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EFFECT OF MARINE GELATIN ON THE RHEOLOGICAL AND PHYSICAL PROPERTIES OF GUMMY CANDY

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EFFECT OF MARINE GELATIN ON THE RHEOLOGICAL AND PHYSICAL PROPERTIES OF GUMMY CANDY

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ABSTRACT

Gummy Candy is the most versatile confectionary, often used to be mineral and vitamin supplement to children, teens and adults. Gelatin is the main ingredients in the formulation of Gummy Candy. However, the problem with commercialized gelatin is the source of the gelatin from cows and pigs. This limits the commercialized gummy candy due to religious beliefs. The main objective of this study was to evaluate different type of gelling agent (commercialized bovine gelatin, commercialized fish gelatin, extracted tilapia gelatin and Arabic gum) with different gelling agent concentration (9, 12, 15%) at different temperature for hydration of gelatin (70, 80, 90°C) on the rheological properties of gummy candy solution and textural properties of gummy candy. Value of pH, °Brix, viscosity and G',G" were studied on the gummy candy solutions and the textural properties are studied on the gummy candy that were chilled in a silicone mold at 10°C for 24 h. The results indicated that the concentration and type of gelling agent used in gummy candy affected the viscosity, gelling and melting temperature of the solution, as well as the hardness, gumminess and adhesiveness of the gummy candy. The result of gelling and melting temperature, hardness, gumminess and adhesiveness is highest in gummy candy made from fish gelatin followed by bovine, tilapia and mixture of Arabic-gum-gelatin. The effect of hydration temperature was only significant in the hardness of gummy candy from 80°C to 90°C that decreases for all gummy candy made from all different type of gelling agents. The textural properties of gummy candy made from 12% tilapia had shown the most similar values to commercialized haribo gummy candy.

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ABSTRAK

Gummy candy merupakan salah satu gula-gula yang serba boleh dan sering digunakan sebagai supplemen mineral dan vitamin kepada kanak-kanak, remaja dan orang dewasa. Gelatin adalah bahan utama dalam formulasi *Gummy candy*. Masalah utama penggunaan gelatin komersial adalah pengekstrakan gelatin dari sumber lembu dan khinzir. Ini menyebabkan limitasi pengambilan *gummy candy* komersial atas kepercayaan agama masing-masing. Objektif utama kajian ini adalah untuk mengkaji ciri-ciri reologi dan fizikal *Gummy Candy* yang diperbuat daripada beberapa jenis ajen perjelan (gelatin lembu komersial, gelatin ikan komersial, gelatin tilapia dan *Arabic gum*) pada beberapa konsentrasi (9, 12, 15%) dan pada beberapa suhu hidrasi (9, 12, 15%). Untuk *Gummy Candy* solusi, nilai pH, °Brix, kelikatan dan G',G" telah dikaji. Manakala, Untuk ciri-ciri teksur, *Gummy Candy* yang telah dituang di dalam acuan silicon dan disejukkan di suhu 10°C selama 24 j telah dikaji. Hasil dapatan adalah apabila konsentrasi dan jenis agen pengejalan menjejaskan kelikatan, suhu jel, suhu cair, kekerasan, kekenyalan dan pelekatan *Gummy Candy*. Hasil kajian didapati bahawa suhu jel, suhu cair, kekerasan dan kekenyalan tertinggi pada *Gummy Candy* diperbuat daripada gelatin ikan komersial diikuti gelatin lembu komersial, gelatin tilapia dan campuran gelatin komersial dan Arabic gum. Seterusnya, kesan suhu hidrasi jelas pada suhu 80°C ke 90°C di mana kekerasan dan kekenyalan *Gummy Candy* berkurangan pada 90°C. Ciri-ciri teksur *Gummy Candy* yang diperbuat daripada 12% tilapia gelatin menunjukkan ciri yang sama dengan *Gummy Candy Haribo*.

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CHAPTER 1

INTRODUCTION

1.1 Overview

Gelatin is a protein that is derived by extraction and partial hydrolysis of collagen which is found in bones, hides, connective tissue, and skins of animals. Although cattle and pigs are the main sources, gelatin from fish may see increased use in the future based on religious concerns. There are two extraction processes: acid extraction produces Type A gelatin whereas Type B gelatin is produced through an alkali extraction.

The properties of gelatin solutions are affected by temperature, pH, and concentration. With time and increasing temperature, gelatin's degradation increases. Holding gelatin solutions at elevated temperatures is undesirable because this degradation of the structure results in loss of Bloom strength and less firm gel. Gelatin solutions are most stable at pH range between 5.0 and 6.0. Either lowering or raising the pH will accelerate degradation and result in loss of Bloom strength. As might be expected, with increasing concentration (increasing molecular weight), the viscosity of the gelatin solution increases.

One of the application of gelatin in food industry is confectionary gels, specifically, gummy candies. Gummy candies are confectionery products and their texture is achieved by using various gelling agents such as gelatin, starch, gums and pectin. Products made of pectin and gum arabic showed similar mechanical properties despite their composition being their moisture and sugars content significantly different. Moreover, their hardness and elasticity were higher than those determined in the gelatin candies (Pizzoni, Compagnone, Natale, Alessandro, & Pittia, 2015). Customers prefer gummy jelly candies due to their unique texture and chewability (Charoen & Savedboworn, 2015). Gelatin provide a network to hold high moisture content sugar syrup in gummy candies.

1.2 Problem Statement

However, the issue of gelatin replacement has existed for many years due to vegetarianism, development of halal and kosher markets as well as the emergence of bovine spongiform encephalopathy (BSE) towards the end of the 20th century (Morrison, Clark, Chen, Talashek, & Sworn, 1999). At the same time, current practice of fish processing generates large amounts of by-products, which can make up to three-quarters of the total weight of a fish. (Regenstein & Zhou, 2007). Typically, fish processing by-products consist of viscera, heads, trim, skins, scales, and bones, as well as fish that are damaged or unsuitable for human consumption or further processing. Thus, fish gelatin has now been identified as a real alternative to mammalian gelatins due to its close resemblance to bovine or pig gelatin in terms of gel strength (Normah & Muhammad Fahmi, 2015).

Tilapia fisheries have a fraction of 33% of the local freshwater in Malaysia (INFOPECSA, 2015). This showed a great opportunity to utilize the waste from tilapia fish

such as scales. Tilapia scales has a large surface area which deemed to provide a large amount of collagen to be hydrolysed to be a gelatin. Several studies have been conducted by Jamilah & Harvinder (2002) that showed the similar properties of gelatin from tilapia skins with mammalian gelatin. However, no studies have been made to study the application of gelatin from tilapia's scales in food products. Thus, it is important to understand the effect of gelatin from tilapia scales in gummy candy to observe its ability to replace mammalian gelatin in gummy candy.

1.3 Objective

The objective of this study are:

1) To evaluate the effect of different types and different concentration of gelatin and gum on the physical properties of gummy candy.
2) To study the effect of temperature of hydration of gelling agent during hydration of gelatin on gummy candy's physical characteristics.
3) To study the rheological properties of gummy candy solution as a function of concentration of gelatin for a given gelatin type.

1.4 Scope of the Study

The scope of this study consists of five chapters. Chapter 1 is about the introduction of gelatin and its application, the problems associated with commercialized gelatin, problem statements and the objective of the study. Chapter 2 is an organized sets of literature review comprising two parts, which is gelatin and the application on gummy candy. In gelatin section, detailed explanation on source, molecular and chemical composition and physical properties of different type of gelatin. Meanwhile, for gummy

candy section, the formulation and its gel formation, manufacturing process and important parameters that needs to be considered for the quality of the gummy candy.

Chapter 3 is about the method used to conduct the experiment with the procedure and equipment used. This consists of formulation of the gummy candy, hydration of the gelatin, cooking the sugar solution and mixing of hydrated gelatin with the sugar solution. The analysis done on the gummy candy solution is physico-chemical analysis such as pH and °Brix, and rheological properties. The gummy candy solution is also poured into mold and left to set for Texture Profile Analysis (TPA). Chapter 4 is result and discussion of the experiments which contains figure, tables and graph. Lastly, conclusion and recommendation of this research is stated in Chapter 5.

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CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Gelatin is a type of protein that does not occur naturally and is derived from the fibrous protein collagen, which is the principal constituent of animal skin, bone, and connective tissue. Gelatin is produced via the partial hydrolysis of native collagen. During the manufacturing of gelatin, raw animal's component is treated with dilute acid or alkali. This in turns causes a formation of partial cleavage of the crosslinks where, the structure is broken down to such an extent that "warm-water-soluble collagen", i.e. gelatin, is formed (Schrieber & Gareis, 2007b)

To date, some 27 different types of collagen have been identified, and a simple classification is shown in Table 2.1. Collagen molecules composed of three α-chains intertwined together that has a 3D structure that provides an ideal geometry for inter-chain hydrogen bonding (Te Nijenhuis, 1997). Each chain in the helix rotates counterclockwise. The triple-helix is approximately 300 nm in length, and the chain has a molecular weight of approximately 105 kDa (Papon, Leblon, & Meijer, 2007). The triple helices are

stabilized by the inter-chain hydrogen bonds. The destruction of the inter-chain hydrogen bond causes collagen denaturation due to separation of the rods and total or partial separation of the chains. This causes a loss of the triple-helix conformation which allow the polymers to exist in a coiled form. Industrial gelatins are mixtures of different compounds: α-chains (one polymer chain), β-chains (two α-chains covalently crosslinked), and γ chains (three covalently crosslinked a-chains) (Papon et al., 2007).

Table 2.1: Classification of collagen (Schrieber & Gareis, 2007b)

Type	Description
Type I	This type occurs widely, primarily in connective tissue such as skin, bone, and tendons.
Type II	This type of collagen occurs practically exclusively in cartilage tissue.
Type III	This type strongly dependent on age: very young skin can contain up to 50%, but in the course of time this is reduced to 5–10%.
Other types	The other types of collagen are present in very low amounts only and organ-specific.

2.2 Source of Gelatin

For gelatin production, the raw material may be any collagen-containing tissue. Hides, skins and bones from mammalian sources such as porcine and bovine are preferred, but gelatins are also produced from the skins of cold and warm water fish species as well as minor quantities from avian sources.

2.2.1 Mammalian Gelatin

Mammalian gelatin is derived from collagen which is the principal constituent of connective tissues and bones of vertebrate animals. Study of two different mammalian gelatins, i.e. from bovine (type B) and porcine (type A) sources revealed that both sources contained components of different molecular weights with wide distribution ranging from 10 to 400 kDa. The result also showed strong correlation between average molecular weight and gel strength of gelatin, with high isoelectric and melting points (Lim & Mohammad, 2011). Mammalian gelatins (porcine and bovine), being the most popular and widely used, are subject to major constraints and skepticism among consumers due to socio-cultural and health-related concerns (Karim & Bhat, 2009). The gelatin can be prepared either by the acid process (type A gelatine), or by the alkaline process (type B gelatine) (Mariod et al., 2011).

2.2.2 Fish Gelatin

Fish gelatin can be obtained from the skin and bones of fish. About 30% of such waste consists of skin and bones with high collagen content that can be used to produce fish gelatin (Sarabia, Gómez-guillén, & Montero, 2000). Extraction of gelatins from fish skins may provide an alternative source that is acceptable for kosher (Jewish) and halal (Muslim) products and serve as an alternative for markets concerned about bovine spongiform encephalopathy (BSE). The yield and quality of gelatin are influenced not only by the species or tissue from which it is extracted but also by the extraction process, which may depend on pH, temperature, and time during both pretreatment and extraction (Sarabia et al., 2000).

2.3 Molecular Structures and Chemical Composition of Gelatin

Gelatin is a type of protein originates from its native self, collagen. In order to understand the functionality of gelatin, the basic chemical and molecular structure of gelatin should be discussed to see how they are as a protein and how its structure build together to give gelatin it's desirable functionality. Generally, there are different degree of structures in protein. This can be classified as primary, secondary, tertiary and quaternary structures.

2.3.1 Molecular Structures of Gelatin

Primary structures of protein describes the unique order in which amino acids are linked together. Amino acids contain an alpha carbon that bonds four different groups of molecules, which is a hydrogen atom, carboxyl group (-COOH), an amino group (-NH2) and also a side chain group, R. The primary structure of type I animal collagen as used in the manufacture of gelatin comprises some 1014 amino acids that are linked in the form of a chain with a molecular weight of approximately 100 000 g mol-1. These so-called alpha chains comprise 334 repetitive units of the general sequence glycine-X-Y.

The secondary structure of protein describes the coiling or folding of a polypeptide chain that gives the protein its 3-D shape. There are two types of secondary structures observed in proteins. One type is the alpha (α) helix structure. This structure resembles a coiled spring and is secured by hydrogen bonding in the polypeptide chain. The second type of secondary structure in proteins is the beta (β) pleated sheet. This structure appears to be folded or pleated and is held together by hydrogen bonding between polypeptide units of the folded chain that lie adjacent to one another. In gelatin, proline and hydroxyproline

are responsible for its unique secondary structure. The amino acids limit the rotation of the polypeptide backbone and thus contributes to the stability of the triple helix. The hydroxyl group of hydroxyproline plays an important factor in stabilizing the triple helix of gelatin.

Tertiary structures describes the comprehensive 3-D structures of the polypeptide chain of a protein. Several bonds and forces that are responsible to keep the 3-D structures in protein's tertiary structure.

R group bonds that form the tertiary structure are non-primary covalent bond such as hydrogen bonding, ionic bonding, dipole-dipole interactions, and London dispersion forces. For example, R groups with like charges repel one another, while those with opposite charges can form an ionic bond. Similarly, polar R groups can form hydrogen bonds and other dipole-dipole interactions. Also important to tertiary structure are hydrophobic interactions, in which amino acids with nonpolar, hydrophobic R groups cluster together on the inside of the protein, leaving hydrophilic amino acids on the outside to interact with surrounding water molecules.

Finally, there's one special type of covalent bond that can contribute to tertiary structure: the disulfide bond. Disulfide bonds, covalent linkages between the sulfur-containing side chains of cysteines, are much stronger than the other types of bonds that contribute to tertiary structure. They act like molecular "safety pins," keeping parts of the polypeptide firmly attached to one another. However, cysteine is usually absent in collagen, therefore, there are usually no disulfide bonds involved in collagen structure although there are some collagens that have cysteine (Engel and Bachinger, 2005). The secondary structure in gelatin, which has alpha-chain that is coiled into a left-handed helix with about

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three amino acids per turn. Then, three of that alpha-chains are then twisted around each other into a right-handed super-helix to form a rigid rope-like structure.

2.3.2 Amino acid compositions

As discussed in section 2.3.1, the amino acid is responsible for the intermolecular interactions in collagen and gelatin.

From Table 2.2, the amino acid content shows a huge difference for gelatin extracted from different sources. The total proline plus hydroxyproline content of collagens and gelatins varies quite markedly between species. It may vary from 156 residues per thousand for a cold water fish gelatin, to 217–223 residues per thousand for gelatin and collagen extracted from warm-blooded mammals, such as pig and cattle. Warm water fish gelatin, as extracted from, e.g. tilapia, has a higher content of proline and hydroxyproline than gelatin from cold water fish species and the total amount of these imino acids was found to be close to 200 by (Sarabia et al., 2000).

Table 2.2: Amino Acid Composition of Gelatin from different Sources of Gelatin (amino acid residues per 1000 residues) (Haug & Draget, 2011)

Amino Acid	Type I Collagen (bovine)	Type A gelatin	Type B gelatin	Cold-Water Fish Gelatin	Warm-Water Fish Gelatin
Alanine	114	112	117	112	123
Arginine	51	49	48	49	47
Aspargine	16	16	46	48	48
Aspartic acid	29	29	-	-	-
Glutamine	48	48	72	72	69
Glutamic acid	25	25	-	-	-

Glycine	332	330	335	347	347
Histidine	4	4	4	11	6
4-Hydroxyproline	104	91	93	60	79
Hydroxylysine	5	6	4	5	8
Isoleucine	11	10	11	11	8
Leucine	24	24	24	21	23
Lysine	28	27	28	28	25
Methionine	6	4	4	3	9
Phenylalanine	13	14	14	13	13
Proline	115	132	124	96	119
Serine	35	35	33	63	35
Theonine	17	18	18	24	24
Tyrosine	4	3	1	9	2
Valine	22	26	22	18	15

According to Haug & Draget (2011), the gelatin and collagen α-chains consist of polar and non-polar regions. The "non-polar" regions are made up from the tripeptide Gly-Pro-R where R is a non-polar amino acid, predominantly hydroxyproline. These "non-polar" regions are scattered with the polar regions which has low content of in both proline and hydroxyproline. The presence and distribution of the charged, polar and non-polar amino acids provides gelatin with unique properties. Gelatin is easily dissolved in water at the right conditions due to the presence of charged amino acids and forms colloidal solutions.

The structure of collagen provides an explanation of why glycine is the most abundant amino acid and why proline and hydroxyproline are found so often in collagen.

Only glycine residues, as the smallest of the hydrophobic amino acids, can fit into the very tight central core between the individual a-chains, while proline and hydroxyproline residues permit sharp twists of the collagen helix allowing for the relatively low three amino acids per turn (Nelson and Cox, 2005).

2.3.3 Molecular Weight Distribution

It is not common for gelatin suppliers to routinely supply the purchaser with other data than those outlined above. Knowledge of the average molecular weight and the molecular weight distribution is, however, in many cases essential and should always be investigated. This can be achieved by the use of chromatographic methods (GPC/SEC) alone, using a polymer standard (polystyrene sulphonates are most commonly used for gelatin characterization), or in combination with multi angle laser light scattering (MALLS) which provides absolute molecular weights.

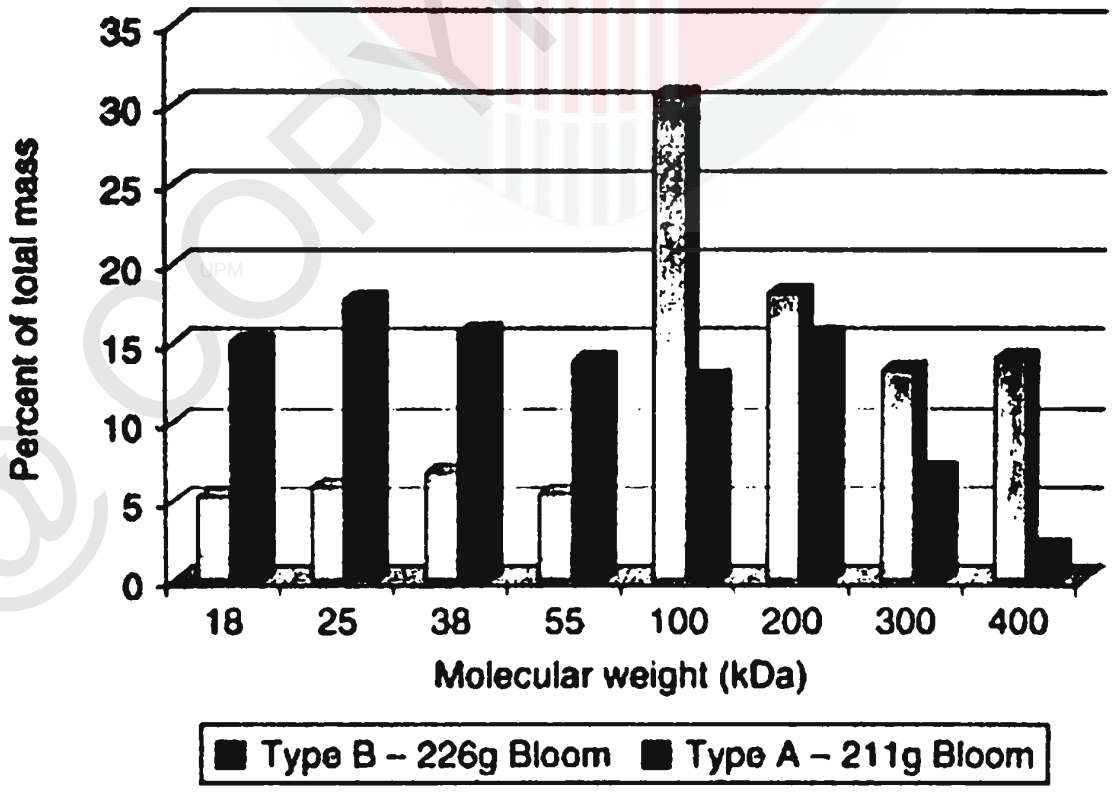

Table 2.3: Molecular Weight Distribution of Two Gelatins (Haug & Draget, 2011)

Figure 2.1 shows the molecular weight distribution for two gelatins with almost similar Bloom values and it is obvious that these gelatins have very different average molecular weights, which is also reflected by the difference in the viscosity values of the two gelatins. The type A gelatin has an average molecular weight of 94 kDa and a viscosity of 2.5 mPas (6.67%, 60 °C). The type B gelatin has an average Mw of 171 kDa while the viscosity is 5.9 mPas (6.67%, 60 °C).

Also, it can be seen that the type B gelatin has a small low molecular weight fraction compared to the type A gelatin in which most molecules weigh less than 100 kDa. By taking the viscosity values into account, it can easily be seen that there is no clear-cut connection between the Bloom values and the viscosity values. The viscosity values represent the hydrodynamic volumes of the molecules in solution and it is therefore obvious that high molecular weight fractions will dominate. Molecular weight distribution appears to play a more important role in the effect on viscosity than it does on gel strength. Some gelatins of higher gel strength may have lower viscosities than gelatins of lower gel strength.

Gelatin is categorized as a physical gel, i.e., the interactions or bonds between the chains that make up the material are physical in nature (van der Waal's interactions and hydrogen bonds, with an E=2 kcal/mole). The functional properties of gelatin are the major physical properties of gelatin gels. These are governed by molecular weight, as well as by complex interactions determined by the amino acid composition and the ratio of a/b-chains present in the gelatin (S. M. Cho et al., 2004).

2.4 Extraction of Gelatin

Gelatin is the water soluble product prepared by processes which involve the destruction of the tertiary, secondary, and partially the primary structure of native collagen present in animal tissues. These changes in the structure of native collagen are usually brought about by chemical means (acidic or alkaline pretreatment) and to a lesser extent by enzymatic modification. Depending on the method in which the collagens are pretreated, two different types of gelatin (each with differing characteristics) can be produced. Type A gelatin (isoelectric point at pH 6–9) is produced from acid-treated collagen, and type B gelatin (isoelectric point at approximately pH 5) is produced from alkali-treated collagen.

2.4.1 Extraction of Mammalian Gelatin

The extraction process of commercial gelatin involves cleaning of the source tissues followed by pre-treatment, extraction of gelatin, filtration/purification/sterilization, concentration, drying and finally milling. Based on figure above, at the beginning the raw material is washed to remove impurities. Bones are processed somewhat differently in that, after washing, crushing and rewashing, the degreased, crushed bone chips are exposed to acidic conditions (usually 4–7% hydrochloric acid) for a minimum of two days. This process is also known as maceration and the result is removal of minerals contained in the bone such as hydroxyl apatite (Ca5(PO4)3(OH)) and calcium carbonate leaving behind a sponge-like bone material called ossein. The concentrated raw materials may be processed directly or dried and stored for later use.

