535.

Jin, S-K., Kim, I-S., Choi, Y-J., & Yang, H-S. (2009). Effects of Number of Washes and pH Adjustment on Characteristics of Surimi-Like Materials from Pork Leg Muscle. *Asian-Australasian Journal of Animal Science, 22*, 584-590.

Karthikeyan, M. Dileep, A.O, & Shamasundar, B.A. (2006). Effect of water washing on the functional and rheological properties of proteins from threadfin bream (*Nemipterus japonicas*) meat. *International Journal of Food Sciences and Technology*, *41*, 1002-1010.

Kim, B.Y., Park, J.W., & Yoon, W.B. (2005). Rheology and texture properties of surimi gels in J.W. Park (2nd Edition), 493-576 pp, CRC Press, Boca Raton.

Kong, W., Zhang, T., Feng, D., Xue, Y., Wang, Y., Li, Z., Yang, W., & Xue, C. (2016). Effects of modified starches on the gel properties of Alaska Pollock surimi subjected to different temperature treatments. *Food Hydrocolloids*, *56*, 20-28.

Kudre, T., Benjakul, S., & Kishimura (2013). Effects of protein isolates from black bean and mungbean on proteolysis and gel properties of surimi from sardine (*Sardinella albella*). *LWT-Food Science and Technology, 50*, 511-518.

Lanier, T.C. (1986). Functional properties of surimi. *Food Technology,* 40(3), 107-124.

Lanier, T.C., Carvajal, P., & Yongsawatdigul, J. (2005). Surimi gelation chemistry In J.W. Park (2nd Edition), 4-29 pp, CRC Press, Boca Raton.

Lemon, P. (2000). Beyond the zone: protein needs of active individuals. *Journal of the American College of Nutrition, 19*(5), 513-521.

Lin, T.M., & Park, J.W. (1996). Extraction of proteins from pacific whiting mince at various washing conditions. *Journal of Food Science, 61*, 432-438.

Liu, Q., Bao, H., Xi, C., & Miao, H. (2014A). Rheological characterization of tuna myofibrillar protein in linear and nonlinear viscoelastic region. *Journal of Food Engineering, 121*, 58-63.

Liu, Q., Chen, Q., Kong, B., Han, J., & He, X. (2014B). The influence of superchiling and cryoprotectants on protein oxidation and structural changes in the myofibrillar proteins of common carp (*Cryprinus carpio*) surimi. *LWT- Food Science Technology, 57,* 603-611.

Liu, Q., Kong, B., Han, J., Chen, Q., & He, X. (2014C). Effects of superchilling and cryoprotectants on the quality of common carp (*Cyprinus carpio*) surimi: Microbial growth, oxidation and physiochemical properties. *LWT-Food Science and Technology, 57,* 165-171.

Liu, R., Zhao, S-M., Xiong, S-B., Qiu, C-G., & Xie, B-J. (2008). Rheological properties of fish actomyosin and pork actomyosin solutions. *Journal of Food Engineering, 85*, 173-179.

Maaruf, A.G., Che Man, Y.B., Asbi, B.A., Junainah, A.H., & Kennedy, J.F. (2001). Effect of water content on the gelatinisation temperature of sago starch. *Carbohydrate Polymer*, *46*, 331-337.

Macdonald, G.A., & Lanier, T.C. (1994). Actomyosin stabilization to freeze-thaw and heat denaturation by lactate salts. *Journal of Food Science*, *59*, 101-105.

Mahawanich, T., Lekhavichitr, J & Duangmal, K. (2010). Gel properties of red tilapia surimi: effects of setting condition, fish freshness and frozen storage. *International Journal of Food Science and Technology, 45*(9), 1777-1786.

Mohan, M., Ramachandran, D., Sankar, T., & Anandan, R. (2008). Physicochemical characterization of muscle proteins from different regions of mackerel (*Rastrelliger kanagurta*). *Food Chemistry, 106*, 451-457.