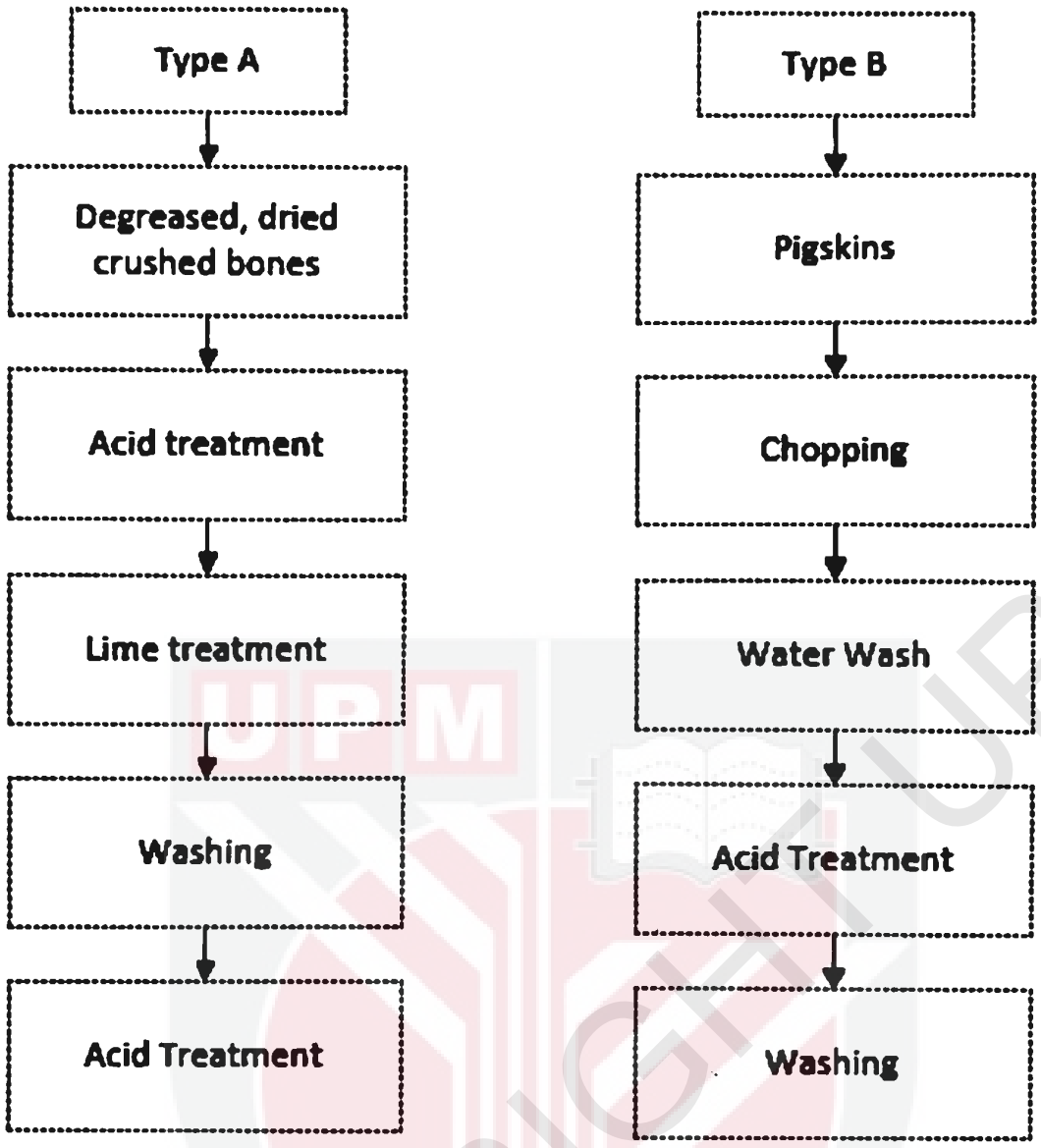

Table 2.4: Extraction of Mammalian Gelatin (GMIA., 2012)

Following the preliminary treatment described in Figure 2.2, the raw material is subjected to either acid or alkaline pre-treatment followed by gelatin extractions depending on the source of the collagen and the required quality of the final gelatin. Acidic treatment is most suitable for the less covalently crosslinked collagens found in pig or fish skins, while alkaline treatment is suitable for the more complex collagens found in bovine hides.

For the production of Type B gelatin, both ossein and cattle hide pieces are subjected to lengthy treatment with an alkali (usually lime) and water at ambient temperature. Depending on previous treatment, the nature of the material, the size of the pieces, and the exact temperature, liming takes 5-20 weeks, usually 8-12. The process is

controlled by the degree of alkalinity of the lime liquor as determined by titration with acid, or by making test extractions. Ossein usually requires more liming time than cattle hides. Additional lime is added to maintain an excess, thereby compensating for any consumed. During liming some deamination of the collagen occurs, with evolution of ammonia. After conditioning, the raw material is thoroughly washed with cold water to remove excess lime; the pH adjusted with acid; and the product extracted with hot water to recover the soluble gelatin.

Porkskin is a significant raw material source for production of edible gelatin in the United States. The short time required for pre-treatment prior to extraction, and the minimization of wastewater generated are important economic factors in the manufacture of gelatin from this raw material. Supplied as either fresh or frozen, porkskins come from slaughter houses and meat processing plants already trimmed of fat, flesh and hair.

Porkskins are usually dehaired by scalding with a hot dilute caustic soda solution. When porkskins are utilized for production of Type A gelatin they are washed with cold water and then soaked in cold dilute mineral acid for several hours until maximum swelling has occurred. Hydrochloric acid and sulfuric acid are most commonly employed. The remaining acid is then drained off and the material is again washed several times with cold water. The porkskins are then ready for extraction with hot water. The pH, time, temperature, and number of extractions varies from processor to processor depending on product needs, type of equipment employed, timing of operations, and economics. Extraction procedures are closely controlled in the manufacture of both Type A and Type B gelatin since they influence both quality and quantity.

2.4.2 Extraction of Fish Gelatin

Fish gelatin has been extracted using a number of different methods, and these are summarized in Table 2.3. The direct procedures used for preparing fish gelatin typically involve a mild chemical pretreatment of the raw material and mild temperature conditions during the extraction process.

According to Gomez-Guillen and Montero (2001), in order to extract gelatin with high gelling strength from fish skins, mild acid is used as pretreatment for collagen swelling, followed by extraction in water at moderate temperatures (45°C) for 24 h. Because of the acid lability of the crosslinks found in fish skin collagen, mild acid treatment is sufficient to produce adequate swelling and to disrupt the non-covalent intra- and intermolecular bonds. High-molecular weight polymers may occur in the resulting gelatin through the possible persistence of crosslinks, depending on the nature and degree of solubilization.

The pretreatment and extraction processing steps of the gelatin manufacture process and the different degrees of cross-linkage of the collagen molecules, which appear to be species dependent have been shown to affect gelatin extraction efficiency, as well as the functional properties of the resulting gelatin (Karim & Bhat, 2009).

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Table 2.5: Procedures to extract fish gelatin (Karim & Bhat, 2009)

Fish Type	Pretreatment	Extraction Procedure	Reference
Channel catfish	The channel catfish skin was soaked in eight volumes (v/w) of 50-mmol/L acetic acid at 15°C (18 h). The treated skin was further washed with distilled water until the pH of solution reached 3.5–4.0.	The gelatin extraction was carried out in distilled water in a water bath at 45°C for 7 h.	(Yang al., 2007)
Black tilapia red tilapia		Fish skins rinsed with excess water followed by soaking in 0.2% (w/v) NaOH (40 min). This was followed by soaking in 0.2% H2SO4 acid and 1.0% citric acid. Rinsing with distilled water was carried out between soakings followed by final extraction in distilled water at 45°C (12 h)	(Jamilah & Harvinder 2002)
ellowfin tuna	Washed, chopped and frozen at 15°C until used. Further treated with 8 volumes (v/w) of alkali solution (1–3% NaOH) at 10 °C with shaking (200 rpm for 1–5 days to remove the non-collagen protein and subcutaneous tissue after they were swollen neutralized with 6 N HCl.	Hot water extraction, six volumes (v/w) of distilled water was added and heated at temperature ranging 40–80°C for 1–9 h. The extracted solution was centrifuged for 30 min (900xg) at 30°C	(S. M. Cho et 2004)

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Table 2.3: Procedures to extract fish gelatin (Karim & Bhat, 2009)

Fish Type	Pretreatment	Extraction Procedure	Reference
Baltic cod	Samples washed with NaCl solution (with the exception of salted and marinated herring skins) and water, followed by gentle stirring with water (1:6, w/v) for 15–120 min at 45, 70 or 100°C. The samples were centrifuged at 10,000 g for 30 min at 15°C	Extracted at 45°C in water	Kolodzieska et al. (2004)

2.5 The effect of processing of extraction condition to fish gelatin quality

There are several factors that significantly affect the properties of gelatin (S. H. Cho, Jahncke, Chin, & Eun, 2006). The raw materials used in gelatin manufacture have obvious effects on gelatin, mostly originating from differences in the amino acid composition of the collagen of the raw material. Also, variations in processing conditions such as extraction time, extraction temperature, and concentration of acid or alkali dramatically affect the product.

The extraction conditions greatly affect the quality of gelatin as shown by many researchers. Therefore, optimization of extraction is of great interest to obtain the best possible product by eliminating and/or minimizing the excessive effects of extraction treatments causing extensive damage to collagen molecules. The most important extraction parameters are acid and/or alkali treatments, more specifically, the concentration of acid and/or alkali and duration of the treatment; extraction temperature and duration, and the conditions of refining and drying processes.

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2.5.1 Alkali and acid treatment

The gel strength of gelatin is greatly influenced by the concentration of acid and/or alkali, the duration of the acid and/or alkali treatment, and possibly the treatment temperature. A study by Gudmundsson and Hafsteinsson (1997) showed that high concentrations of alkali or acid increased the gelatin yield while decreasing gel strength.

Acid treatment is also important for the sensory aspects of gelatin, appearance and smell, as the acid treatment effectively removes odors and color that originate from the raw material (Boran and Regenstein, 2009; Zhang et al., 2007). Alkali treatment is, similarly, important and responsible for removal of possible impurities from the raw material and also for weakening the collagen structure, leading to higher yield and superior quality. In addition, alkali treatment causes glutamine and asparagine to lose their amine groups, converting them to glutamic and aspartic acid residues, respectively, lowering the isoelectric point of collagen (Johns and Courts, 1977). Therefore, both acid and alkali treatments need to be optimized for pH, duration, and temperature of extraction. The example of optimization of acid and alkali treatments are stated in Table 2.3.

In term of the extraction stage, which is normally done in water can be done at using mild acid or alkali conditions. Acid or alkali treatments are useful for a more effective extraction, increasing the yield and shortening the extraction time. Zhou and Regenstein (2005) showed that acidic conditions are more favorable for higher gelatin yield. However, acidic conditions also cause low gel strength, which is not desired in most gelatin applications. The isoelectric point of collagen is around 6–6.5, depending on the amino acid composition, specifically the content of the acidic and basic amino acids of collagen,

which vary both due to source and to processing conditions, for example, due to the impact of processing on glutamine and asparagine. According to the application in which gelatin is used, the effect of pH on gelatin needs to be carefully considered and the pH of the extraction solution needs to be adjusted to get a high quality gelatin. For example, as type-A gelatin has a higher isoelectric point, its use is favorable in those applications that require low pH at which the gelatin would be conducive to forming gel networks. Similarly, as type-B gelatin has a low isoelectric point, it is used in those applications that require a higher pH at which the gelatin is readily available for formation of the gel network.

2.5.2 Extraction temperature and duration

Different temperatures and duration are used in gelatin manufacturing but most extractions are between 45 and 60°C. Temperatures from 50 until 80°C can promote intramolecular bond formation between strands and consequently gelatin with stronger gelling ability can be obtained (Djagny et al., 2001). Higher temperatures over 80°C, however, result in fracturing of intramolecular chains giving gelatin having a weaker gelling ability. Lower extraction temperatures, on the other hand, lead to low yields but a superior quality. Similarly, longer extraction times give better yield, while the extracted material suffers from low gel strength and viscosity due to excessive damage to collagen fractions with longer heating and possibly extraction of other proteins. Therefore, it is necessary to balance both extraction temperature and duration of the extraction, to get the best possible outcome. Some of the example of the optimized duration has been summarized in Table 2.3.

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2.6 Physical Properties of Gelatin

The chemical composition of the gelatin such as amino acid sequences of its molecules, the molecular mass distribution contributes to the physicochemical behavior of gelatin. The physicochemical behavior of gelatin is studied primarily prior to its application.

2.6.1 Solubility and viscosity

Gelatin has a very good solubility and displays high water-binding capacity due to its structure and the presence of ionic charges along the chain, Gelatin swells either in cold or warm water and can bind up to 10 times its own weight of water. Highly concentrated solutions, up to 50%, can be obtained using various methods. To reach total solubilisation, gelatin requires only low temperatures between 50°C and 60°C or a short time at 90–95◦C. In production of gummy candy, 1:2 ratio of gelatin to water was used to dissolve the gelatin (Jiamjariyatam, 2018).

Gelatin solutions have a low viscosity and are easy to handle, even at low temperatures of 50–70°C, compared to other hydrocolloids which have more limited conditions for use. The viscosity of gelatin solutions depends on temperature, concentration, ionic content, pH and molecular weight. Gelatin solutions exhibit the properties of a Newtonian fluid above 40°C. A standard method is used to characterise the viscosity of gelatin which the viscosity of a 6.67% solution of gelatin is determined at 60°C by measuring the flow.

Table 2.6: Viscosity of Different Type of Gelatin

Type of Gelatin	Viscosity	Reference
Mammalian Gelatin		
1. Bovine 2. Porcine	2.0-7.0 cP	Ninan, Jose, & Abubacker, 2009
Fish Gelatin		
1. Channel Catfish Skin	3.23 cP	Yang et al., 2007
2. Black Tilapia Skin	7.12 cP	Jamilah & Harvinder, 2002
3. Red Tilapia Skin	3.20 cP	Jamilah & Harvinder, 2002
4. Rohu skin	6.06 cP	Ninan, Jose, & Abubacker, 2009
5. Common Carp Skin	5.96 cP	Ninan, Jose, & Abubacker, 2009

The commercial gelatin's viscosity ranges from 2.0-7.0 cP from Table 2.4 and it can be seen that the gelatin derived from marine sources has similar value around the ranges which was comparable to that of mammalian gelatins, implying that fish gelatins behave like the mammalian with respect to their molecular weight and hydrodynamic volume. All gelatins had low viscosities as they behave as random coils in solutions at higher temperature. As the temperature is lowered, the gelatin molecules begin to form triple helical junction zones and partly revert to the collagen structure. Thus, as more physical cross-linking occurs, some network structure eventually develops and the viscosity rapidly increases in value (Gomez-Guillen et al., 2002).

2.6.2 The Bloom Gel Strength

A gelatin solution sets below a given temperature dependent on the gelatin type, the concentration, its gel strength and viscosity. This gelation process is due to a rearrangement of individual molecular chains into an ordered network of helical arrangements (Michon, Cuvelier, Relkin, & Launay, 1997).

A standard AOAC method is used to characterize the gel strength of gelatin. A 6.67% solution of the gelatin sample is prepared in a standard test bottle at 60°C, cooled to 10°C and kept for 17 h for maturation at this temperature. The resulting gel is tested using a texture analyser. The gel strength, often expressed as Bloom, is the mass necessary to depress a standard plunger with a plane surface and diameter 12.7 mm (1/2 inch) to a distance of 4 mm into the gel. Gel strength measured in these conditions is called Bloom and expressed in grams. Bloom ranges generally from 50 to 300 g.

Table 2.7: Bloom Strength of Different Type of Gelatin

Type of Gelatin	Bloom Strength (g)	Reference
Mammalian Gelatin		
1. Bovine	266.9 ± 6.67	Raja Nhari, Che Man, Ismail, & Anuar, 2011
2. Porcine	350.42 ± 1.05	
Fish Gelatin		
3. Channel Catfish Skin	276	Yang et al., 2007
4. Black Tilapia Skin	181	Jamilah & Harvinder, 2002
5. Red Tilapia Skin	128	Jamilah & Harvinder, 2002

For commercial products, gelatin is commonly divided in three categories: Low Bloom gelatin (less than 125 Bloom), Medium Bloom gelatin (150–200 Bloom), High Bloom gelatin (above 220 Bloom) and a higher Bloom value represents among others a gelatin with a higher gelling and melting temperature and a stronger gel.

According to Table 2.5, fish gelatins have lower gel strength than mammalian gelatins. Previous researchers indicated that warm-fish gelatin has physical properties more like beef and pork than that of cold-water gelatin (S. M. Cho, Gu, & Kim, 2005). As a point of reference, commercial pork or bovine gelatins often present gel strength values in the range of 200–300 g (Giménez & Montero, 2011).

The gel strength is dependent on concentration, pH, thermal history (temperature and time) and ionic content. The lower the ionic content, the higher the physicochemical properties such as viscosity and gel strength. Gelatin gels are viscoelastic materials with the properties of a solid and a liquid. Resilience and hardness of gelatin are given, respectively, by Bloom and viscosity, which can be modified by the presence of other substances, such as sugar, proteins and plasticizer.

2.6.3 Stability

As gelatin is a protein, it can undergo hydrolysis, which can be caused by numerous factors such as acid, alkali, temperature, bacteria, enzymes and irradiation. Once in solution, gelatin can suffer a loss in gel strength and viscosity when exposed to elevated temperatures for any prolonged period of time. This loss can be magnified under acidic conditions arising from the presence of fruit juices and organic acids. As with other proteins, the presence of proteolytic enzymes can also degrade gelatin solutions. The loss

of gel strength and viscosity is a result of hydrolysis of the gelatin molecules. The rate of hydrolysis is a function of temperature, time and the pH of the solution. To avoid unnecessary hydrolysis, the addition of acid to a product formulation should be delayed until the latest possible time in the process.

2.6.4 Setting point and melting point

Rheological measurements of both melting and gelling temperatures give highly reproducible results. A temperature sweep test is performed for this purpose. Heating or cooling is required to determine the melting and gelling temperature, respectively.

Other rheological tests, including time sweep, frequency sweep, stress sweep, and strain sweep, have also found applications in determination of gelatin quality, as they allow researchers to discriminate the gelatin gels according to their strength and elasticity. Stress and strain sweep tests are used to determine the linear viscoelastic region of the gels. Frequency sweep tests are useful to determine if the gelatin gels change with the changing frequency of the stress applied. Time sweep tests are used to determine if the gelatin gels' viscoelastic properties change with time at a controlled temperature and at a set level of stress applied

The setting point is commonly determined by evaluating the viscosity of a 10% solution, and observing the temperature at which gelation starts. However, the setting point can be estimated at different concentrations using more sophisticated methods based on the viscoelastic character of gelatin. The setting points of commercial gelatin vary from 20°C to 29°C. Meanwhile, melting point is the temperature at which a gelatin gel softens sufficiently and allowing carbontetrachloride drops to sink through it. Factors such as the

maturing temperature and the concentration of the gelatin gel tend to affects its melting point (Gomez-Guillen et al., 2002). When heated to temperatures over 25–35°C, for example when placed in the mouth, gelatin gels melt. The melting point of gelatin is above the setting point by 2–5°C (GMIA., 2012).

Based on Table 2.6, it can be seen that Cold-Water Fish Gelatin has the lowest setting and melting temperature, followed by Warm-Water Fish Gelatin and Mammalian Gelatin. This shows that Cold-Water Fish Gelatin does not exhibit a gel-like properties at a room temperature and melts at a range of temperature lower than a room temperature, meanwhile Warm-Water Fish Gelatin shows a similar characteristics to a Mammalian Gelatin. This shows the suitability of Warm-Water Fish Gelatin to be used to replace the mammalian gelatin in different application commercially.

Table 2.8: Setting and Melting Temperature of Gelatin (Haug & Draget, 2011)

Physical Property	Mammalian Gelatin (220 Bloom)	Cold-Water Fish Gelatin	Warm-Water Fish Gelatin (220 Bloom)
Setting Temperature (°C)	26–27 °C	4–8 °C	21–22 °C
Melting Temperature (°C)	33–34 °C	14–16 °C	28–29 °C

2.7 Application of Gelatin

Gelatin is a versatile hydrocolloid and is widely applied in food, pharmaceutical, cosmetic, medical and photographic products. Commercially, gelatins of porcine and bovine sources is used for these applications. Although, cold-water fish gelatin does not show the characterization that is suitable to be used as a replacement of the commercialized gelatin, several studies have suggested that warm water fish gelatins can in most cases also be used (Haug & Draget, 2011, (Giménez & Montero, 2011). The most important properties of gelatin are: thermoreversible gel formation texturing, thickening, high water-binding capacity, emulsion formation and stabilization, foam formation, protective colloidal function and adhesion/cohesion.

2.7.1 Applications in foods

Gelatin is typically used in the food industry. The thermoreversible gel formation and the "melt in mouth" texture of gelatin is favourable for desserts, increases emulsifying capacity of milk by binding to the surface of the fat droplets and thereby reduce the surface tension towards the aqueous phase, preventing syneresis in gel, and to bind whey in dairy product such as yogurts, curds and cream cheese. Furthermore, gelatin decreases the surface tension of water, enabling formation of foam by mechanical whipping or injection of gas. Gelatin is widely used in production of ice cream to give better texture and mouthfeel by changing the size and distribution of ice crystals formed. Besides, gelatin usage in meat processing industry is also common. Gelatin increased the ability to bind water and meat juices, and to secure good texture and taste is very important. Gelatin is also widely used in low-fat (fat replacer), low-carb (binding agent) and low-calorie (fat replacer and binding agent) food products.

2.7.2 Applications in confectionery

The confectionery industry uses gelatin not only for its thermoreversible gelling properties, but also for foam formation and stabilization, binding, emulsification and controlling sugar crystallization. Gelatins with unique properties are developed especially for the confectionery industry. Examples of products containing gelatin are gummy candy, mallows, meringues, caramels, bar products and sugar-coated candies.

Table 2.9: Example of Application of Gelatins

Application	Gel Strength (g Bloom)	Concentration (%)	Function
Desserts	200-260	2.5-3.0	Gel formation Texture Transparency
Gummy Candy	200-280	6.0-15.0	Gel formation Texture Elasticity Transparency
Marshmallows	160-260	1.0-3.0	Foam formation Foam stabilization Gel formation
Pastilles	160-220	1.0-2.0	Binding agent Texture Melting properties

In Table 2.7, it can be seen that there are different recommended gel strength of gelatin with its concentration for one specific application. As discussed in Section 2.6, the

physical properties of gelatin is uniquely origin from the raw materials itself. External factors such as effect of extraction parameters may alter the physical properties but each type of raw materials have their specific amino acid composition which is the main factors in determining the gel strength of the gelatin.

2.8 Applications in Gummy Candy

Gummy candy has its amorphous structure and is categorized as an amorphous candy and gelatin is used to provide gel formation and texture to the candy. Gelatin provide a network to hold high moisture content sugar syrup. Starch and pectin are other hydrocolloids that are commonly used aside of gelatin and each of them has its own unique texture and organoleptic properties to the candy. Several commercialized gummy candy such as Haribo Gummy Candy, mainly uses porcine and bovine derived gelatin. Bovine and porcine gelatin other than giving desired texture, has excellent clarity, and is neutral in color and flavor. In most cases, the use of a high-Bloom type A gelatie is recommended. Confections such as gummi bears contain a relatively high percentage of gelatin at 9-15% (GMIA., 2012). These candies dissolve more slowly thus lengthening the enjoyment of the candy while smoothing the flavor.

In the sugar and confectionery category, gummy candies stick to the top of the heap when it comes to new product launches. Some of this growth can be attributed to the crossover of traditionally confectionery gummies to health and wellness for the use in adult and children's vitamins. Vitamin, mineral and supplement gummies grew 48.5% from 2012-2014 with vitamins having the largest share but supplements experiencing the largest increase as more users become interested in gummy formats.

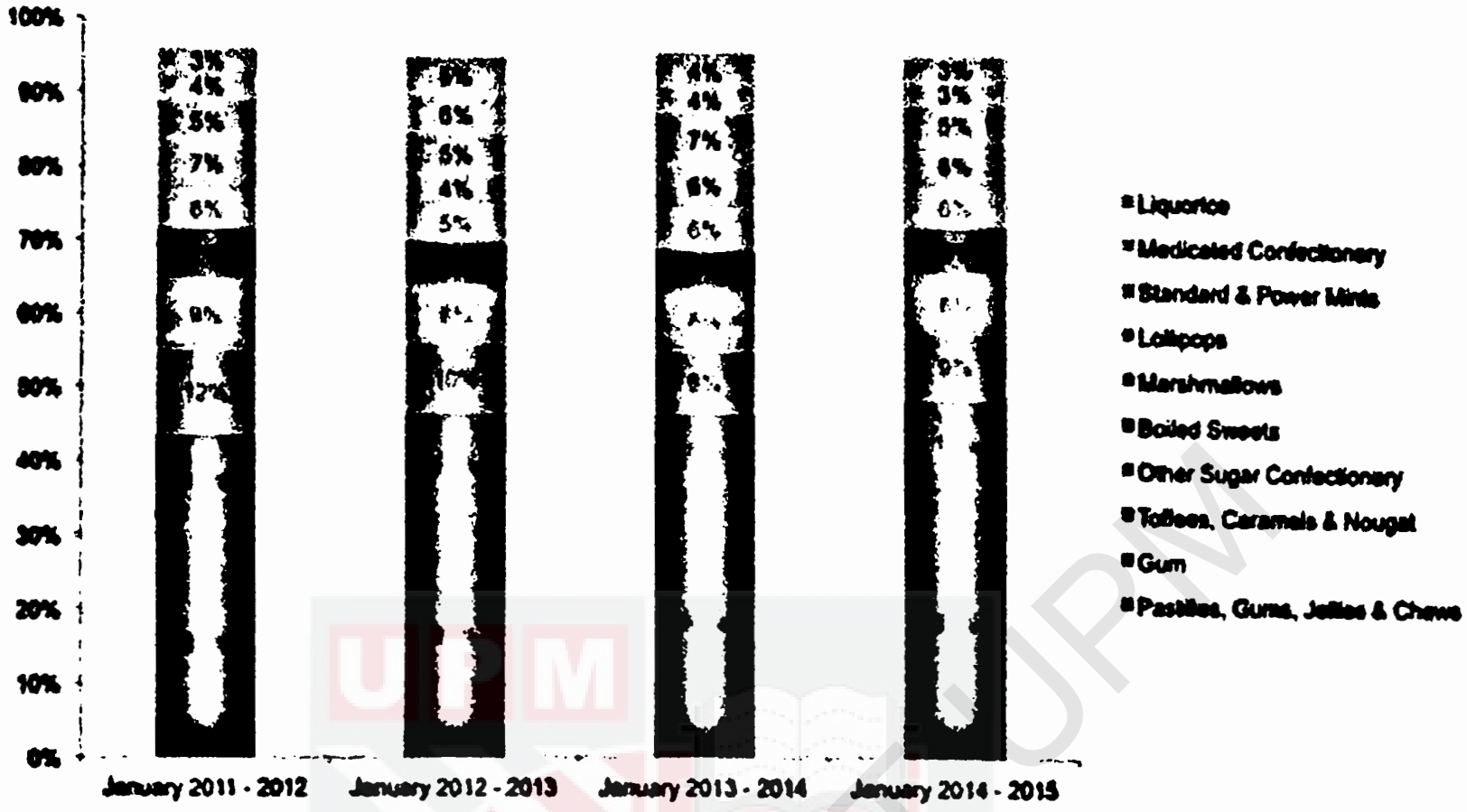

Table 2.10: New product launches in North America

New product launches for the gummy market (defined as pastilles, gums, jellies & chews) in North America (Figure 2.3) have been consistently the largest for the past five years with a modest growth from 31% in 2010 to an expected 37% in 2016 (Fona International, 2016).

2.9 Ingredients and Formulations of Gummy Candy

Gummy Candy main ingredients is the sweeteners, hydrocolloids and minor ingredients such as organic acids, flavourings and food colouring. The physicochemical properties of these candies arises due to the interactions of the ingredients such as sweeteners and hydrocolloids together, forming a cross-linking network of entrapped gel giving it the semi-solid texture.

2.9.1 Sweeteners

Sugar may be utilized as the primary ingredient of a formulation. Candy-making primarily is dependent on the concentration of sugar in boiled sugar syrups and on controlling or preventing crystal formation.

There are two major types of candies which are crystalline and amorphous candies and gummy candy is a type of amorphous candies as they do not have a crystalline pattern of molecules. In general, they contain a high sucrose concentration and a large amounts of agents that interfere with or prevent the crystal formulation. They have a low moisture level, and are more viscous as syrup than crystalline candies. Sucrose molecules are able to align and form large lattice arrangements of crystals. Other sugars, such as the monosaccharides glucose and fructose (or invert sugar), has different shapes that interfere with aggregation and crystal development, thus, a candy with too much invert sugar will fail to harden. High-fructose corn syrup, honey, and invert sugar are examples of sugars that are added to syrup in candy-making in order to prevent the formation of large crystals. Amorphous candies have a higher sugar concentration than crystalline candy because more sugar is incorporated and more water has evaporated at the higher temperature. The candy mixture is so viscous that crystals cannot form (Vaclavik, 2008).

Choosing the correct ratio and type of sweeteners are important as the technology of candy-making is based largely on manipulating sugar, the principal ingredient in candy, to achieve special textural effects. This is accomplished primarily by controlling the state of crystallization of the sugar and the sugar-moisture ratio. When the sugar in confections is crystalline, the crystals may be large or small, and the non-crystalline is amorphous or

glasslike. Invert sugar can prevent or help control the degree of sucrose crystallization. A mixture of sucrose and invert sugar has greater solubility in water than sucrose alone, increased solubility is equivalent to less crystallization. Furthermore, invert sugar is hygroscopic which helps to prevent chewy candies from drying out and becoming overly brittle. In terms of sweetness, the components of invert sugar differ from sucrose, thus a mixture of invert sugar and sucrose is sweeter than sucrose alone.

Other ingredients often used in candy making may influence sucrose crystallization, although this effect may be secondary to the main reason for their use. Thus, besides the thickening and chewiness properties, these ingredients interfere with sucrose crystal formation. This is due to adsorption of these materials onto crystals surfaces during formation. A barrier between attractive forces of the crystal lattice and sucrose molecules in solution is produced limiting the crystals from growing in size. Some softer candies owe their chewiness in part to pectin, gums and gelatin. Besides, common humectants such as glycerol and sorbitol is added to hold moisture within such confections. (Potter, 1995).

2.9.2 Hydrocolloids

Gummy candy could exists with different textural properties, primarily due to the type and amount of hydrocolloid employed. The type and nature of the sweeteners and the final water content also play important roles in determining product characteristics. The most commonly used hydrocolloids include starch, pectin, and gelatin, although gum arabic and agar can be used as well.

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Table 2.11: Hydrocolloids and its advantages and disadvantages

Type of hydrocolloids	Advantages	Disadvantages
Gelatin	• Multifunctional • Melts at body temperature with rapid and intense release of flavor • Unique texture, elasticity and brilliance • Transparent	• Low stability to heat • Low gelation temperature • Slow gelation • Soluble only at higher temperature • Animal source • BSE discussion
Pectin	• Higher melting temperature • Excellent flavor carriers • Rapid gelation	• Gelation difficult to control • No elasticity • Poor melting behavior • Possible pesticide contamination
Carrageenan	• Gels rapidly • High melting point • Gels vary in firmness and elasticity depending on the type used • The iota texture is similar to that of gelatin in confectionery applications	• Turbid gels if not refined • Allergenic potential • Poor release of flavor (iota) • Syneresis may occur during storage
Alginates	• High melting point • Soluble in cold water	• Short, brittle texture • Poor release of flavor due to poor melting behavior • Restricted applications at low pH • Syneresis may occur during storage
Arabic Gum	• Not genetically modified • Can be processed in high concentrations and thus used as fiber. • Prebiotic, cholesterol lowering, prevent cancer • Soluble in cold water	• Non transparent • Does not form gels • Allergenic potential
Agar-agar	• High melting point • Small quantities required • High gelling power	• Low gel elasticity • Poor release of flavor due to higher melting point • Low transperancy • Soluble in hot water only

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The hydrocolloids that can be used to manufacture gummy candy is summarized to Table 2.8. It states that each hydrocolloids have their own respective technological properties. Nonetheless, hydrocolloid X cannot simply be replace with hydrocolloid Y. But with that, mixture of hydrocolloids with gelatin can be done to expand their range of properties and possible applications.

As discussed in Table 2.8, the advantages of gelatin as hydrocolloid is melts at body temperature and intense release of flavor, besides its unique texture. This is attractive for gummy candy consumers as they can be used as pharmaceuticals for the treatment of the oral cavity, mouth infection products or medical devices containing essential oils and plant extracts, or supplements containing vitamins and minerals.

However, one distinct problem is the source of gelatin itself, where there are some religion against the use of cow-derived, pig-derived product, the benefits of gummy candy from gelatin could be a limitation for some part of the community. The use of marine gelatin might be able to counter the problem.

Texture Profile Analysis is used in confectionery factories to evaluate texture characteristic so that, for example, gummy jellies can be handled without risk of deformation or breakage (P. Delgado & Banon, 2015). Several TPA parameters is summarized in Table 2.9. The TPA heavily relies on the formulations, sample preparations and the setting of the Texture Analyzer itself. Besides, different area of sample size could be contributing to the TPA as well.

Table 2.12: TPA properties of Gummy Candy

Type of Gelatin	Formulation	% Gelatin	Hardness (g)	Adhesiveness (g.s)	Gumminess	Springiness	Cohesiveness	Reference
Porcine Gelatin (240 Bloom)	23.5% water, 33% sugars 31.5% glucose syrups, 3% citric acid	9	917.75	ND	ND	ND	ND	(Jiamjariyatam, 2018)
		12	1121.69					
		15	1325.63					
Chinese Herring Skin Gelatin (161 Bloom)	36.6% sugars, 24.4% glucose syrups, 3.9% pineapple juice, 0.05% ascorbic acid, red pitaya fruit puree 20%	7	1269	ND	ND	ND	ND	(Hani, Romli, & Ahmad, 2015)
Fresh grass carp Skin Gelatin	1-20% stevia	6.67	2200-1600	0.91-0.94	1800-1400	0.93-0.94	2-1.4	(Cai, Feng, Regenstein, Lv, & Li, 2017)
Porcine (260 Bloom)	40% water, 50-54% sugar (4:6 of sucrose:glucose syrup)	6	2710	ND	2440	0.95	0.91	(Periche, Heredia, Escriche, Andrés, & Castelló, 2014)
		8	4430		4040	0.965	0.912	
		10	6210		5620	0.969	0.905	

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Table 2.9: TPA properties of Gummy Candy

Type of Gelatin	Formulation	% Gelatin	Hardness (g)	Adhesiveness (g.s)	Gumminess	Springiness	Cohesiveness	Reference
Sutchi catfish skin gelatin (360 Bloom)	60 ml water, 10 ml lemon juice, 26 ml glucose syrup	5	912.90	2.66	867.97	2.68	0.86	(Normah & Muhammad Fahmi, 2015)
Bovine gelatin (217.37 Bloom)			897.88	0.87	763.37	2.67	0.88	
Bovine gelatin	Honey (*% Gelatin is based on weight of honey)	15	1047	0.04	1009	1.07	0.93	(Mutlu, Tontul, & Erbaş, 2018)
		20	1442	0.05	1401	1.01	0.93	
		25	1730	0.08	1604	0.93	0.94	
Bovine gelatin (240 Bloom)	24.5% water, 33% sugar, 31.5% glucose syrup, 3% citric acid, 1% crude extract	8	ND	ND	1273	0.97	0.90	(Charoen & Savedboworn, 2015)
Porcine gelatin	38.3% glucose syrup, 26.3% water, 20.8% sucrose, 0.9% citric acid, 0.5% lactic acid, 0.2% vitamins, 0.9% starch	3.5	2800	ND	1988	4 mm* (different unit from the rest)	0.74	(Delgado & Bañón, 2017)

2.10 Gel formation in Gummy Candy

Gelatin is a mixture of polymer chains of different lengths. Because of that, colloidal solutions or sols are formed instead of the real solutions. Heating the solutions will retain them as a liquid sols and by cooling, the sols are converted to gels. The structural bond that is important for gel formation is hydrogen bond (Johns 1977).

Gelatin and other polysaccharides can form gel by several structural bonds such as hydrogen bonding, hydrophobic association, cation-mediated crosslinking and also covalent crosslinking even though it's uncommon but it's possible. The nature of the intramolecular bonding is dependent on the type of hydrocolloid, or their combination, used. In some cases, hydrocolloid molecules may be covalently bound through strong chemical interactions, whereas in other cases, the hydrocolloid molecules form a physical structure, without covalent bonding. Differences in bonding structures among the hydrocolloids are what impart the different textural attributes for the wide range of gummy and jelly confections on the market. Mixing hydrocolloids in gummy and jelly candies provides even greater variation in product texture due to the complex interactions in mixed, or composite, gels. The setting rates and physical properties of the hydrocolloid candies are also dependent, to some extent, on the nature of the sweeteners used in the formulation since the sweeteners may impact gelation properties.

Gel formation occurs due to the cross-linking of polymer chain, creating 3D network which entraps water within it forming a semi-rigid condition that has resistant to flow. Gelatin gel, just like most of natural polymer, exhibits viscoelastic behavior. This means, they show characteristics of both liquid and solids depending on the applied deformation stress. By

using a rheometer, the viscous and elastic properties can be characterized by the storage, G' and loss modulus, G''. It is said that in dilute system, molecule entanglement does not occur thus they have G'' greater than G'.

- **Gummy Candy manufacturing process**

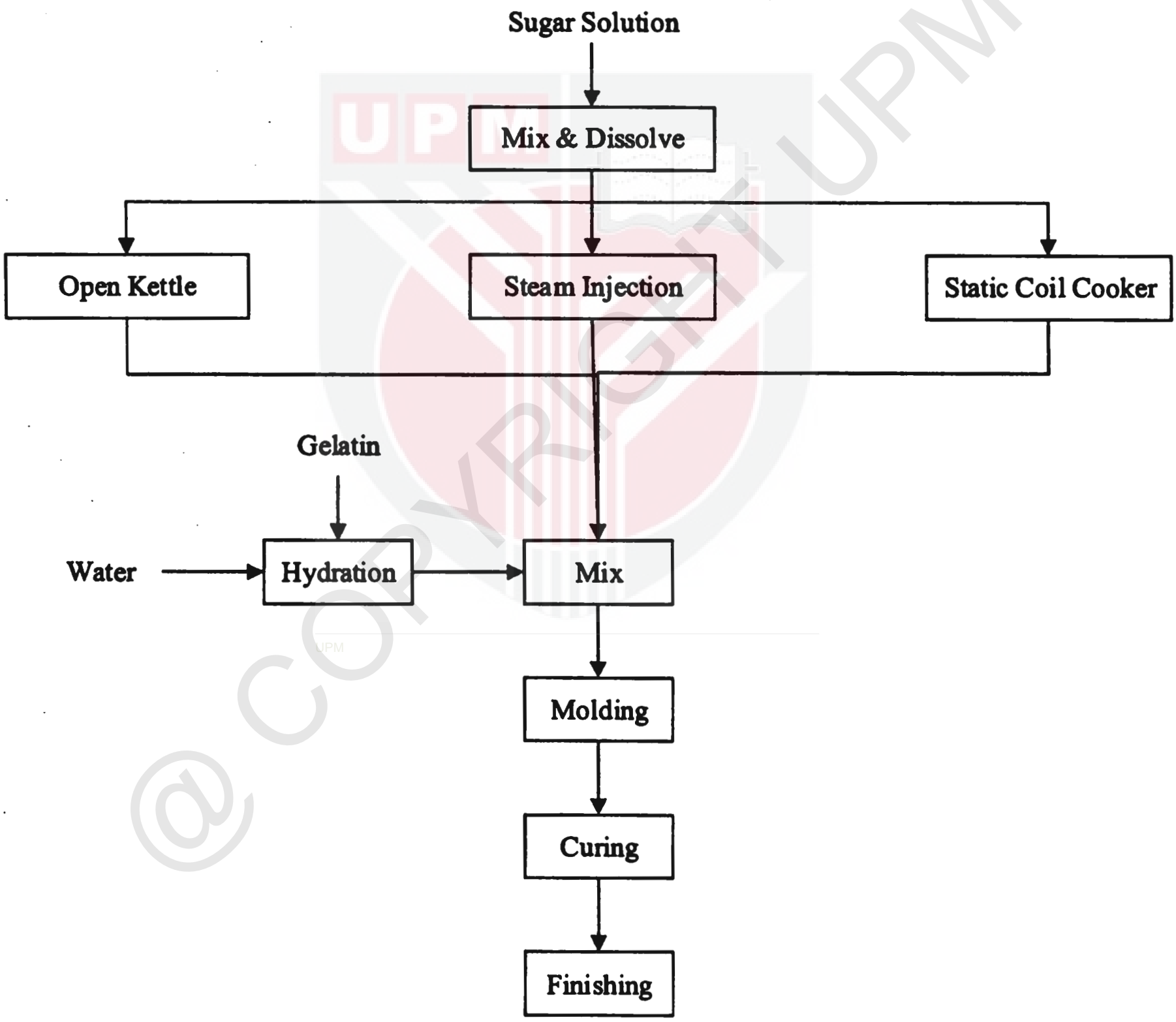

Table 2.13: Industrial Manufacturing Process of Gummy Candy

The flowchart in Figure 2.4 shows the industrial production of gummy candy. The production starts with mixing and dissolving the sugar solution before cooking it in either four type of cooking method. Then, a separate process of hydrating the gelatin is done before they are mixed together with the sugar solution. Next is molding and curing and finishing of the gummy candy. Each process will be discussed in details in next section.

2.10.1 Dissolving and cooking

First, production of gummy starts with mixing the sweeteners and then cooked to the appropriate temperature to get the desired moisture content. This is to ensure all the crystalline sucrose are completely dissolve, evaporate the water, deaerate the syrup and ensure the appropriate viscosity for the forming or molding step. Several methods can be used for cooking the sugar solution such as batch kettle cooking, vacuum cooking, swept surface heat exchanger and coil cooking. The defects of gummy candy sometimes may come from the cooking process where the colour of the gummy candy may become brown-ish or yellow-ish. This is due to Maillard browning due to long exposure of the sugar solution to heat.

Batch kettle cooking is the simplest method of cooking the mixed sugar solution. Either steam, electric heat or gas flame can be used to provide heat for cooking the sweetener. It is equipped with scrapping blade to prevent scorching on surface. Meanwhile, pressure cooking generally used to speed cooking process, with elevated pressure, the boiling point temperature increases, thus maintain the water in liquid form. A static cooker is often used to heat and evaporate the liquid sweetener mixture for gummy candies. The static cooker is simply a metal coil contained within a steam chest filled with high-pressure

steam. The sweetener slurry containing the hydrocolloid is pumped through the coil with condensation of steam on the outside of the coil causing rapid heating of the slurry. Steam condensate is continually removed from the bottom of the steam chest and either used for heat recovery schemes to recover valuable energy or returned to the steam boiler. By use of a back-pressure regulator, pressure within the heating coil maintains the water in the feed slurry in liquid state despite the high temperatures. The residence time within the coil depends on the type of candy being made, although typical residence times are on the order of a minute or two. Once the super-heated slurry exits the back-pressure valve, the water is flashed off in an expansion chamber with the concentrated candy syrup exiting the bottom of the chamber, ready for further processing. Flashing off the water also helps to deaerate the sugar mass. The static cooker is often used for gelatin because it gives a very rapid cook with minimal degradation of the protein (due to the short residence time) and minimal air entrainment. Thus, hydrated gelatin can be added directly to the batch prior to cooking, making this an efficient and convenient process for gummy candies.

2.10.2 Hydration of gelatin

The hydration process consists of a number of process steps: dispersion of the powder gelatin, swelling of the dry gelatin particles, and their dissolution by warming. The hydration of gelatin can be done in two methods, cold-method and warm-method. In cold method, the cold-swollen gelatin is placed in a water bath at 60-70 °C. At temperature above 50°C, the structural bonds are broken down and disperse molecular of a random coil structure is formed. In warm-method, the gelatin is added to water at 80°C under vigorous stirring. The gelatin immediately swells and dissolves. Comparisons of both method is summarized in Table 2.11.

Cold-method is said to be the traditional technique of hydration of gelatin. Gelatin is pre-swell with water before placed in a water bath at 60–70°C or a in a vessel heated by an external mantle or heating coil to a similar temperature. The solution that is prepared can then be further processed. Meanwhile warm-method do not necessitate a pre-swelling step. In such a one-step hot-dissolution process, the powder gelatin is added to water at 80°C (Schrieber & Gareis, 2007a) under vigorous stirring.

Table 2.14: Comparisons of hydration technique

Hydration technique	Advantage	Disadvantage
Cold-method	• Less thermal degradation	• Slow hydration method • Possibility of lump formation
Warm-method	• Rapid swelling, pre-swelling process can be skipped • Bubble-free solution	• Expensive apparatus • Solutions can be very hot so they have to be processed immediately to avoid thermal degradation

Hydrating gelatin is one of the crucial stage that may affect the quality of the gummy candy at the end of the process. This is because gelatin is sensitive to heat, and excessive exposure to heat may causes low gelling ability due to degradation of the structural bond (Ling, 1977).

As summarized in Table 2.9, the benefits on cold hydration method is that it has less thermal degradation as they are usually heated lower than 70°C. But the underside of

this is that it does take longer time and more steps to achieve, thus is not very efficient for the industrial scale. In order to prevent that, warm-method is done to reduce the time of hydration. These one-step dissolution processes were previously recommended only for concentrations up to a maximum of 15%. Today, however, in the interest of shortening production processes, even highly concentrated solutions are prepared using the hot-melt process. However, this method come up with a risk of degradation of the peptide chain, thus it must have been done rapidly.

Another aspects that can be discussed at this stage of gummy candy is the lump formation. Lump formation is undesirable in the end-product as it disrupt the uniformity of the candy produced. Lump formation can occurs if the gelatin is not hydrated first and is mixed together with sweeteners. This is because they will compete with available water as sugars have a high affinity to the available water. Thus, separate hydration of gelatin should be done to avoid lump formations. However, another causes of lump formation is also due to increasing concentration of gelatin. Higher concentration of gelatin will cause them to swell slower due to competition against each other. It is noted that it's possible to hydrate a set of high gelatin concentration gels but foaming is expected to occur.

The gelatin to water ratio is 1:2 (Charoen & Savedboworn, 2015). A study was made to compare the different gelatin to water ratio at 1:1, 1:1.5 and 1:2 and it is discovered that as the water ratio increases, the hardness of the gummy candy formed decreases (Hani et al., 2015).

2.10.3 Molding

One of the best methods to make candies with detailed shapes and patterns is by depositing the liquid candy mass into specially shaped molds. Molds can be made of plastic or polymeric materials, but quite often molds are made from dried starch powder (usually corn starch) shaped into the desired form. Once the candy has been cooled and dried (for starch depositing) to solidify, it is removed from the mold and finished prior to packaging. The equipment specialized for this is Mogul machine. After mixing the sugar solution with the hydrated gelatin, the mixture is then fed into the molding chambers of the machine and filled into the negative forms pressed into the starch trays.

To achieve optimal molding, the following criteria must be fulfilled: the solution must be fluid enough to flow easily through the valves and completely fill the molds, and it must separate cleanly from the nozzle at the end of each molding step. Viscosity is the important parameter in this section of processing. Prior to be filled in, if the molding mass is too viscous, i.e. too cold or too highly concentrated, ''tailing'' may occur as a result of the solution not separating cleanly when the valve is closed. This brings about the formation of gel fibers, causing the products to adhere to each other and form long chains. As a result, the starch is contaminated as the latter cannot be completely removed when the gummies are subsequently separated from the starch. Molding starch is also inexpensive, easy to work with and shapes can be quickly changed simply by replacing the press boards. However, dust can be a problem in manufacturing plants with starch depositing, and powder explosions are a continual concern.

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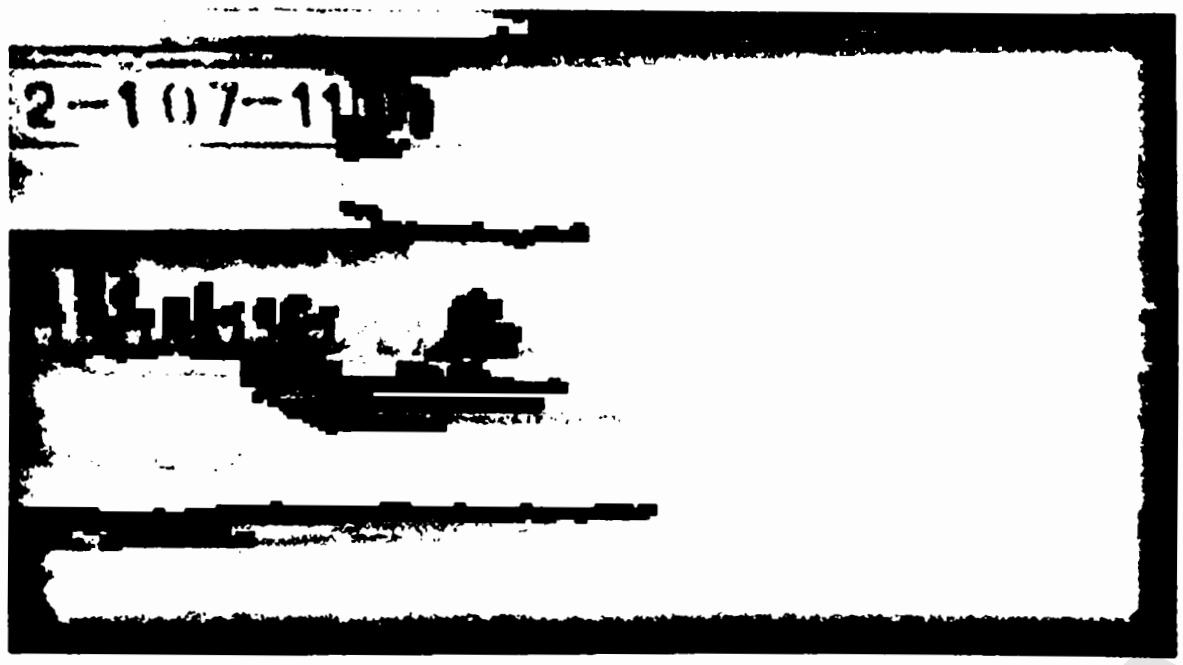

Table 2.15: Depositing of gummy candy mass into a starch mold

The mixed solution is deposited into the depression (Figure 2.5) and allowed to cool and dry from several hours to 1 day plus. The fluid candy deposited into the starch typically has higher water content than desired in the final product in order to ensure sufficiently low viscosity so that the candy completely fills the mold, filling all the small points that give the desired detail. Thus, it is important to ensure the formulations of the gummy candy will give the right viscosity for it to be allowed to flow into the molds.