Moosavi-Nasab, M. (2003). Protein structural changes during preparation and storage of surimi. PhD Thesis. McGill University,Montreal, Canada.

Morrissey, T.M., Lin, J., & Ismond, A. (2005). Waste management and by-product utilization In J.W. Park (2nd Edition), 281-316 pp, CRC Press, Boca Raton.

Nabors, L, O'B. (2001). Alternative sweeteners. Third edition, revised and expanded. Marcel Dekker, Inc., New York.

Niwa, E., (1992). Chemistry of surimi gelation. *Surimi technology*, 389-427.

Nopianti, R., Huda, N., Fazilah, A., Ismail, N., & Easa, A.M. (2012). Effect of different types of low sweetness sugar on physicochemical properties of threadfin bream surimi (*Nemipterus* spp.) during frozen storage. *International Food Research Journal,19*(3), 1011-1021.

Núñex-Flores, R., Cando., D, Borderías, A.J., & Moreno, H.M. (2018). Importance of salt and temperature in myosin polymerization during surimi gelation. *Food Chemistry*, *239*, 1224-1234.

Ohkuma, C., Kawai, K., Viriyarattanasak, C., Mahawanich, T., Tantratian, S., Takai, R., & Suzuki, T. (2008). Glass transition properties of frozen and freeze-dried surimi products: Effects of sugar and moisture on the glass transition temperature. *Food Hydrocolloids, 22*, 255-262.

Okada, M. (1992). History of surimi technology in Japan. In C.L. Tyre and M.L Chong (eds), Surimi Technology, 3-21 pp. Marcel Dekker, Inc., New York.

Park, J.W. (2005). Ingredient technology for surimi and surimi seafood in J.W. Park (2nd Edition), 649-720 pp, CRC Press, Boca Raton.

Park, J.W., & Lin, T.M.J. (2005). Surimi: Manufacturing and evaluation in J.W. Park (2nd Edition), pg.33-98. CRC Press, Boca Raton.

Park, J.W., Lanier, T.C., & Green, D.P. (1988). Cryoprotective effects of sugar/ polyols and/or phosphates on Alaska Pollock surimi. *Journal of Food Science, 53*(1), 1-3.

Parvathy, U., & George, S. (2014). Influence of cryoprotectant levels on storage stability of surimi from *Nemipterus japonicas* and quality of surimi-based products. *Journal of Food Science and Technology, 51*(5), 982-987.

Pérez-Mateos, M, & Lanier, T.C. (2006). Comparison of Atlantic menhaden gels from surimi processed by acid or alkali solubilisation. *Food Chemistry, 101*, 1223-1229.

Pietrowski, B.N., Tahergorabi, R., Matak, K.E., Tou, J.C & Jaczynski, J. (2011). Chemical properties of surimi seafood nitrified with -3 rich oils. *Food Chemistry, 129*, 912-919.

Pietrowski, B.N., Tahergorabi, R., & Jaczynski, J. (2012). Dynamic rheology and thermal transition of surimi seafood enhanced with -3-rich oils. *Food Hydrocolloids, 27,* 384-389.

Pipatsattanuyawong, S. (1996). Alternative products from pacific whiting: fresh surimi and texturized mince. Masters of Science Thesis. Oregon State University.

Priyadarshini, B., Xavier, K.A.M., Nayak, B.B., Dhanapal, K., & Balange, A.K. (2017). Instrumental quality attributes if single washed surimi gels tilapia: Effect of different washing media. *LWT – Food Science and Technology*, *86*, 385-392.

Ramírez, J.A., Uresti, R.M., Velazquez, G., & Vázquez, M. (2011). Food hydrocolloids as additives to improve the mechanical and functional properties of fish products: A review. *Food Hydrocolloids, 25*, 1842-1852.

Saeed. S., & Howell. N.K. (2001). Effect of lipid oxidation and frozen storage on muscle proteins of Atlantic mackerel (*Scomber scombrus). Journal of the Science of Food and Agricultural, 82*, 579-586.