Another type of molding is starch-less molding where the hot mixture is deposited into a plastic or polymeric material with the desired shape, often with a small hole in the base to facilitate product. Since no drying takes place in a polymeric mold, the water content of the deposited candy must be that of the end product.

2.10.4 Drying or cooling

The filled starch molded trays are stacked and taken to the drying room where the molding solution solidifies as the gelatin sets. At the same time, the products lose water; this water is either absorbed by the starch or vented to the atmosphere. Depending on the storage temperature and storage time, the water content of the finished product is reduced

to 14–19%. The drying parameters are dependent on the type of hydrocolloid being used. Products containing gelatin, pectin, and agar-agar are normally dried for up to 48 h at room temperature. Gums that are molded with a higher water content must be stored longer, up to 72 h, at the same temperature. For special products (pastilles, jelly babies, etc.) temperatures of 35–70 °C are used. The more water that the gel releases during this production step, the firmer the structure becomes. In this way, the texture can be varied by the drying process in starch molds. However, the final water content must be low enough to guarantee microbiological stability.

One of the main advantages of starch-free molding is that a separate drying step is no longer necessary; hence the production time and storage costs can be reduced. Products manufactured using starch-free molds have sharp contours and are extremely uniform. Instead of drying, they will undergoes chilling process to set the gummy candy. One disadvantage is that the manufacturer is restricted to using one shape of mold. If other product contours are to be produced, all the molds have to be replaced. This is a considerable investment and also requires refitting of the machines. When starch is used, it is only necessary to replace a few gypsum positive molds to produce and imprint new product shapes. Thus, starchless molding is oftenly used for laboratory scale.

2.10.5 Finishing

As soon as the product has attained its final texture, it is sieved to remove the starch powder or separated from its starch-free mold by special pins or compressed air. To prevent the product from sticking to the starch-free mold, the molds are coated with a release agent such as vegetable oil. Care should be taken to ensure that the oil used does not become

rancid during storage, as this can affect the taste of the product. The same is true of the starch process, as some oil is also added to improve the sharpness of the contours of the negative molds during imprinting and to reduce the generation of dust. The product is now subjected to a number of additional processes. It is moistened with steam, either sugar or a mixture of sugar and citric acid is sprinkled over it, or it is oiled with wax to give it a shiny surface. This oiling process takes place in an oiling drum and should last at least five to six minutes, as the oil must have time to be worked into the product and become stable. If this does not occur, the wax tends to run off the product and collect in the packaging, and the product becomes somewhat turbid after a short period of time. Subsequently, the product is packaged and delivered to the customer.

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CHAPTER 3

METHODOLOGY

3.1 Overview

Generally, in gummy candy preparation, sucrose, glucose syrup, citric acid and gelling agents are used. The gummy candy was prepared with different type of gelling agents, which was commercial bovine gelatin, commercial fish gelatin, extracted tilapia gelatin, Arabic gum, and mixture of bovine gelatin with Arabic gum and fish gelatin with Arabic gum and were hydrated at different temperature, at 70°C, 80°C and 90°C. This chapter presents the preparation of gummy candy by several section and analysis done.

3.2 Raw Materials

Commercial fish gelatin was obtained from Phywon System Ingredient Sdn Bhd Shah Alam. The commercial bovine gelatin was obtained from Halagel Distributor Sdn. Bhd. Shah Alam, Selangor. Arabic gum was obtained from Mahnaz Food, Shah Alam, Selangor. Tilapia fish scales were collected from a local wet market at Jerantut, Pahang. These wastes were immediately washed with tap water and frozen until further used.

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Generally, Tilapia fish scales samples were thawed at room temperature prior to the extraction procedure

3.3 Extraction of Gelatin from tilapia sp. Scales

Gelatin was extracted from tilapia scales according to the method of (Limpisophon, Tanaka, Weng, Abe, & Osako, 2009) with some modifications. Scales were soaked in a mined solution containing 0.05 M NaOH and 25 % Methanol with a scale/solution ratio of 1:4 (w/v), then placed at 10 °C overnight to remove lipids and non-collagenous proteins. Alkaline treated scales were washed with tap water to achieve the neutral pH. Demineralisation of tilapia scales was carried out using 5:1 of 0.05 M HCl solution /scale for 2 h at room temperature. This to ensure that at the end of extraction process only protein was extracted. Then, the demineralised tilapia scales were washed to neutral pH with tap water. After pre-treatment, the swollen scales were soaked in distilled water at 80°C, for 60 minutes to extract the gelatin. The extract was centrifuged at 15,000×g, 20 °C for 30 min with a centrifuge (Avanti J-25; Beckman, California, USA). The supernatant of gelatin extract was freeze-dried and the gelatin powder obtained was stored at −18 °C until use. Figure 3.1 is the summary of this process.

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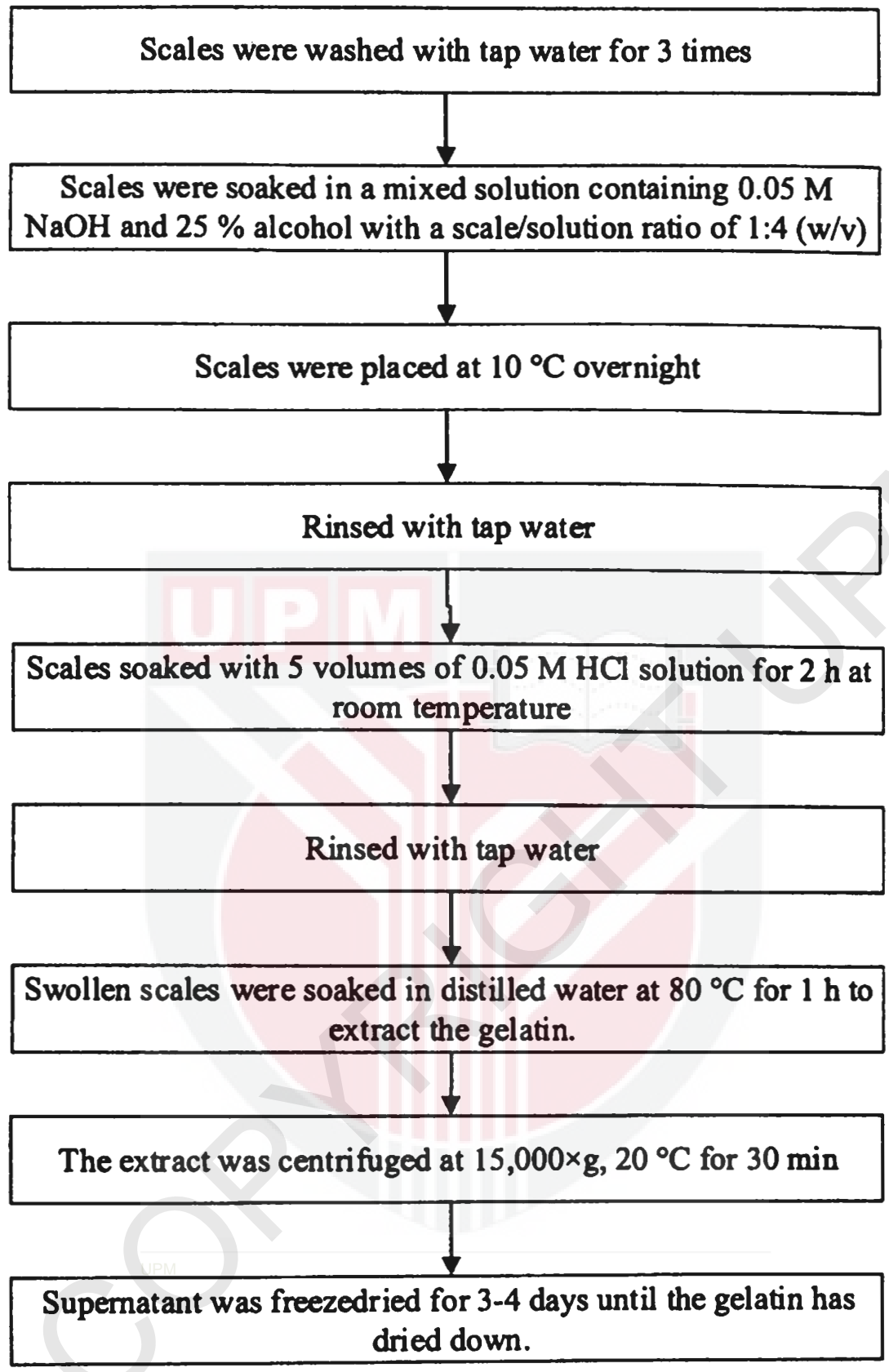

Figure 3.1: Extraction of Gelatin from Tilapia Scales

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3.4 Gelling Agents Preparation

Four different gelling agent were used, commercial bovine gelatin, commercial fish gelatin, extracted tilapia sp. gelatin and Arabic gum. The concentration of the gelling agent is varies at 9, 12 and 15% (w/v) (Jiamjariyatam, 2018). The gelling agents were hydrated with different temperature (60, 70, 80 °C) at ratio of 1:2 of gelatin to water (Charoen & Savedboworn, 2015) and 1:1 of Arabic Gum to water (Pizzoni et al., 2015) in a waterbath for about 15 minutes.

Another set of samples which were mixture of gelatin and Arabic gum with ratio of 1:1 were prepared. The concentration of mixed gelling agents were varied at 9, 12 and 15%. The mixed gelling agents were hydrated with different temperature (60, 70, 80 °C) at ratio of 1:2 of gelatin to water and with 1:1 of Arabic Gum to water (Pizzoni et al., 2015) in a waterbath for about 15 minutes.

3.5 Gummy Candies Preparation

The gummy candies are prepared following the method of Pizzoni et al., 2015 with some modifications. 32% (w/w) of sugar, 40% (w/w) of glucose syrup and 2% (w/w) of citric acid was added to 18% (w/w) of water and was mixed in beaker on a hot electrical plate until the solutions are completely dissolved. Then, the sugar mixture is poured into the hydrated gelling agent and is stirred slowly to incorporate all the ingredients in the waterbath, minimum stirring is needed to ensure no air entrapment occurs during this stage of processing. This process is summarized in Figure. 3.2.

Removal of lipids and non-collagenous protein
- Soaking of washed scales in 0.05 M of NaOH and 25% alcohol with ratio of scales to solution at 1:4

- Place overnight at 10 °C

Demineralisation
- Soaking of rinsed scales with 5 volumes of HCl solution for 2 h at room temperature

Gelatin extraction
- Soaking of rinsed swollen scales in distilled water at 80 °C for 1 h
- Centrifuge the extract at 15, 000 x g, 20°C for 30 min

Drying
- Freeze drying of supernatant for 3-4 days until gelatin has dried down

Tilapia scales gelatin

Gelling Agent Preparation
- Weigh different type of gelling agent (bovine gelatin, fish gelatin, tilapia gelatin, arabic gum) at different concentration (9%, 12%, 15%)

- Filling of distilled water in beaker with ratio of gelatin to water at 1:2 and arabic gum to water 1:1

- Mixing of gelling agent inside the beaker
- Put in water bath at different temperature (70°C, 80°C, 90°C for 15 minutes

Hydrated gelling agent

Sugar Solution Preparation
- Mixing of 32% sucrose, 40% glucose syrup and 18% water
- Heat on hot plate at 90 °C until all dissolved

Addition of 2% citric acid is added

Sugar solution

Mixing of hydrated gelling agent and sugar solution

Mixture of gummy candy solution

Molding of Gummy Candy
Pour mixed sugar solution into mold and chilled at 5°C for 24 hours

TPA

Physicochemical analysis

Brix

pH

Rheological analysis

Viscosity

Temperature sweep

Figure 3.2: Summary of experimental design

3.6 Physicochemical Properties Analysis

The total soluble solids content was measured in °Brix using a digital refractometer (AR200, ABBE Refractometer, Kruss, Germany). The pH was measured with a pH-meter (s40 SevenMulti, Mettler Toledo, Switzerland).

3.7 Texture Profile Analysis

The TPA of samples were evaluated at room temperature using a texture analyzer (TA-XTplus, Stable Micro Systems, UK) equipped with a 75 mm diameter aluminum cylindrical probe and it was used until the deformation reach 30% at a speed of 1.0 mm/s. The pause between the first and second compression were 3 s (Normah & Muhammad Fahmi, 2015). The measurement was in triplicate for all samples. From the force-time curve of TPA, the hardness, springiness, cohesiveness, adhesiveness, gumminess and chewiness were obtained.

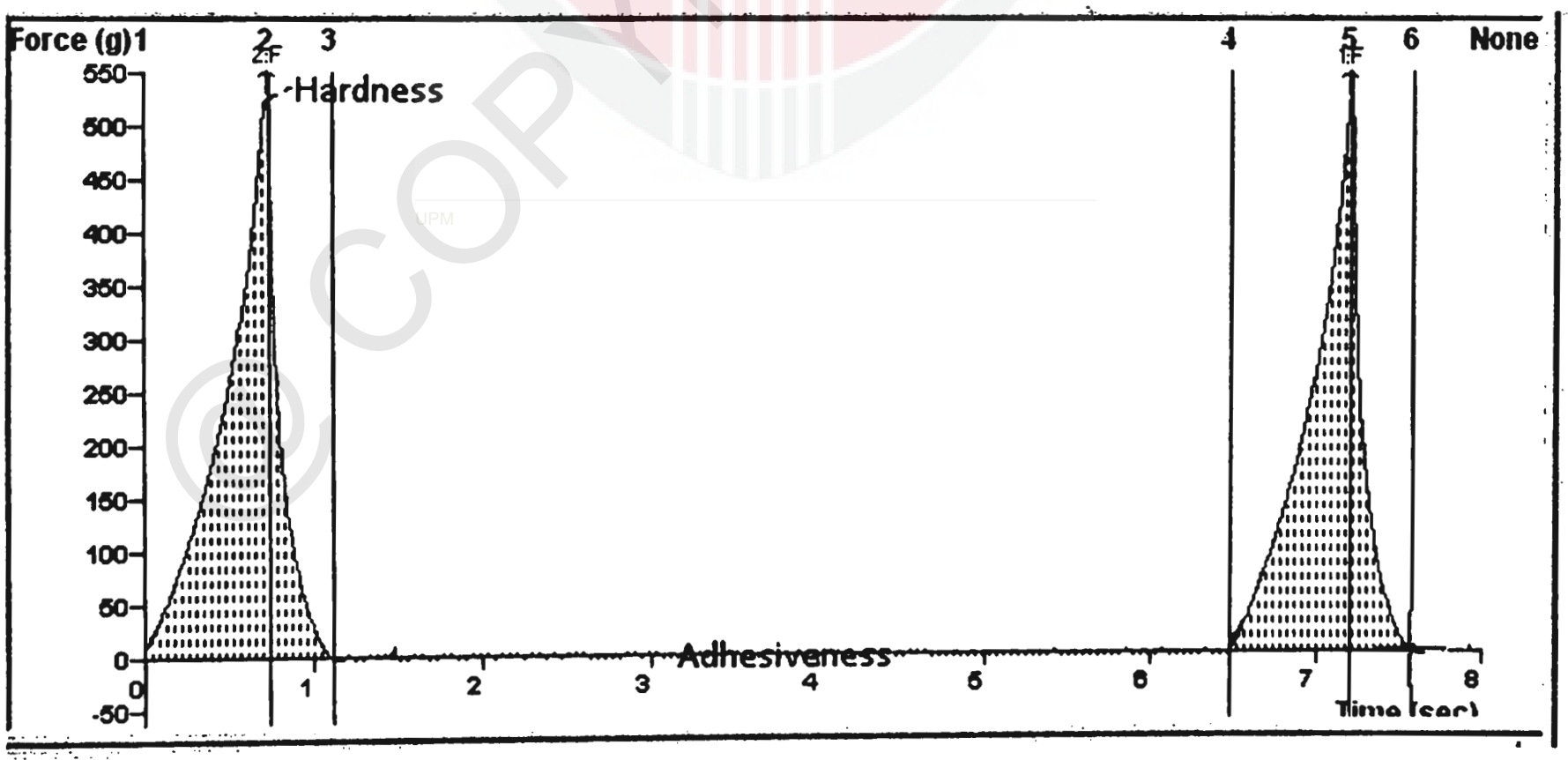

Figure 3.3: Force-time curve TPA

Hardness is defined as the force required for a complete penetration and biting through the sample when placed between molars (Gunasekaran & Ak, 2002) which is determined by Force 2. Cohesiveness is defined as the strength of the internal bonds making up the body of the product. It can determine the energy of sample during the second compression in relation to the first compression (Area 4:6/Area 1:3) (Gunasekaran & Ak, 2002). Gumminess is defined as the energy needed to disintegrate a semisolid food for ease of swallowing and it is determined by (Hardness*Cohesiveness) (Gunasekaran & Ak, 2002). Meanwhile, adhesiveness is defined as the stickiness of sample in the mouth through mastication and force required to remove the sample from the palate during eating (Zoon, 1991) which is determined from the negative force for the duration of the first bite. Springiness is the degree at which sample goes back to its unique shape after partial compression between the tongue and palate. It can be determined by the distance recovered by the sample during the time between end of first bite and start of second bite area (Time 4:5/Time 1:2). Lastly, resilience is defined as the ability of a material to absorb energy when it is deformed elastically and to release that energy upon unloading.

3.8 Small deformation oscillatory measurements

Small deformation oscillatory measurements were done according to the method of (Mhd Sarbon, Badii, & Howell, 2013) with a slight modification. The temperature sweep and steady state condition of different concentrations of gelling agents (9%, 12%, 15%) (w/v) were performed on a Rheometer (ARG2, TA Instruments, New Castle, DE, USA) using 60 mm parallel plate geometry with a 0.7 mm gap. The two main parameters determined in a dynamic rheological test are the storage or elastic modulus (G') describing

the amount of energy that is stored elastically in the structure and the viscous or loss modulus (G'') indicating the amount of energy loss or the viscous response.

3.8.1 Temperature sweep

A dynamic temperature sweep rheological test was used to determine the gelation and melting temperature of the gelatin samples. The stress and frequency used were 0.1 Pa and 1 rad/s respectively. For gelation, the sample was initially maintained at a temperature of 40 °C for 10 min to allow for equilibration. Gelatin samples were cooled on a Peltier plate from 40 °C to 10 °C and heated back to 40 °C both at a scanning rate of 2 °C/min. The gelation temperature was taken to be the temperature at which the elastic modulus began to dramatically increase in value. The temperature at which the G'/G" cross over occurred during cooling is close to the sol-gel transition or the gel formation point (Gudmundsson, 2002). The test for determining melting temperature immediately followed after the gelation test. After the sample reached 10 °C, the temperature was raised back to 40 °C. Melting occurred when the elastic modulus (G') began to decrease and loss modulus (G") began to increase in value. Changes in the elastic or storage modulus (G') and loss modulus (G") were determined as a function of temperature and were recorded.

3.8.2 Steady-state shear

The steady-state flow from 0.1 to 55.8 s^{-1} shear rate is done at 60°C and the value of viscosity at 55.8 s^{-1} shear rate is tabulated in graph for all samples (Harvinder, 2006).

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Introduction

Chapter 4 compares the physico-chemical, texture and rheological properties of gummy candy with different type of gelatin and mixture of gelatin with Arabic gum at different concentration. Comparisons of the effect of hydration temperature of the gelling agents on those three properties are also reported. However, it was discovered that gummy candy prepared from Arabic gum (9%, 12%, 15%) does not form a gummy candy after chilling at 5°C for 24 hours, thus they were not included in the results.

4.2 Effect of different type and concentration of gelatin on rheological properties of gummy candy solution.

The gummy candy is prepared by mixing the sugar solution with citric acid and gelling agent (gelatin and mixture of gelatin with Arabic gum). The brix, pH and viscosity

of gummy candy solution is tabulated in Table 4.1, Table 4.2 and Table 4.3 at 70°C, 80°C and 90°C hydration temperature respectively.

Table 4.1: Brix, and pH Value for gummy candy solution at 70°C hydrating temperature

Type of gelling agent	Concentration (w/v %)	°Brix	pH	Viscosity (Pa.s)
Commercial bovine gelatin	9	68.3±0.5	4.64±0.11	0.37±0.09
	12	71.9±0.3	3.99±0.13	0.42±0.02
	15	71.1±0.5	4.27±0.07	0.87±0.02
Commercial fish gelatin	9	67.8±0.3	4.31±0.03	0.23±0.01
	12	63.1±0.3	4.44±0.03	0.51±0.07
	15	68.4±0.2	4.00±0.03	0.68±0.02
Extracted tilapia gelatin	9	72.7±0.3	3.75±0.03	0.11±0.01
	12	69.4±0.4	4.07±0.06	0.25±0.06
	15	74.5±0.3	3.92±0.02	0.54±0.08
Mixed bovine gelatin and Arabic gum	9	70.5±0.3	3.71±0.04	0.02±0.009
	12	71.1±0.4	3.81±0.01	0.06±0.06
	15	72.1±0.2	3.91±0.01	0.17±0.07
Mixed fish gelatin and Arabic gum	9	73.2±0.3	4.07±0.05	0.05±0.01
	12	74.0±0.4	3.83±0.05	0.09±0.04
	15	69.7±0.2	4.05±0.04	0.31±0.04

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Table 4.2: Brix, and pH Value for gummy candy solution at 80°C hydrating temperature

Type of gelling agent	Concentration (w/v %)	°Brix	pH	Viscosity (Pa.s)
Commercial bovine gelatin	9	67.8±0.6	4.23±0.11	0.31±0.20
	12	63.1±0.3	4.29±0.10	0.88±0.67
	15	68.9±0.8	4.04±0.07	1.18±0.92
Commercial fish gelatin	9	69.7±0.2	4.26±0.01	0.44±0.01
	12	72.8±0.4	4.29±0.02	0.51±0.03
	15	70.9±0.2	4.32±0.01	0.78±0.04
Extracted tilapia gelatin	9	74.2±0.1	4.04±0.06	0.20±0.05
	12	71.6±0.2	3.95±0.02	0.28±0.08
	15	70.4±0.5	4.13±0.02	0.31±0.02
Mixed bovine gelatin with Arabic gum	9	70.3±0.2	3.93±0.04	0.02±0.004
	12	72.5±0.2	4.16±0.03	0.18±0.01
	15	71.3±0.2	4.33±0.02	0.29±0.01
Mixed fish gelatin with Arabic gum	9	71.5±0.1	3.94±0.03	0.06±0.06
	12	73.4±0.5	3.79±0.03	0.44±0.01
	15	72.0±0.3	3.71±0.03	0.50±0.04

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Table 4.3: Brix, and pH Value for gummy candy solution at 90°C hydrating temperature

Type of gelling agent	Concentration (w/v %)	°Brix	pH	Viscosity (Pa.s)
Commercial bovine gelatin	9	68.4±0.5	4.30±0.02	0.40±0.27
	12	67.4±0.3	4.15±0.03	0.54±0.09
	15	66.0±0.4	4.01±0.09	0.78±0.09
Commercial fish gelatin	9	72.7±0.3	4.30±0.07	0.36±0.23
	12	73.3±0.2	4.17±0.04	0.53±0.43
	15	71.4±0.3	4.22±0.01	1.26±0.30
Extracted tilapia gelatin	9	72.7±0.3	4.30±0.07	0.25±0.02
	12	73.3±0.2	4.17±0.04	0.33±0.06
	15	71.4±0.3	4.22±0.01	0.36±0.17
Mixed bovine gelatin with Arabic gum	9	72.4±0.3	3.98±0.03	0.15±0.03
	12	71.6±0.5	3.73±0.05	0.26±0.03
	15	73.4±0.2	4.25±0.02	0.40±0.001
Mixed fish gelatin with Arabic gum	9	72.3±0.3	4.09±0.03	0.15±0.03
	12	71.8±0.3	3.92±0.04	0.21±0.04
	15	69.7±0.4	4.25±0.05	0.33±0.001

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4.2.1 The pH value of Gummy Candy Solutions

pH is a scale used to specify how acidic a solution is. At neutral pH (6–7), most proteins are stable. At both higher and lower pH values, the stability toward denaturation decreases as the net charge on the proteins change. It is for this reason that in the manufacturing of an acid gelatin candy that the acid is added last to avoid excessive denaturation. Gelatin solutions are most stable at pH range between 5.0 and 6.0. Either lowering or raising the pH will accelerate degradation (Hartel & Hofberger, 2018). From Table 4.1, 4.2 and 4.3, the pH of the all the gummy candy is around 3.71-4.33. The value of pH is higher than that of gummy candy made by Jiamjariyatam (2018) which is around 2.8-3.1 and lower than that of gummy candy made by Periche et al (2014) around 5.1-6.1.

The pH level is contributed from the amount of citric acid added to the formulations. The differences may come from the different amount added in different formulations. The citric acid is added for tanginess and flavor of the gummy candy. Besides, the citric acid added is has an important role as interfering agent, which inhibits the formation of crystallization in the gummy candy (Strauss, 2014). The crystallization of sugar and roughness in gummy jelly would provide dissatisfaction among consumers (Jiamjariyatam, 2018).

4.2.2 The °Brix value of Gummy Candy Solution

Another set of characterization that can be made for the gummy candy solution is the degree Brix value. Degree Brix is referred as the sugar content in an aqueous solution. One degree Brix is 1 gram of sucrose in 100 grams of solution and represents the strength of the solution as percentage by mass. If the solution contains dissolved solids other than

pure sucrose, then the °Brix only approximates the dissolved solid content. From Table 4.1, 4.2 and 4.3, the value of dissolved solid content of the gummy candy solution ranges from 67-74 °Brix. The gummy candy made in the study by Periche et al (2014) has 73 °Brix, another study has adjusted the gummy candy around 68-75 °Brix (Charoen & Savedboworn, 2015) and 80 °Brix by Delgado et al (2017).

The value is largely contributed from the sucrose and glucose syrup mixture. The usage of sucrose alone will lead to a formation of crystallization thus, the glucose syrup is added as the invert sugar component in the gummy candy formulation. Sucrose molecules are able to align and form large lattice arrangements of crystals. Other sugars, such as the monosaccharides glucose and fructose (or invert sugar), has different shapes that interfere with aggregation and crystal development, thus, a candy with too much invert sugar will fail to harden.

4.2.3 The viscosity of Gummy Candy Solution

Viscosity is defined as a measure of a fluid's resistance to flow. It describes the internal friction of a moving fluid. Based on Table 4.1, 4.2 and 4.3 it was observed that as concentration increases, the viscosity increases. This is similar to study by Sarbon (2015). However, there are differences between the viscosities of gummy candy solution made up of different gelling agent. The viscosity of gelatin is partially controlled by molecular weight and its polydispersity of the peptides. Higher molecular weight fraction increases the viscosity (Jamilah & Harvinder, 2002). A study have shown that there is no linearity of viscosity with gel strength of gelatin (Foster & Hill, 2012). Furthermore, the viscosity analysis was done at 60°C (refer section 3.8.2), the solutions were in a random coiled order

instead of the 3D triple helix which is related to the gel strength. In order to study the effects of each gelatin's molecular weight on the viscosity of a solution, a dilute solution which will give the value of viscosity-average molecular weight (Sperling, 2005). The average molecular weight is affected by the chain length of the gelatin. Higher chain length allows for entanglement which help hold the whole solution together under stress (Sperling, 2005). In the random coiled state of gummy candy solution, chain entanglements cause the viscosity to be raised very significantly also. The chain length of gelatin is affected by the extraction method.

4.2.4 The gelling and melting temperature of gummy candy solutions.

Gelling and melting temperature was taken at which G'/G" crossover occurred during cooling and heating, respectively (Mhd Sarbon et al., 2013). Figure 4.1, 4.2, 4.3 shows the gelling temperature and Figure 4.4, 4.5, 4.6 shows the melting temperature of gummy candy made up of different type and concentration of gelling agent at 70°C, 80°C and 90°C hydration temperature.

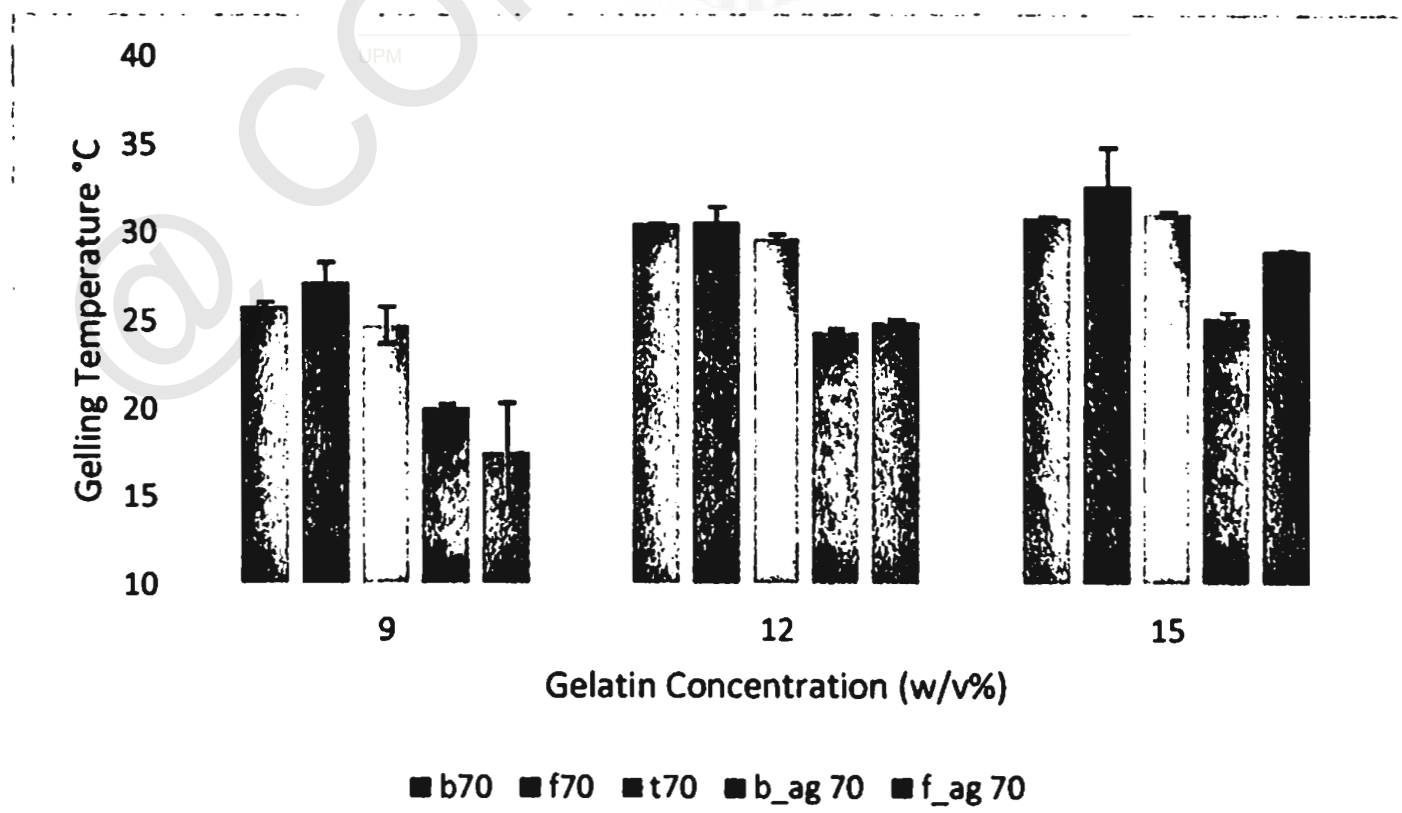

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Figure 4.1: Gelling temperature of Gummy Candy at 70°C hydration temperature

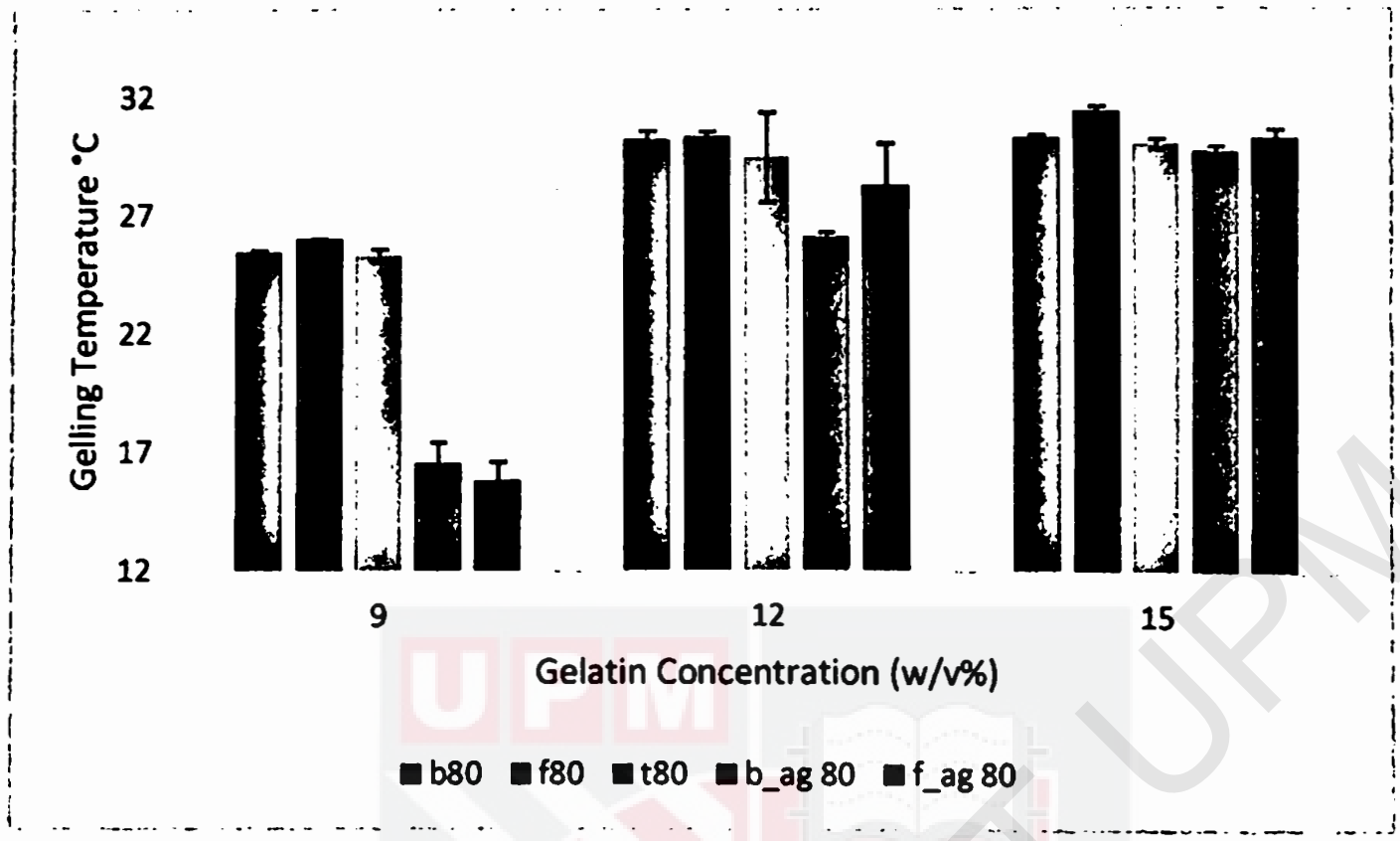

Figure 4.2: Gelling temperature of Gummy Candy at 80°C hydration temperature

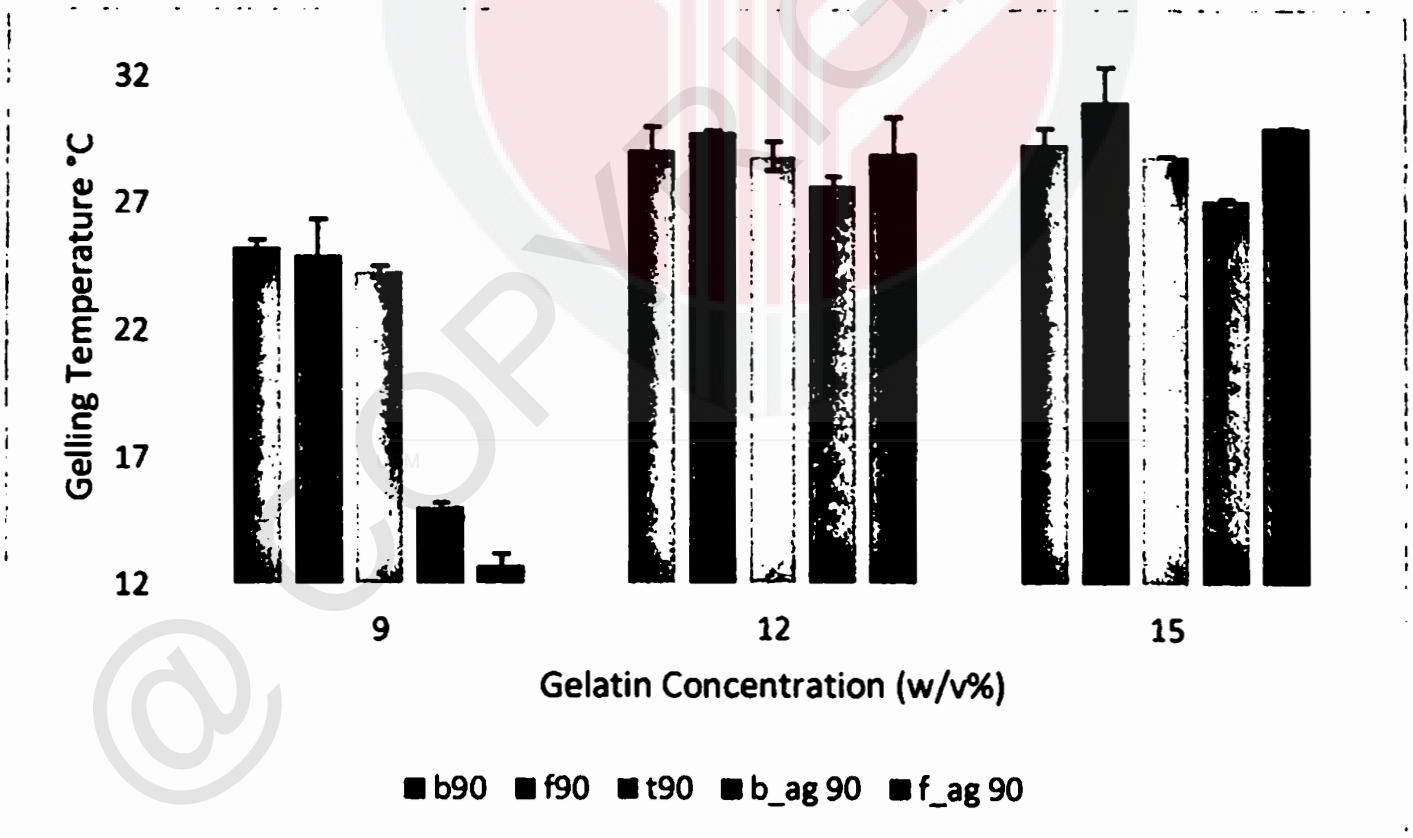

Figure 4.3: Gelling temperature of Gummy Candy at 90°C hydration temperature

From Figure 4.1, 4.2 and 4.3, it can be observed that gelling temperature of gummy candy made from bovine, fish and tilapia increases as the concentration of the gelatin increases at all hydration temperature. This is because the gelling temperature is dependent

of the cross-linking of hydrogen bonds in the system. By increasing the gelatin concentration in the system, the junction sites for hydrogen bond between the hydroxylated amino acid and water (Arnesen & Gildberg, 2007). This is proven by a similar study by Mhd Sarbon et al., (2013) that stated higher gelling temperature yielded from increasing the gelatin concentration.

Besides that, the effect of the type of gelatin used in the gummy candy can also affects its gelling temperature. This can be observed in terms of the pure gelatin and mixed gelatin and Arabic gum used in the gummy candy. From Figure, 4.1, 4.2, 4.3, there are notable difference between the gummy candy from bovine, fish and tilapia gelatin, compared to bovine-arabic gum and fish-arabic gum. It is later after molding that it is found that the gummy candy made from bovine-arabic gum and fish-arabic gum failed to harden and become a gel. The low gelling temperature and failed to harden in gummy candy from that mixture might be because the cross-linking could not occur due to a low concentration of Arabic gum is used in this study. Typically, the concentration of Arabic Gum utilized in gummy candy formulation is around 54% (Lees & Jackson, 1973). However, it is observed that the gummy candy made from bovine, fish and tilapia gelatin showed small differences in their gelling temperature.

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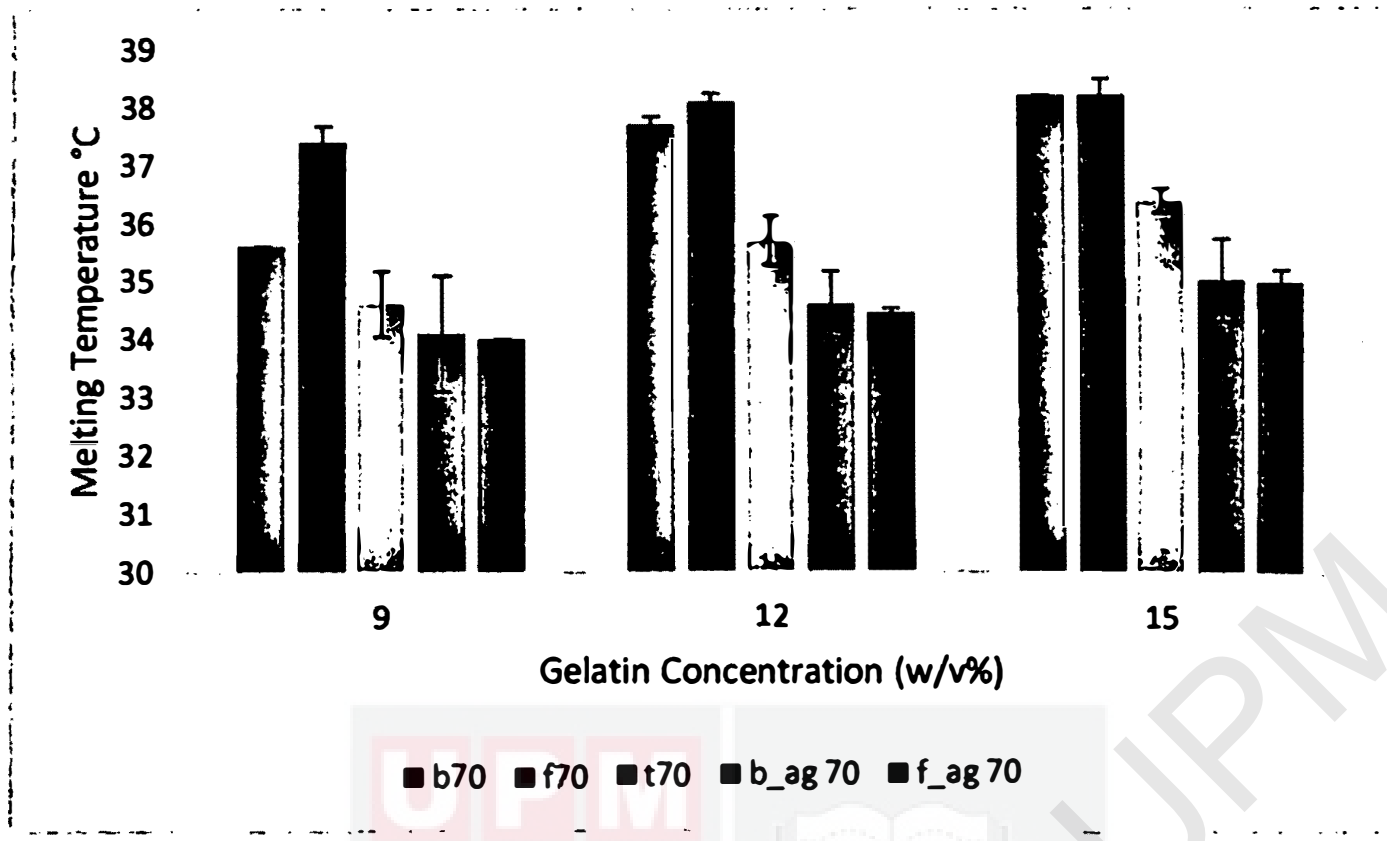