Sánchez-González, I., Carmona, P., Moreno, P., Borderías, J, Sánchez-Alonso, I., Rodríguez-Casado, A., & Mercedes, C. (2008). Protein and water structural changes in fish surimi during gelation as revealed by isotropic H/D exchange and Raman spectroscopy. *Food Chemistry, 106*, 56-64.

Sarabia, A.I., Gomez-Guillén, M.C., & Montero, P. (2000). The effect of added salts on the viscoelastic properties of fish skin gelatin. *Food Chemistry, 70,* 71-76.

Scott, D.N., Porter, R.W., Kudo, G., Miller, R. & Koury, B. (1988). Effect of freezing and frozen storage of Alaska Pollock on the chemical and gel-forming properties of surimi. *Journal of Food Science, 53*(2), 353-358.

Shenouda, S.Y.K. (1980). Protein denaturation in frozen fish. *Advances in Food Research*, *26*, 175-311.

Solo-de-Zaldívar, B., Tovar, C.Z, Borderías, A.J., & Herranz, B. (2014A). Effect of deacetlyation on the glucomannan gelation process for making restructured seafood products. *Food Hydrocolloids, 35*, 59-68.

Solo-de-Zaldívar, B., Herranz, B., Borderías, A.J., & Tovar, C.Z (2014B). Effect of freezing and frozen storage on restructured FISH prototypes made with glucomannan and FISH mince. *Food Hydrocolloids, 41,* 233-240.

Somjit, K., Ruttanapornwareesakul, Y., Hara, K., & Nozaki, Y. (2005). The cryoprotectant effect of shrimp chitin and shrimp chitin hydrolysate on denaturation and unfrozen water of lizardfish surimi during frozen storage. *Food Research International, 38,* 345-355.

Sow, L.C., & Yang, H. (2015). Effects of salt and sugar addition on the physicochemical properties and nanostructure of fish gelatin. *Food Hydrocolloids, 45,* 72-82.

Steffe, J.F. (1996). Rheological Methods in Food Processing Engineering Second Edition. Freeman Press, Michigan.

Sultanbawa, Y., & Li-Chan, E.C.Y. (1998). Cryoprotective effects of sugars and polyol blends in ling cod surimi during frozen storage. *Food Research International*, *31*(2), 8.

Sung-Hwan, E., Jung-Ae, K., Byoung-Yil, S., Dong, H.Y., Jeong, M.H, Jung-Hwan, O., Bomg-Yeun, K., & Chang-Suk, K. (2013). Effects of carrageenan on the gelatinization of salt-based surimi gels. *Fisheries and Aquatic Sciences, 16,* 143-147.

Sych, J., Lacroix, C., Adambounou, L.T., & Castaigne, F. (1991A). The effect of low- or non-sweet additives on the stability of protein functional properties of frozen cod surimi. *International Journal of Food Science and Technology, 26*, 185-197.

Sych, J., Lacroix, C., & Carrier, M. (1991B). Determination of optimal level of lactitol for surimi. *Journal of Food Science*, *56*, 285-290.

Tabilo-Munizaga, G., & Barbosa-Cánovas, G. (2005A). Rheology for the food industry. *Journal of Food Engineering*, *67*, 147-156.

Tabilo-Munizaga, G., & Barbosa-Cánovas, G. (2005B). Pressurized and heat-treated surimi gels as affected by potato starch and egg white: microstructure and water-holding capacity. *LWT – Swiss Society of Food Science and Technology*, *35*, 45-57.

Tahergorabi, R., & Jaczynski, J. (2012A). Physicochemical changes in surimi with salt substitute. *Food Chemistry, 132,* 1281-1286.

Tahergorabi, R., & Jaczynski, J. (2012B). Salt substitution in surimi seafood and its effects on instrumental quality attributes. *LWT – Food Science and Technology, 48,* 175-181.