Figure 4.4: Melting temperature of Gummy Candy at 70°C hydration temperature

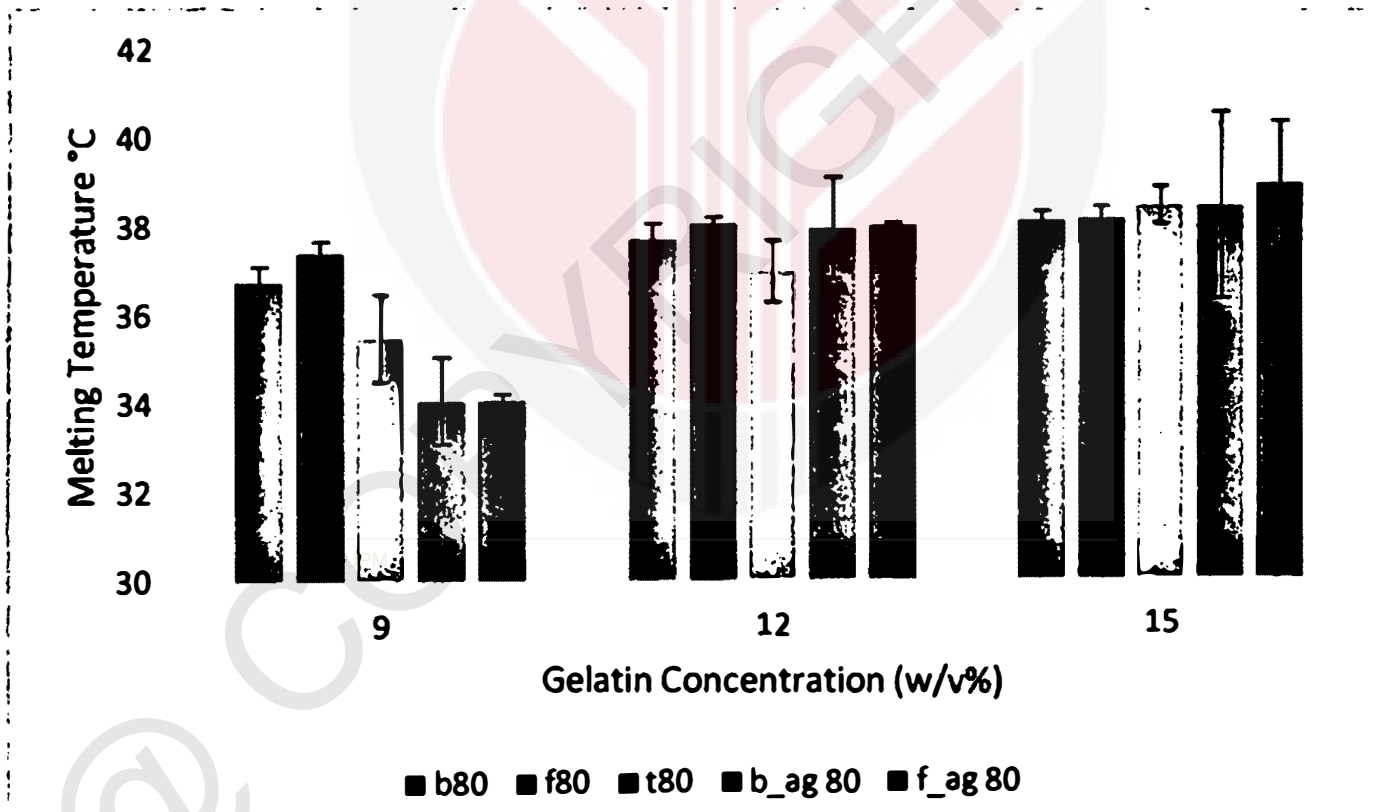

Figure 4.5: Melting temperature of Gummy Candy at 80°C hydration temperature

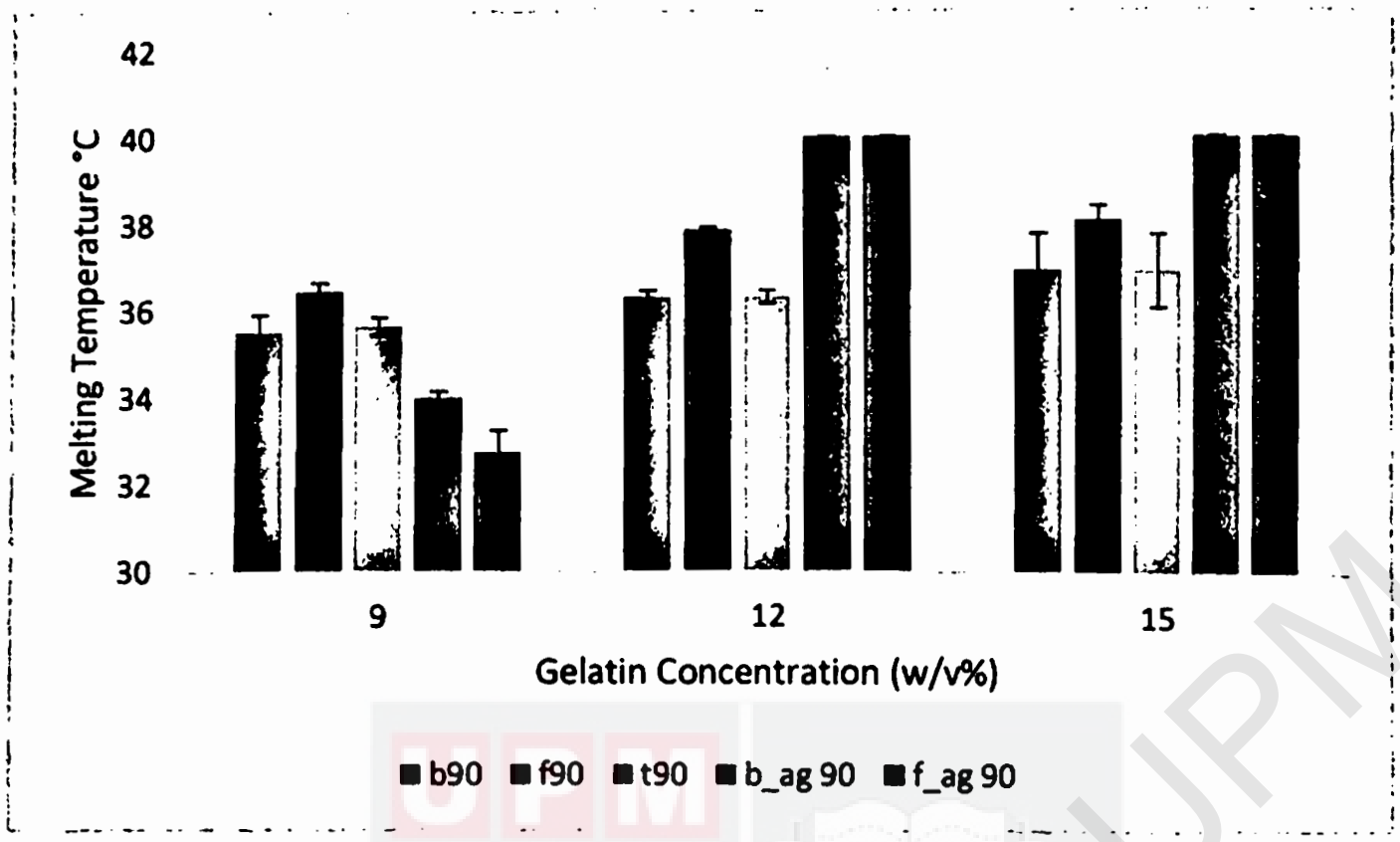

Figure 4.6: Melting temperature of Gummy Candy at 90°C hydration temperature

Similar to Gelling temperature of Gummy Candy, the Melting temperature of Gummy Candy increases as the concentration of gelatin increases. Melting temperature is an important character of gummy candy, as the melt-in-mouth properties is one of the advantage of using gelatin in the formulation of gummy candy.

Generally, the melting temperature of fish gelatin is much lower than that of mammalian gelatin (Karim & Bhat, 2009). However, the results in Figure 4.4, 4.5 and 4.6 contradicts to that as it can be seen that Gummy Candy made from fish gelatin has higher melting temperature at 9, 12, 15% concentration compared to bovine gelatin. At the same time, Gummy Candy made from tilapia gelatin showed lower melting temperature than that of bovine gelatin. The difference in thermostability of gummy candy from different gelatin may be attributed from the variations of processing methods of the gelatin itself (Jamilah & Harvinder, 2002) which in turn, affect the imino content of the gelatin (Schrieber & Gareis, 2007b).

Besides, gummy candy made from mixture of gelatin-arabic gum showed a superior melting temperature. This is because of mixture of polysaccharide and gelatin increases its melting temperature. Similar result can be seen from a study, a gel with mixture of gelatin and agar has higher melting temperature than that of a gel with gelatin only (Somboon, Karrila, Kaewmanee, & Karrila, 2014b)

4.3 Effect of different type and concentration of gelatin on textural properties of gummy candy

Texture of gummy candy can be measured by Texture Profile Analysis (TPA). In this section, the TPA of gummy candy made from different type of gelling agent is discussed. In relation to this study, a TPA of a commercialized gummy candy is made as a control sample to compare the gummy candy that is made with different type of gelatin and different concentration of gelatin.

Table 4.4: TPA of Haribo Gummy Candy

TPA Parameters	Average Value±Std dev
Hardness	514.705±71.016 g
Cohesiveness	0.971±0.002
Gumminess	500.061±69.479 g
Springiness	1.027±0.038
Adhesiveness	0.172 g.sec

4.3.1 Hardness of Gummy Candy

Hardness is defined as the maximum force required to compress food between molar teeth (Chandra & Shamasundar, 2015). Figure 4.1, 4.2 and 4.3 shows the hardness of gummy candy made of different type of gelatin with 9, 12, 15% concentration at 70°C, 80°C and 90°C hydration temperature respectively.

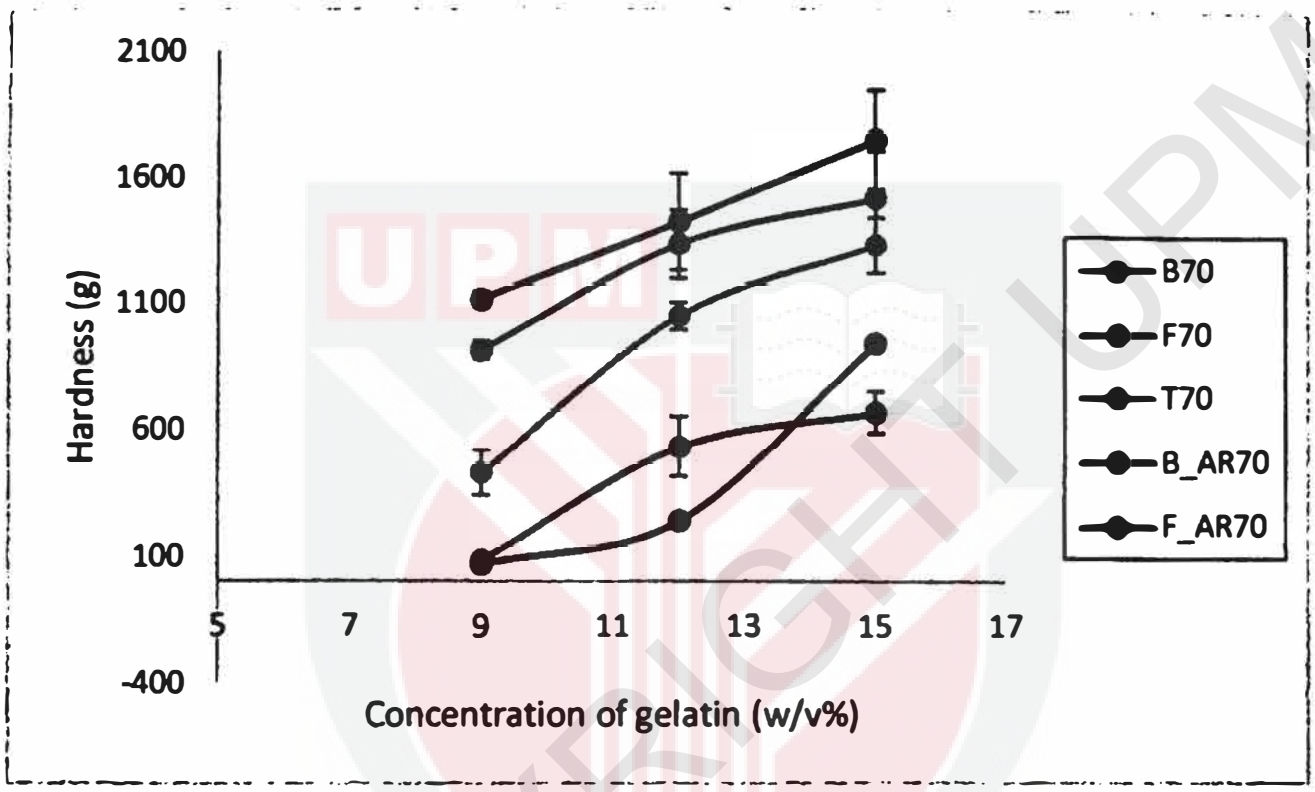

Figure 4.7: Hardness of Gummy Candy with Different Gelatin Concentration at 70°C

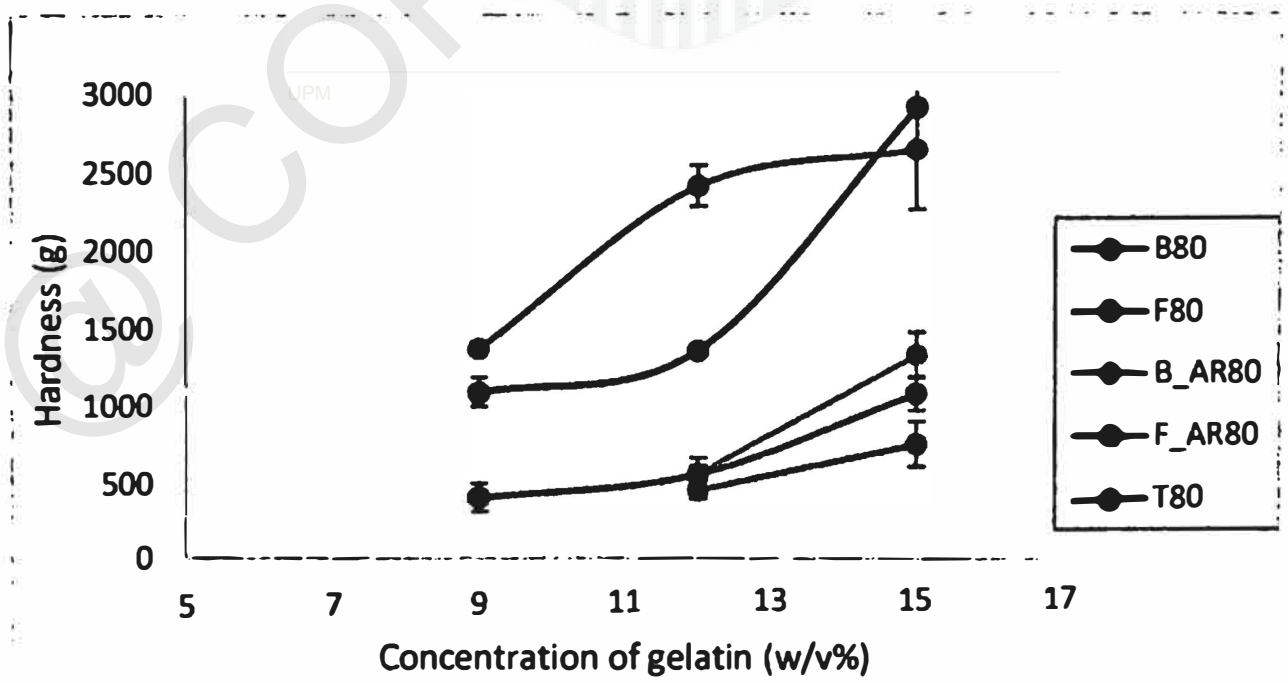

Figure 4.8: Hardness of Gummy Candy with Different Gelatin Concentration at 80°C

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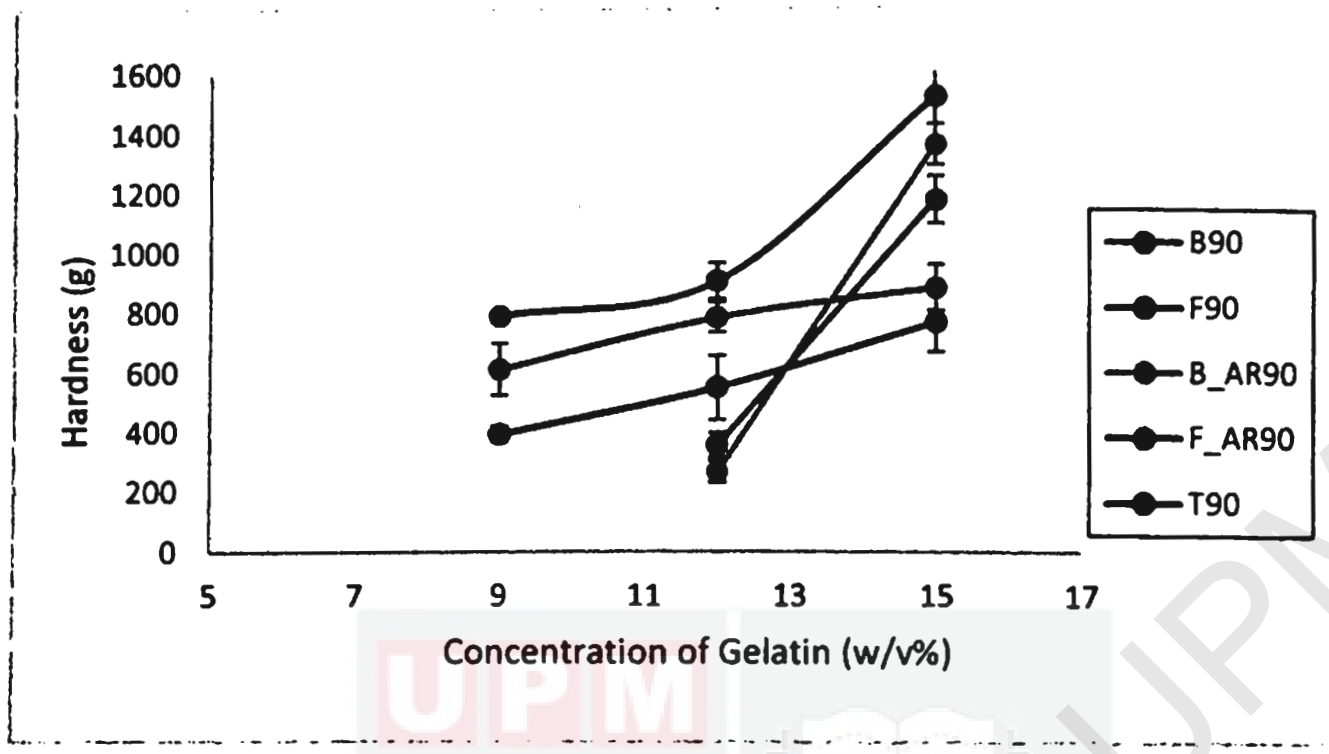

Figure 4.9: Hardness of Gummy Candy at Different Gelatin Concentration at 90°C

4.3.1.1 Effect of different type of gelatin on the hardness of gummy candy

The hardness of Gummy Candy made from all type of gelling agent increased as the concentration increases at all hydration temperature. This is because by increasing the gelatin concentration will increase the hydrogen bond formed between the gelatin molecules. As the constituents of the gelatin increased, a higher number of available gelling sites were occupied which caused a harder gel texture (Jiamjariyatam, 2018).

In comparisons to the hardness of commercialized Haribo Gummy Candy (514.705±71.016 g), the concentration of bovine and commercialized fish gelatin have to be lower than 9% as at the least gelatin concentration, both of them had hardness value higher than 500 g at all gelatin hydration temperature. In contrast, tilapia gelatin showed a promising as result as at 9% concentration, the hardness of the gummy candy were similar to that of Haribo Gummy Candy at all hydration temperature. However, the downside of this result is that it is known that Haribo uses bovine gelatin in their gummy candy, and

from this study, the minimum bovine gelatin concentration (9%) showed double value of hardness than that of Haribo. Thus, higher amount of tilapia gelatin is needed to give the desired hardness value.

4.3.1.2 Effect of different gelling agent on the hardness of gummy candy

Gummy Candy made from fish gelatin had higher hardness value at all hydration temperature compared to other gelling agent, except for at 80°C hydration temperature where Gummy Candy from bovine gelatin has higher hardness value at 9 and 12% concentration. However, Gummy Candy containing Tilapia Gelatin showed the lowest hardness value at all hydration temperature than that of Gummy Candy from commercialized fish and bovine gelatin.

The hardness of the gummy candy is contributed from the structural bond of the gelatin with the sugar solution. The structural bond are mostly affected by the amino acid composition of the gelatin. The type of amino acids that are responsible for the formation of structural bond is the imino acids, proline and hydroxyproline (Boran & Regenstein, 2010). Both proline and hydroxyproline stabilize the triple-helix of gelatin structure by the formation of hydrogen bond between free water molecules and the hydroxyl group of the hydroxyproline of the gelatin (Badii & Howell, 2006; Gomez-Guillen et al., 2002). The content of hydroxyproline is 30%, 22% to 25% and 17% for mammalian gelatins, warm water fish gelatins cold-water fish gelatins respectively (See, Hong, Ng, Wan Aida, & Babji, 2010). This shows that mammalian gelatin could have better structural network formed contrary to other type of gelatin. However, that is not the case for this result as the commercial bovine gelatin that is bought for this research might have undergone severe

extraction process which affects the content of proline in the collagen which leads to a weaker gel (Eysturskard, Haug, Elharfaoui, Djabourov, & Draget, 2009). Similar to a study by (Normah & Muhammad Fahmi, 2015), the hardness of the gummy candy from sutchi catfish gelatin is higher than that of bovine gelatin. Meanwhile, the content of proline and hydroxyproline for the extracted tilapia gelatin is only 10% and 14% respectively (Asshazali, 2018). This is comparatively lower than the hydroxyproline content of warm-water fish gelatin and cold-water fish gelatin. Theoretically, tilapia is a type of warm-water fish and they should have exhibited a higher gel strength as the collagen of warm-water fish was more stable compared to that of cold-water fish (Gilsenan & Ross-Murphy, 2000).

The Gummy Candy made from the mixture of Arabic gum and the two commercialized fish and bovine gelatins had exhibits lowest hardness value for all hydration temperature. This is probably due to a low percentage of the Arabic gum concentration used in this study. Arabic gum is one of the hydrocolloid that is used to bind the sugar solution in gummy candy and pastilles (Schrieber & Gareis, 2007b). A sugar solution with 50% Arabic Gum alone would produce a hard gums and a is reduced to be mixed with gelatin to produce a softer candy (Lees & Jackson, 1973). However, a uniform concentration of gelling agents is chosen in this study to compare the gummy candy in term of various concentration.

As discussed earlier, the content of hydroxyproline affects the hardness of gummy candy. As fraction of hydroxyproline increases, the physical crosslinking of the gummy candy increases with the formation of hydrogen bond network linking the hydroxyproline to the main-chain carbonyl group (Zaupo, 2010). The more hydrogen bond formation in

the gummy candy, the higher the dissociation energy of the gummy candy. Thus, a higher force is needed to break the gummy candy cross-linked structures, yielding a high hardness value for gummy candy made from a higher value of hydroxyproline.

4.3.1.3 Effect of different hydration temperature on the hardness of gummy candy

The thermal degradation occurrence during hydration of gelatin generates gelatin-derived peptides and their constituent amino acids (Qi et al., 2018). The increase level of peptides and free amino acids in the gelatin solution could be coming from the degradation of α and β subunits of the gelatin. This can be further discussed by the dissociation of structural bonds in the tertiary and secondary structure of the gelatin.

The triple helix structure of the gelatin decreased upon FT-IR investigation due to thermal effects (Qi et al., 2018). This is because the hydrogen bonds that stabilizes the triple helix structure of the gelatin can be broken when the heating temperature is greater than the gel melting point (Payne & Veis, 1988). Furthermore, the unfolding of triple helix structures could increase the surface hydrophobicity. Related to that, the proline residues was said to be more than 30% (Payne & Veis, 1988) which is caused by the breakdown of internal hydrogen bond in the helix (secondary structure).

All of this leads to a decrease in hardness value at all concentrations as the hydration temperature. It can be seen from Figure 4.8 and 4.9, there was a decrease of hardness from 80 °C to 90 °C of the hydration temperature at all concentration of different type of gelling agents. Furthermore, the gummy candy made from 9% of Arabic gum-gelatin mixture did not form a gel at 80 °C and 90 °C. Any materials with gelatin's concentration less than 5%, is degraded when heated at 80 °C and above around 10 minutes (Schrieber & Gareis,

2007b). At 9% of Arabic gum-gelatin mixture, the gelatin to Arabic gum ratio is 1:1, which is at 4.5% w/v gelatin concentration in the gummy candy. This showed that the hydration temperature above 80 °C isn't suitable for the mixture of Arabic gum-gelatin in the gummy candy.

The decrease in hardness value for gummy candy made from bovine, fish and tilapia gelatin was due to the loss of the ability of the solution to physically cross-linked. However, the degradation of the gelatin was not severely as no free amino acid was observed. This correlated with Table 4.2 and 4.3 as there was no significant decrease in pH value that indicates higher acidity due to free amino acids from the thermal degradation.

It can be seen from Figure 4.8 and 4.9, the hardness of gummy candy from Arabic gum-gelatin mixture maintained at 15% gelling agent concentration around 1200 g, but a decrease of hardness can be observed in gummy candy from bovine and tilapia gelatin at 15% gelatin concentration. This showed that the gelatin alone had undergone the degradation but the Arabic gum did not and thus giving higher hardness value at 90 °C at 1300 g compared to bovine and tilapia at 830 and 760 g respectively. Arabic gum is a polysaccharide that has a high melting point value (Anvari & Chung, 2016) thus it did not degraded at 90 °C

4.3.2 Cohesiveness of Gummy Candy

Cohesiveness can be interpreted as the tensile strength of a gummy candy where it refers to the degree to which a food can be deformed before it ruptures (Chandra & Shamasundar, 2015) or disintegrate under mechanical action.

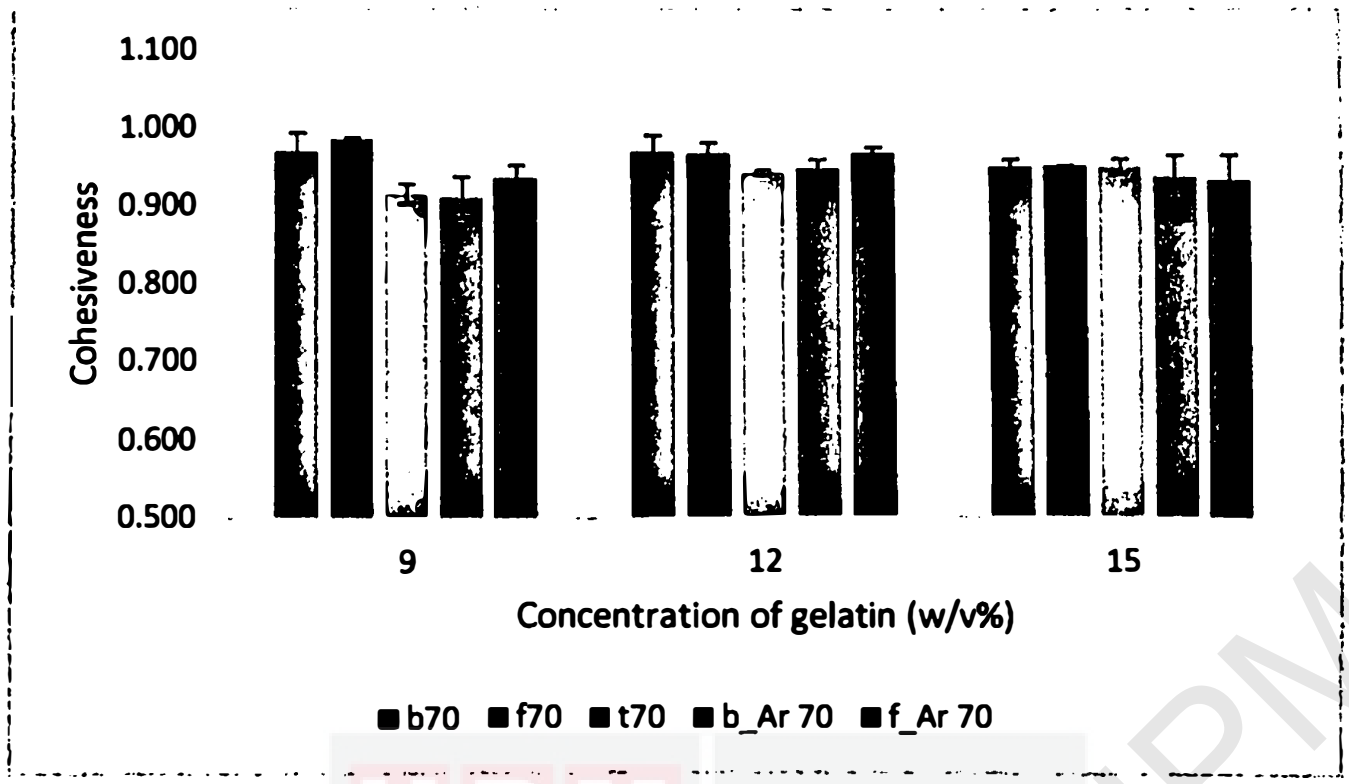

Figure 4.10: Cohesiveness of Gummy Candy for Different Gelatin Concentration at 70°C

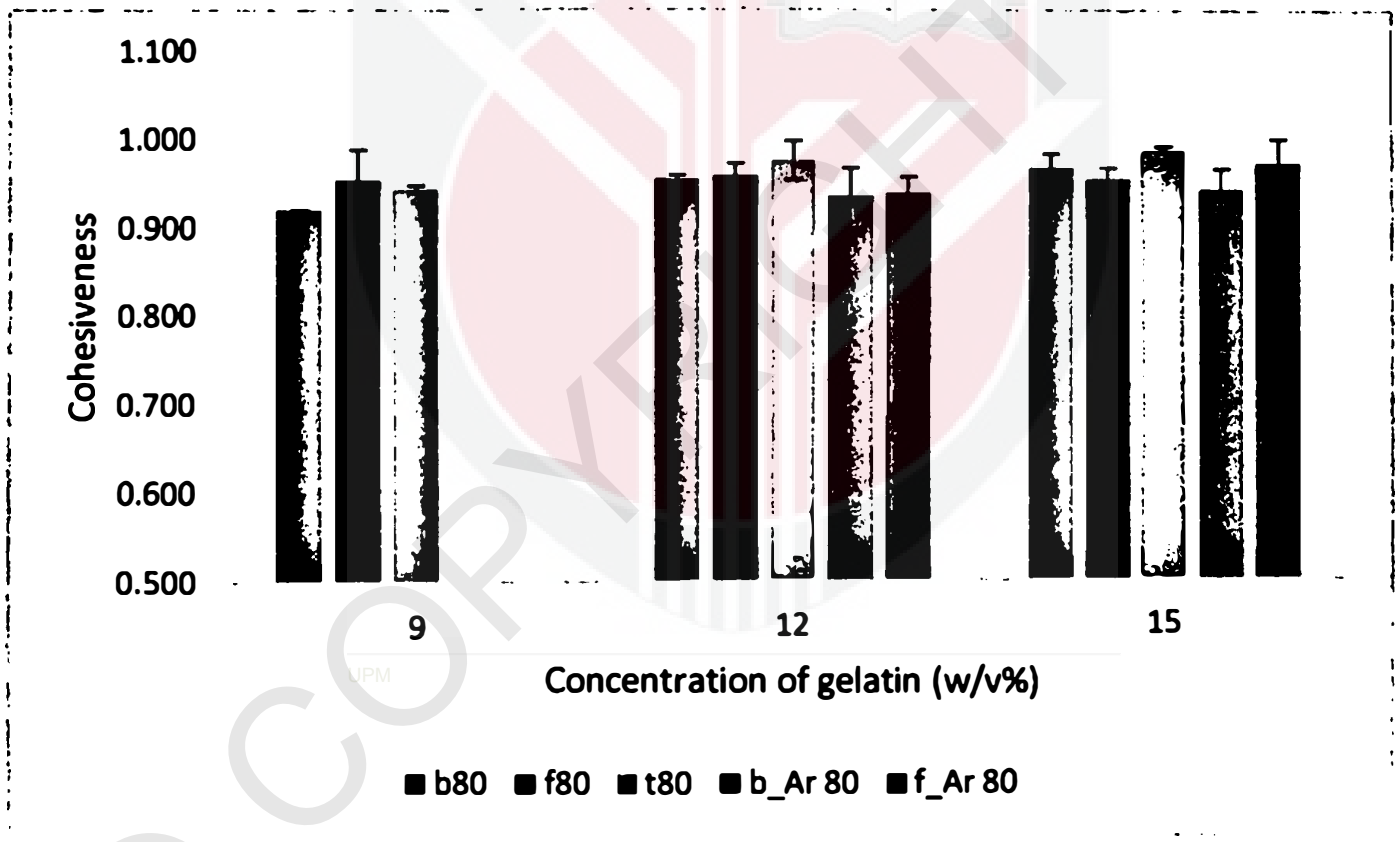

Figure 4.11: Cohesiveness of Gummy Candy with Different Gelatin Concentration at 80°C

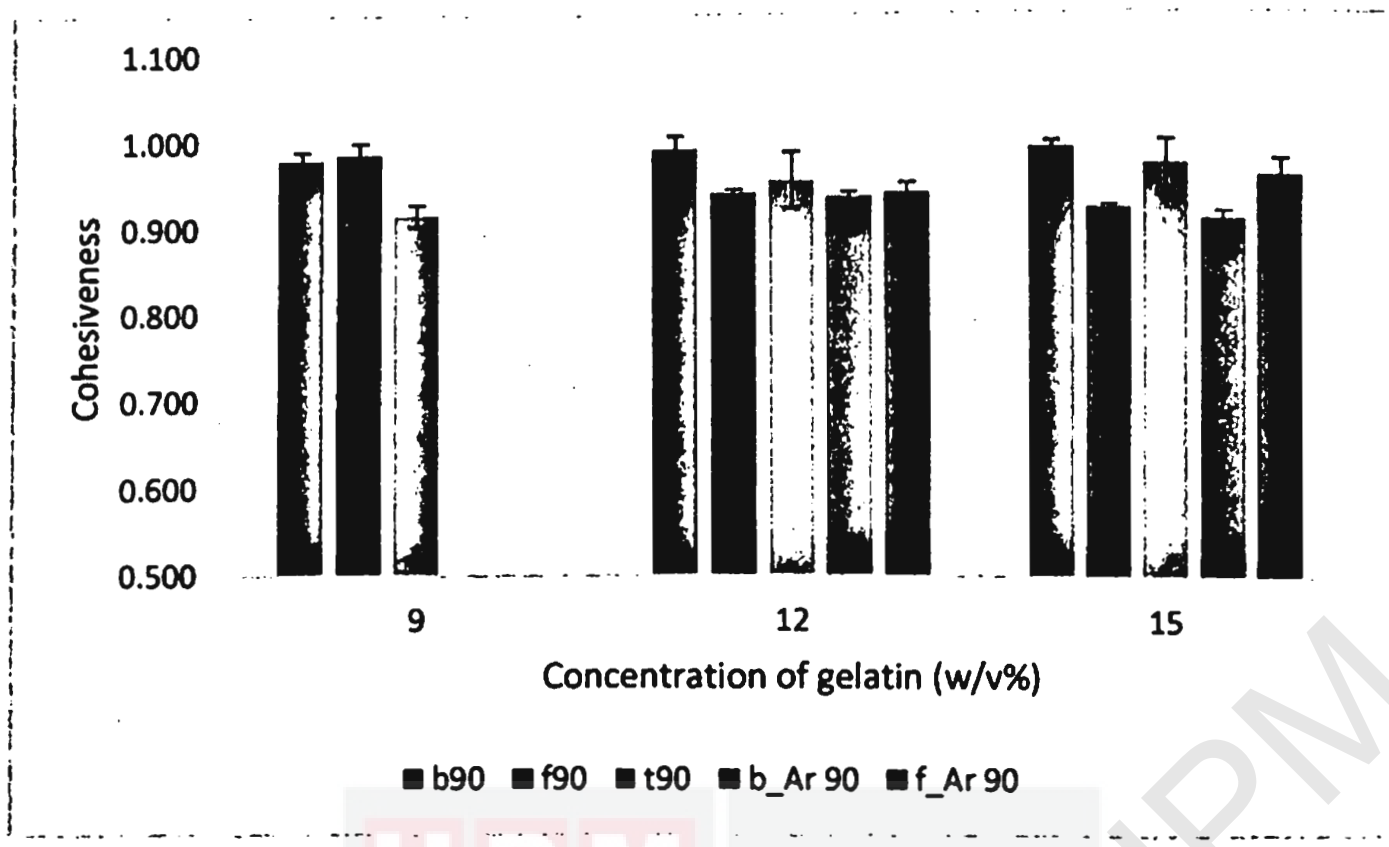

Figure 4.12: Cohesiveness of Gummy Candy with Different Gelatin Concentration at 90°C

It can be seen from Figure 4.10, 4.11 and 4.12 that as concentration of gelatin increases, there are no significant change in the cohesiveness value of the gummy candy. The same trend is displayed as the hydration temperature increases. Similar to a study, where gels made from different concentration of porcine, bovine and fish gelatin had shown no significant difference at range around 0.3 to 0.5 (Rahman & Al-mahrouqi, 2009). However, that study showed a much lower ranges of cohesiveness value due to the formulation of the gel which consisted 10, 20, 30% of gelatin concentration and water only.

Generally, the ranges value of cohesiveness is 0.9 to 0.98, 0.92 to 0.98 and 0.91 to 0.98 from Figure 4.10, 4.11 and 4.12 respectively. Cohesiveness in TPA is generated through the difference on the second compression area to the first compression area. This imitates the effect of gummy candy at the act of first bite to second bite. The high value of cohesiveness reflects to a stronger internal bond of gummy candy. This is due to the

presence of sugar such as sucrose and glucose syrup in the formulation of the gummy candy. Sucrose and many monosaccharides have a stabilizing effect on a gelatin gel, most likely because they facilitate hydrogen bonding (Schrieber & Gareis, 2007a). This can be supported by several studies that has the same ranges value such as the result which is at 0.8 to 1 cohesiveness value (Charoen & Savedboworn, 2015; Normah & Muhammad Fahmi, 2015; Periche et al., 2014). This value is comparable to Haribo Gummy Candy at 0.971.

The high value of cohesiveness of gummy candy made from all type of gelatin used in this study means that they require stronger force before they break, or deform. This is also the reason why there is no fracturability detected in this study as the lower the cohesiveness of a material, the more brittle it will be (Phet & Si, 2016).

4.3.3 Gumminess of Gummy Candy

Gumminess is the textural parameter related to semi-solid food. It is represented as the energy required to masticate food (Chandra & Shamasundar, 2015)

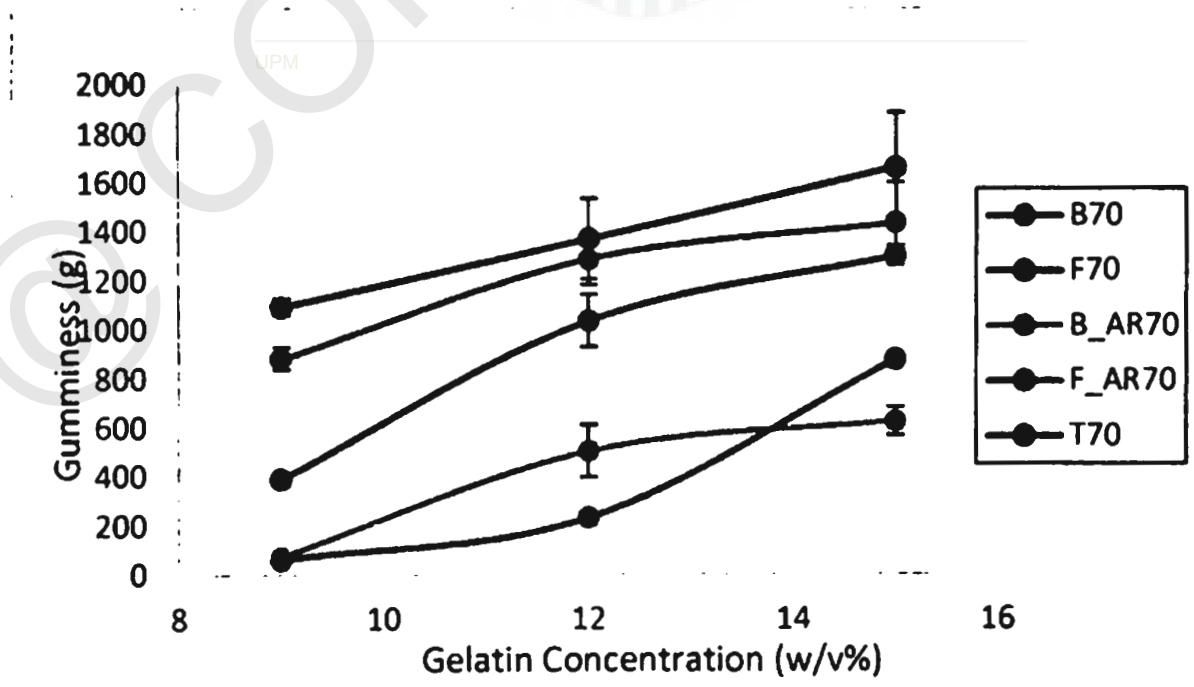

Figure 4.13: Gumminess of Gummy Candy with Different Gelatin Concentration at 70°C

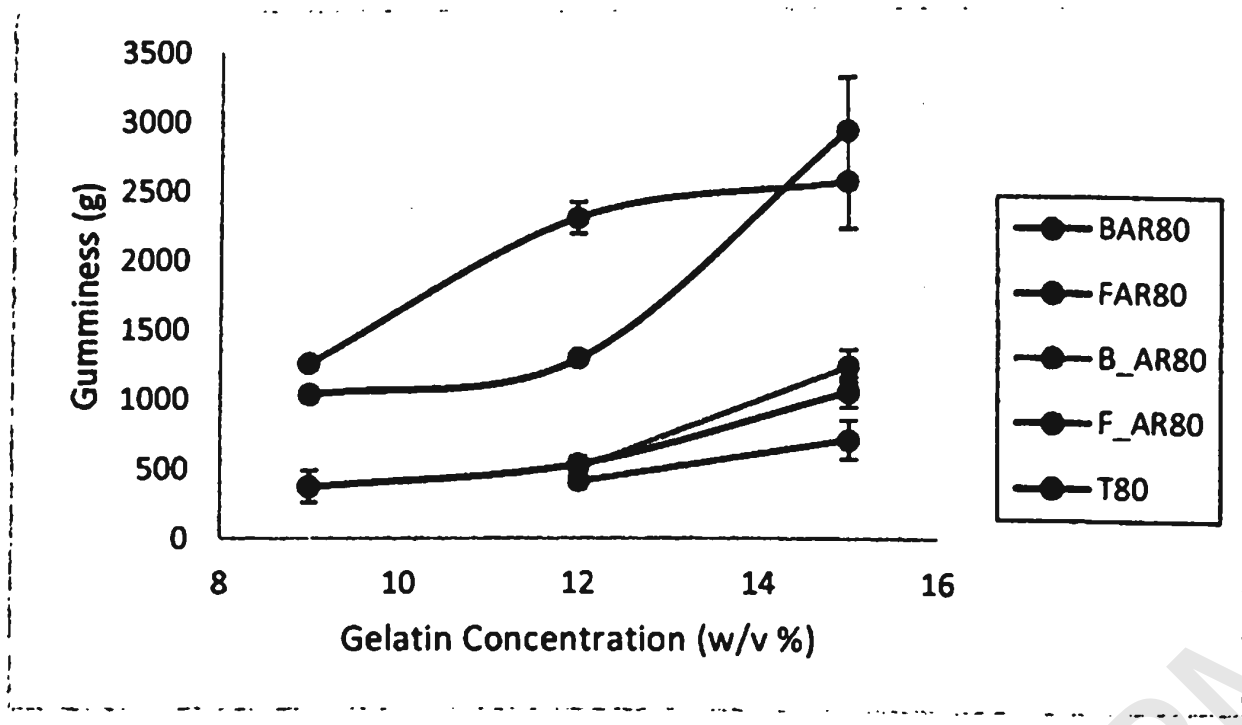

Figure 4.14: Gumminess of Gummy Candy with Different Gelatin Concentration at 80° C

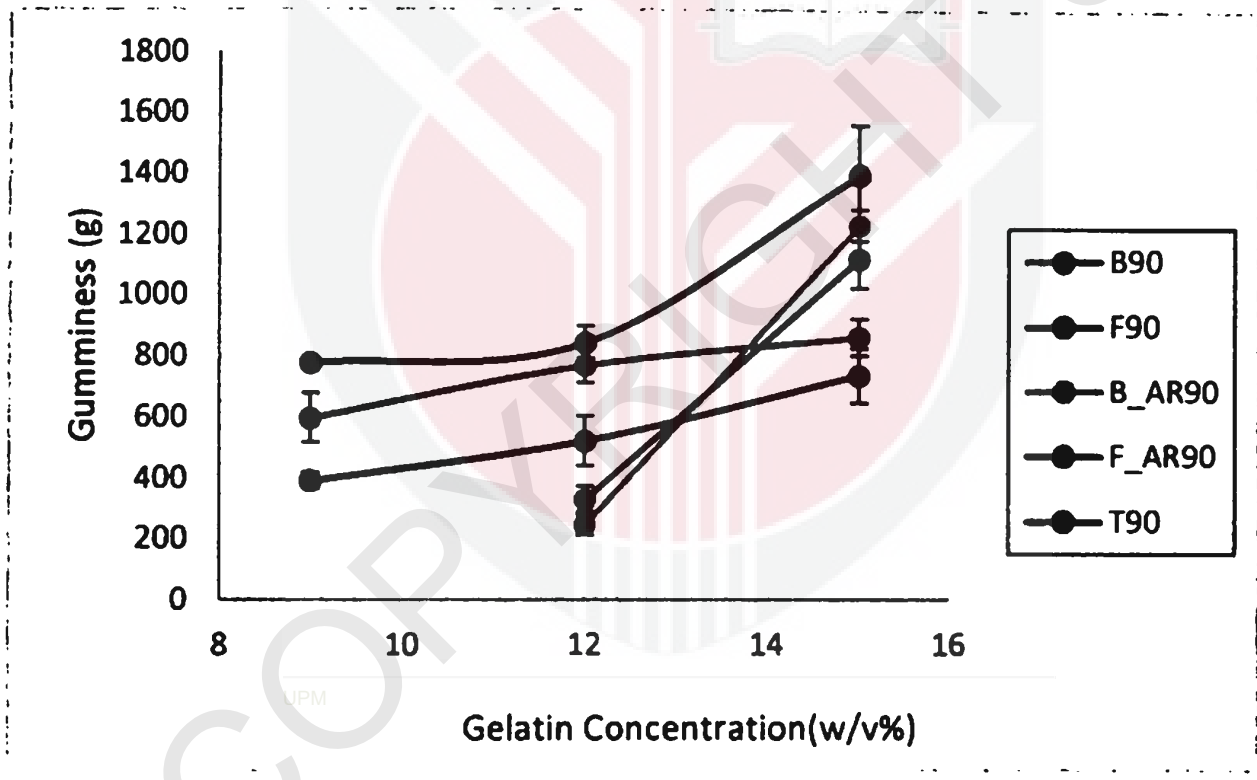

Figure 4.15: Gumminess of Gummy Candy with Different Gelatin Concentration at 90 °C

4.3.3.1 Effect of different concentration on the gumminess of gummy candy

From Figure 4.13, 4.14 and 4.15, it can be observed that as concentration of gelatin increases, the gumminess of gummy candy increases at all hydration temperature. The increasing value of gumminess has arisen from the increasing value of hardness. As

discussed in Section 4.3.1.1, increasing concentration of the gelatin will increase the hydrogen bond formation between the gelatin molecules in the gummy candy.

The gumminess of Haribo Gummy Candy is at 500.061±69.479 g which was comparable with Gummy Candy made of 9% of Tilapia gelatin showed its potential to be utilized as replacement of the conventional mammalian gelatin that is used in commercialized gummy candy.

4.3.3.2 Effect of different types of gelling agent on the gumminess of gummy candy

Gumminess is defined as the energy required to disintegrate a semi-solid food until it is read to be swallowed. Similar trend of the effect of different type of gelling agent on hardness (Section 4.3.1) is observed in that of gumminess of gummy candy. This is because gumminess is the product of hardness and cohesiveness, which is the maximum force needed to compress the gummy candy and the strength of internal bonds of the gummy candy respectively. As discussed in Section 4.3.2, the cohesiveness of gummy candy is considered high compared to other food product such as jelly which is at 0.5, has lower gumminess (720 g) value even though its hardness is quite high (1480 g) (Rao, Cooley, Walter, & Downing, 1989).