Tahergorabi, R., Beamer, S.K., Matak, K.E. & Jaczynski, J. (2013). Chemical properties of ω-3 fortified gels made of protein isolate recovered with isoelectric solubilisation/precipitation from whole fish. *Food Chemistry, 139*, 777-785.

Techarang, J., & Apichartsrangkoon, A. (2015). Physical, chemical and rheological parameters of pressurized *swai*-fish (*Pangasisus hypophthlmus*) emulsion incorporating fermented soybeans. *Food and Bioproducts Processing*, *94*, 649-656.

Temesgen, T., Bui, T.T., Han, M., Kim, T., & Park, H. (2017). Micro and nanobubble technologies as a new horizon for water-treatment techniques: A review. *Advances in Colloid and Interface Science*, *246*, 40-51.

Teng, L., Chin, N., & Yusof, Y. (2011). Food hydrocolloids rheological and textural studies of fresh and freeze-thawed native sago starch-sugar gels. I. Optimisation using response surface methodology. *Food Hydrocolloids*, *25*, 1530-1537.

Teng, L., Chin, N., & Yusof, Y. (2013). Food hydrocolloids rheological and textural studies of fresh and freeze-thawed native sago starch-sugar gels. II. Comparisons with other starch sources and reheating effects. *Food Hydrocolloids*, *31*, 156-165.

Ushikubo, F.Y., Furukawa, T., Nakagawa, R., Enari, M., Makino, Y., Kawagoe, Y., Shiina, T., & Oshita, S. (2010). Evidence of the existence and the stability of nano-bubbles I water. *Colloids and Surface A: Physicochemical and Engineering Aspects, 351*, 31-37.

Widodo, R.T., & Hassam, A. (2015). Compression and mechanical properties of directly compressible pregelatinized sago starches. *Powder Technology*, *269*, 15-21.

Yang, H. (1998). Effects of starch on rheological, microstructural, and color properties of surimi-starch gels. Masters of Science, Oregon State University.

Yin, T., & Park, J.W. (2015). Textural and rheological properties of Pacific whiting surimi as affected by nano-scaled fish bone and heating rates. *Food Chemistry, 180*, 42-47.

Yin, T., & Park, J.W. (2014). Effect of nano-scaled fish bone on the gelation properties of Alaska Pollock surimi. *Food Chemistry, 150*, 463-468.

Yoon, K.S, & Lee, C.M. (1990) Cryoprotectant effects in surimi and surimi / mince-based extruded products. *Journal of Food Science,* 55, 1210-1216.

Yoon., W.B., Gunasekaran, S., & Park, J.W. (2004A). Characterization of thermorheological behaviour of Alaska pollock and pacific whiting. *Journal of Food Science, 69*(7), E338-E343.

Yoon, W.B., Gunasekaran, S., & Park, J.W. (2004B). Evaluating viscosity of surimi paste at different moisture contents. *Applied Rheology, 13*, 133-139.

Zhang, L., Li, Q., Shi, J., Zhu, B., & Luo, Y. (2018). Changes in chemical interactions and gel properties of heat-induced surimi gels from silver carp (*Hypophtalmichthys molitrix*) fillets during setting and heating: Effects of different washing solutions. *Food Hydrocolloids*, 75, 116-124.

Zhang, L., Xue, Y., Xu, J., Zhaojie, Li., & Xue, C. (2013). Effects of high temperature treatment (≥ 100 °C) on Alaska Pollock (*Theragra chalcogramma*) surimi gels. *Journal of Food Engineering, 115*, 115-120.

Zhou, A., Benjakul, S., Pan, K., Gong, J., & Liu, X. (2006). Cryoprotective effects of trehalose and sodium lactate on tilapia (*Sarotherodon nilotica*) surimi during frozen storage. *Food Chemistry, 96*, 96-103.

Zimmerman, W.B., Tesař, V., & Bandulasena, H.C.H. (2011). Towards energy efficient nanobubble generation with fluidic oscillation. *Current Opinion in Colloid & Interface Science*, *16*, 350-356.