4.3.4 Springiness of gummy candy.

Springiness is defined as the height that the food recovers at the end of the first bite and the start of second bite (Chandra & Shamasundar, 2015)

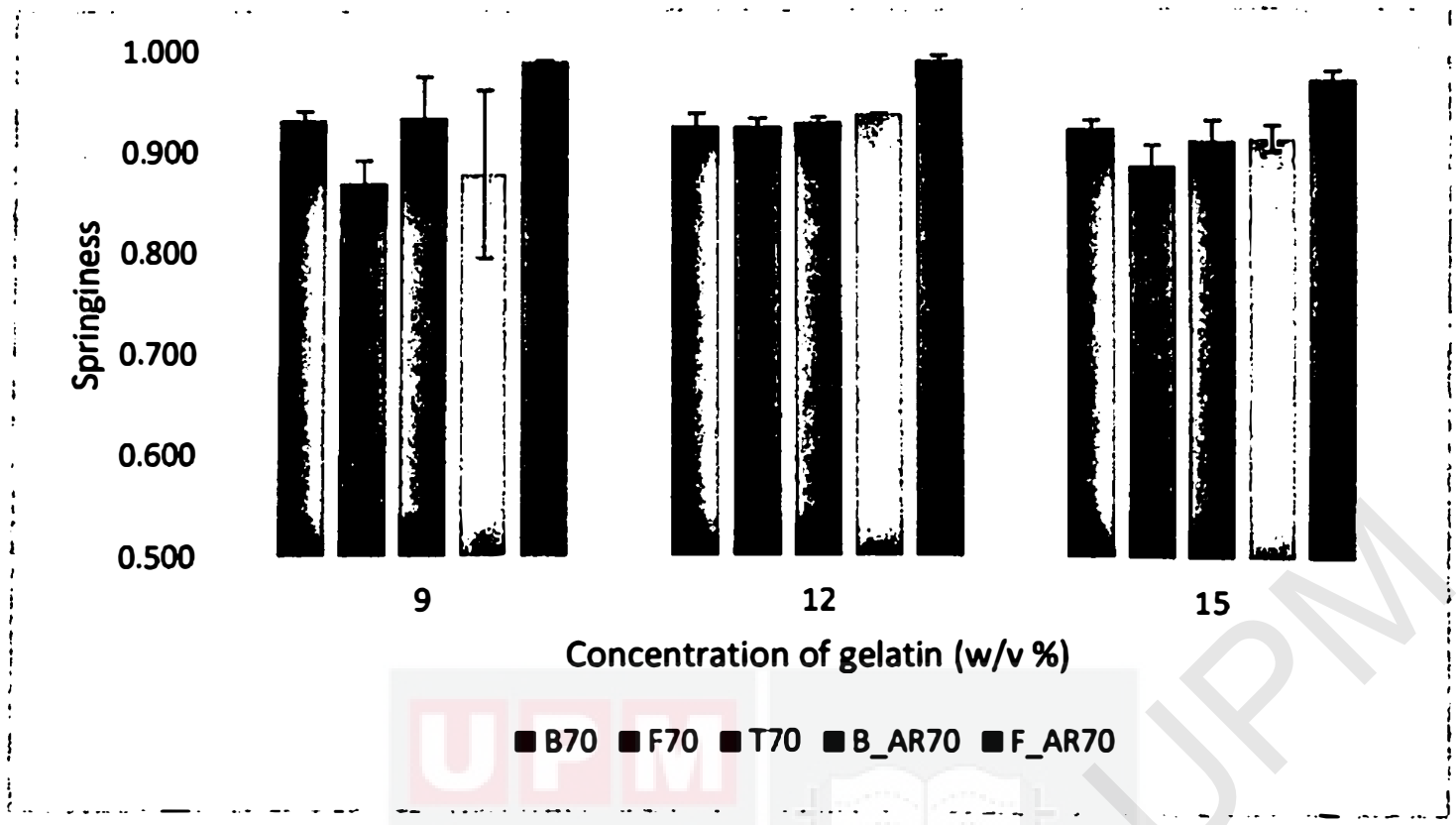

Figure 4.16: Springiness of Gummy Candy with Different Gelatin Concentration at 70 °C

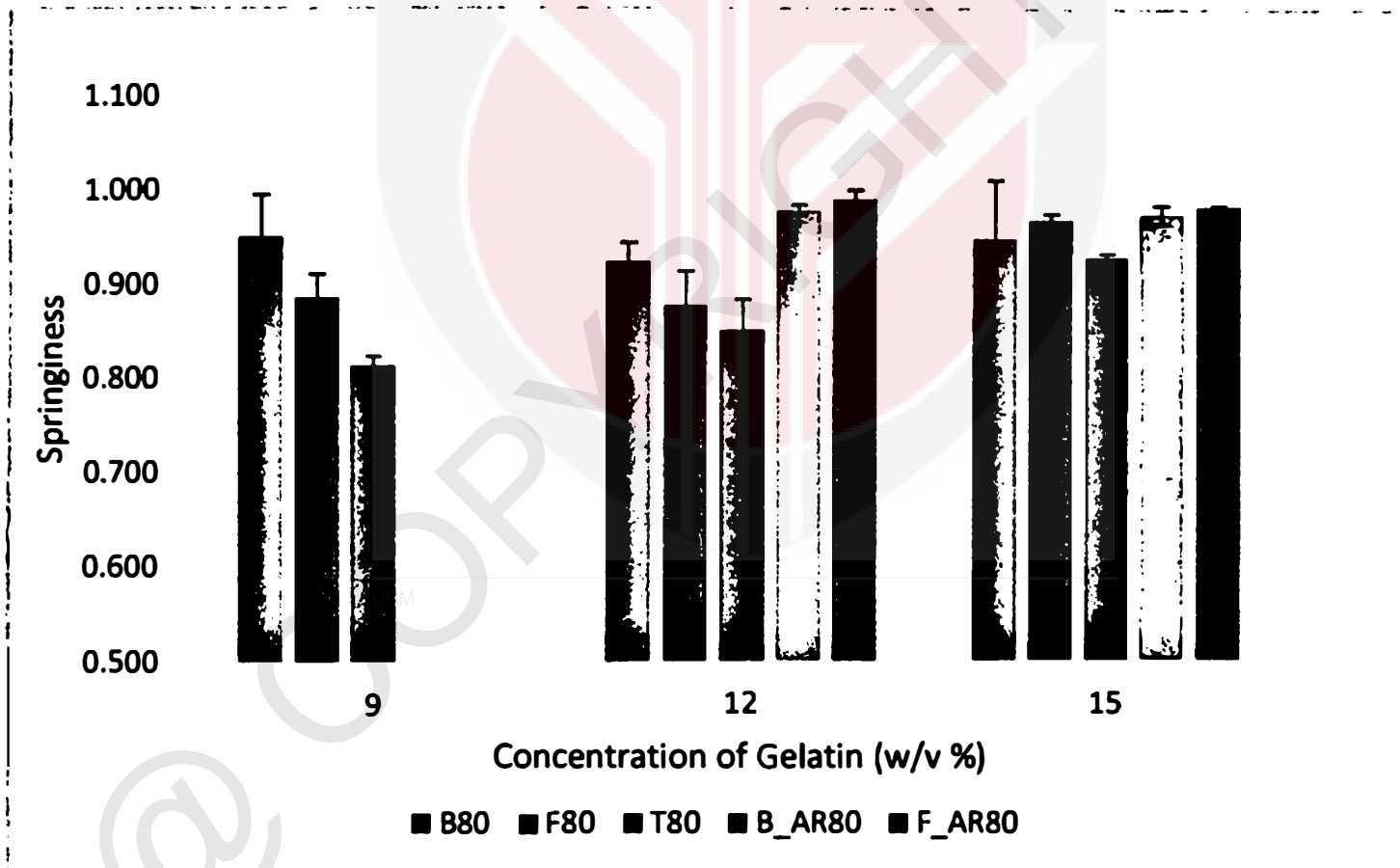

Figure 4.17: Springiness of Gummy Candy with Different Gelatin Concentration at 80°C

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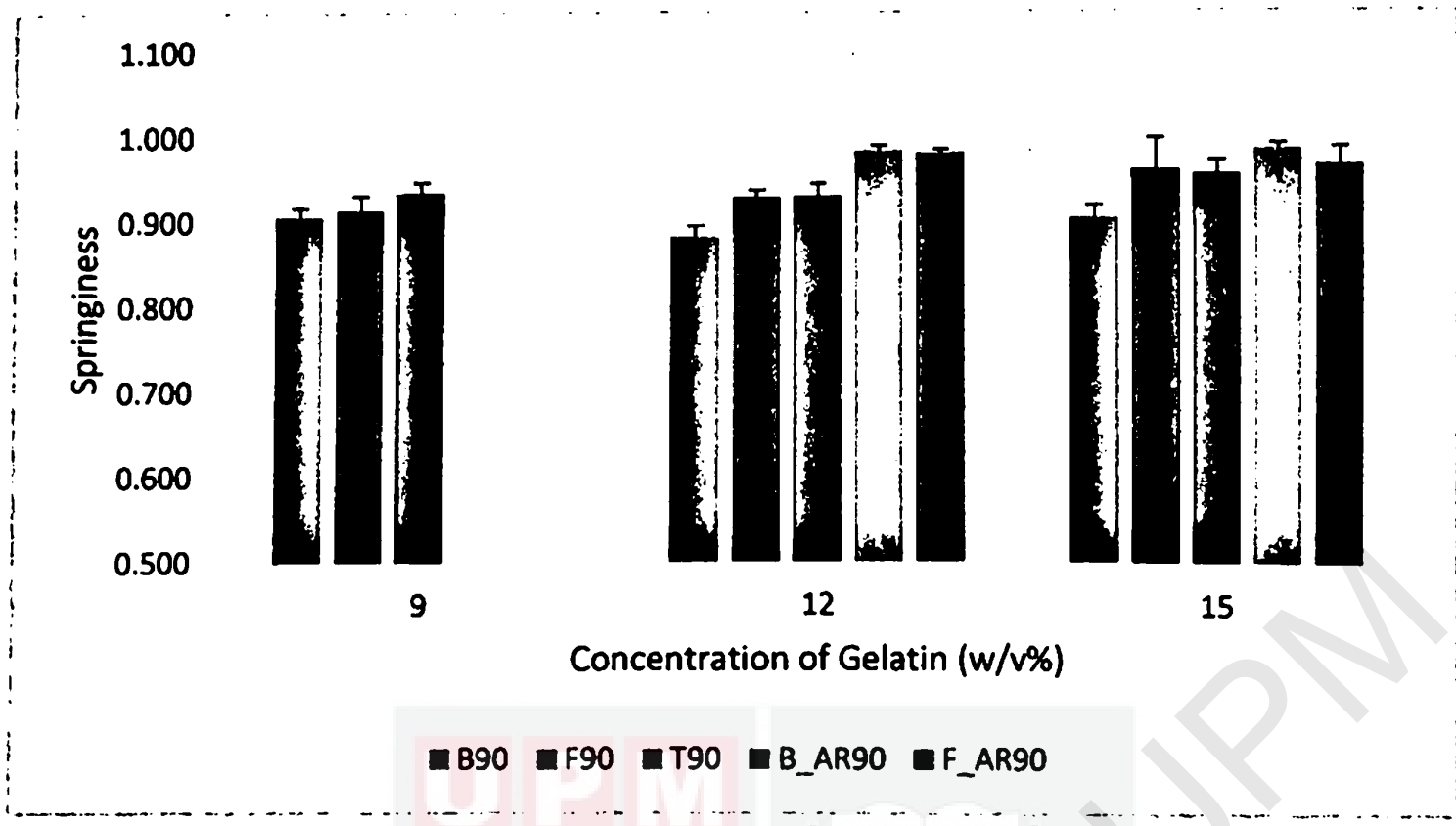

Figure 4.18: Springiness of Gummy Candy with Different Gelatin Concentration at 90 °C

Springiness, similar to cohesiveness value, had showed no significant difference upon increasing gelatin concentration and hydration temperature. The ranges value of springiness is 0.87 to 0.99, 0.81 to 0.99 and 0.88 to 0.98 from Figure 4.16, 4.17 and 4.18 respectively Similar ranges of value can be seen which is 0.8-1.0 to several studies (Charoen & Savedboworn, 2015; Normah & Muhammad Fahmi, 2015; Periche et al., 2014).

Even though cohesiveness value is similar to springiness, they are two different thing as springiness refers to the height recovery after the first bite, and the latter means degree of deformation that the gummy candy can withstand before it breaks. Springiness is the degree at which sample goes back to its unique shape after partial compression between the tongue and palate (Bourne, 2011). It can be determined by the distance recovered by the sample during the time between end of first bite and start of second bite area. High .value of springiness means that gummy candy requires higher mastication

energy. This is the essence of gummy candy as they would have to be springy and can be chewed several times before it completely ready to be swallowed. This can be supported from a study of a candy from mixed agar and gelatin where the controlled sample which have 0% of gelatin had shown a lower springiness value compared to a sample with 15% of gelatin at 0.2 and 0.7 respectively (Somboon, Karrila, Kaewmanee, & Karrila, 2014a).

4.3.5 Adhesiveness of Gummy Candy

Adhesiveness is to overcome the attractive forces between the surface of a food and the surface of other materials which the food comes into contact with (Chandra & Shamasundar, 2015)

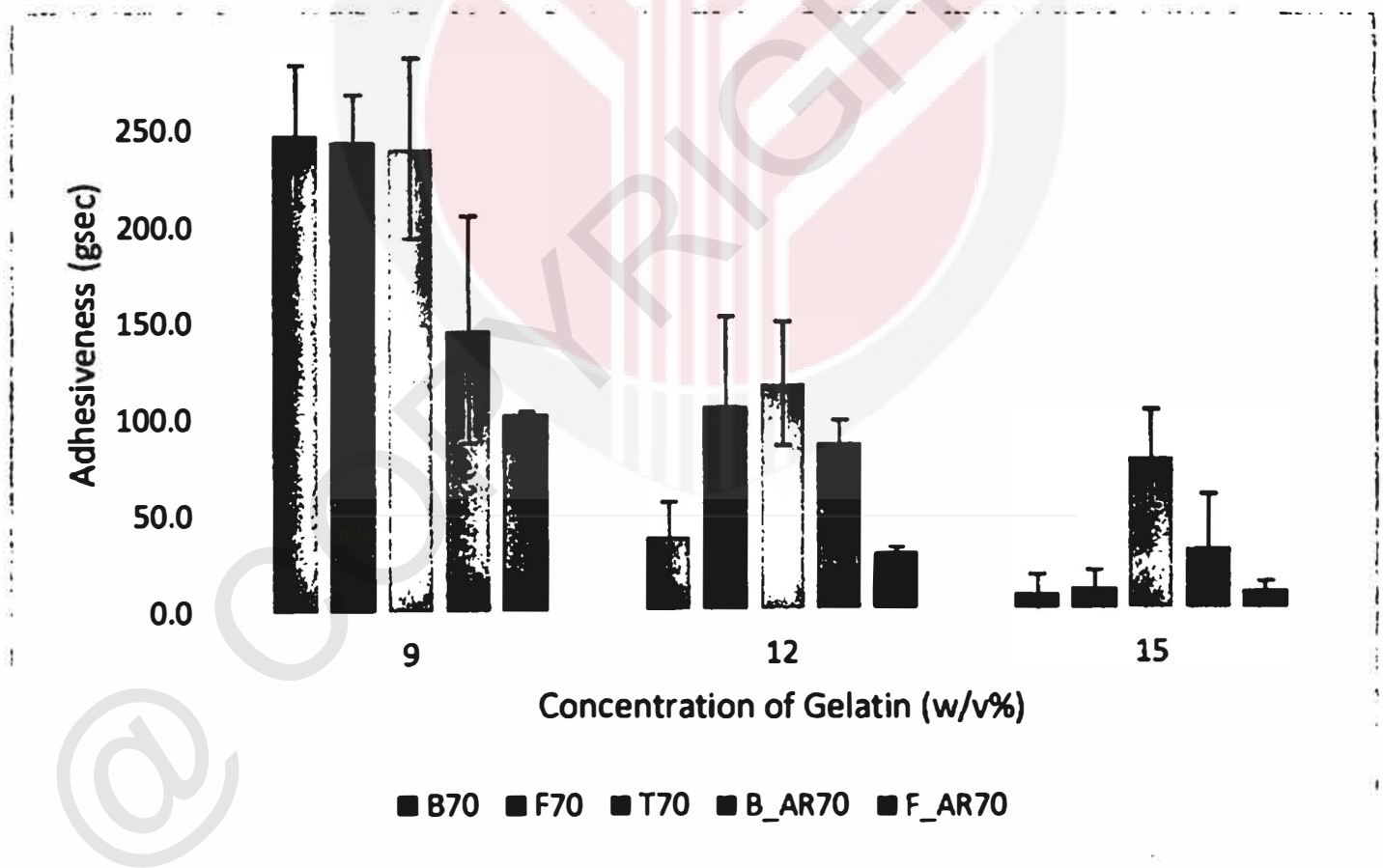

Figure 4.19: Adhesiveness of Gummy Candy with Different Gelatin Concentration at 70 °C

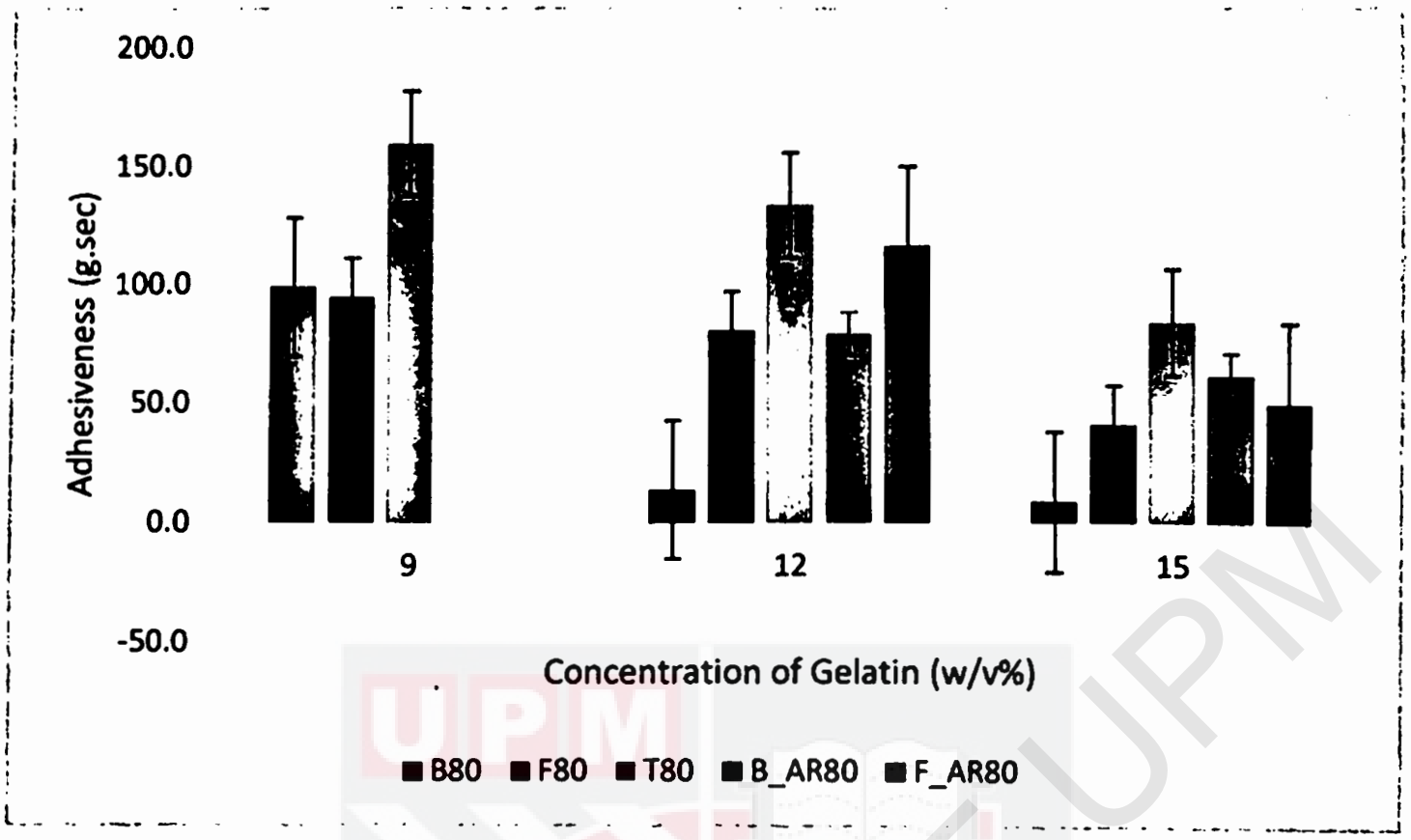

Figure 4.20: Adhesiveness of Gummy Candy with Different Gelatin Concentration at 80 °C

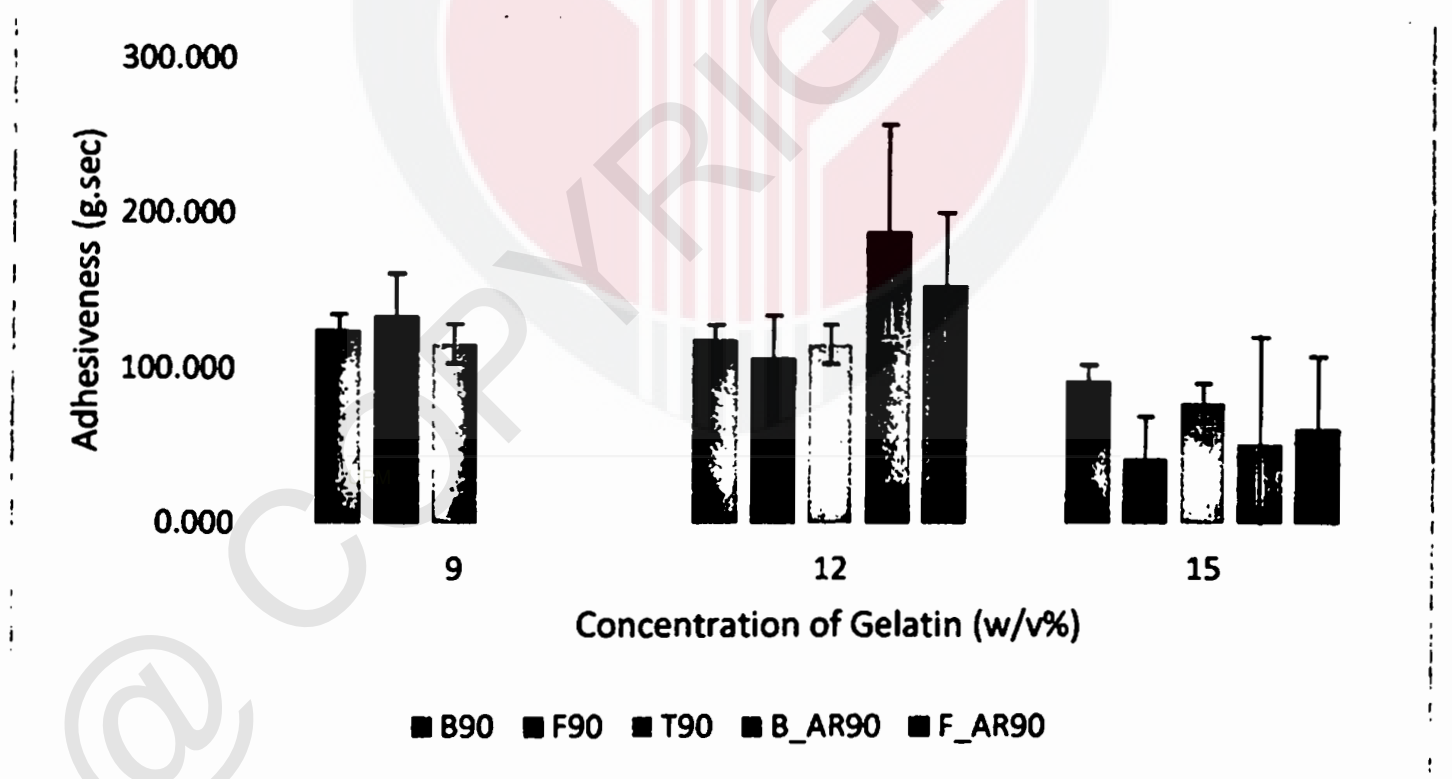

Figure 4.21: Adhesiveness of Gummy Candy with Different Gelatin Concentration at 90 °C

Based on Figure 4.19, 4.20 and 4.21 it can be seen that adhesiveness decreases as the concentration increases at all hydration temperature. The trend is the same for all type of gelatin and mixed gelatin and Arabic gum. Adhesiveness is said to be the stickiness of

sample in the mouth through mastication and force required to remove the sample from the palate during eating (Zoon, 1991) or in term of gummy candy, adhesiveness is the stickiness after the first bite using incisor teeth.

Increasing the gelatin concentration may affect the adhesiveness due to its relation to increasing hardness parameters, which is due to a higher number of available gelling sites were occupied which caused a harder gel texture (Jiamjariyatam, 2018). Similar study showed that increasing the concentration of gelatin reduces the adhesiveness value (Rahman & Al-mahrouqi, 2009), however there are a study that showed a contradiction whereby they stated that increasing the gelatin concentration has significantly increases the adhesiveness value (Mutlu et al., 2018). One notably differences is the formulation of the gummy candy, which is composed of honey only. This can be the important factor in explaining the increasing adhesiveness value. The formulation of gummy candy is usually a combination of sucrose and glucose syrup. Both of this is a requirement due to its functionality when combined together. Sucrose molecules are able to align and form large lattice arrangements of crystals which greater susceptibility against deformation. Thus, upon first bite, it still can retain the structure compared to honey gummy candy, which has lower degree of crystallinity.

Besides that, the adhesiveness value of the gummy candy in this study is ranged from 7 to 250 g.sec. Similar study had showed several ranges of adhesiveness value but they're all are more than 30 g.sec (Normah & Muhammad Fahmi, 2015; Rahman & Al-mahrouqi, 2009). The value is significantly higher than that of the Haribo Gummy Candy (0.172 g.sec). This is due to different finishing technique is done for all the experimental

gummy candy. One of the ingredient in Haribi Gummy Candy is beeswax, which is used to prevent pieces from sticking in the package (Hartel & Hofberger, 2018) and this in turn, decreases the adhesiveness value of Haribo Gummy Candy.

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CHAPTER 5

CONCLUSION AND RECOMMENDATION FOR FUTURE WORK

5.1 Conclusion

The effects of addition gelatin from different source such as commercialized bovine and fish gelatin, extracted tilapia gelatin, Arabic gum and their mixture with said commercialized bovine at varying range of concentrations (9, 12, 15 %) on Gummy Candy were studied. The effect of hydration of temperature of the gelatin was also studied. The analysis done was separated by two part, on the Gummy Candy Solution which was about the rheological properties and on the textural properties of the Gummy Candy.

The studied of rheological properties showed that the pH and °Brix of Gummy Candy Solution were not affected as different type and concentration of gelatin were used. This is because both of them relied on the other part of the formulation such as sugar solution and citric acid. The viscosity of the gummy candy solution is higher at higher concentration of gelatin. As for the gelling and melting temperature, it was discovered that as concentration of gelatin increases, both of the parameters increases. The different type of gelatin used showed small difference in gelling and melting temperature, but a significant changes were

observed at gummy candy made from mixture of Arabic gum-bovine gelatin and Arabic gum-fish gelatin.

Textural characteristics studied in this study is hardness, cohesiveness, gumminess, springiness, and adhesiveness. After molding, it is found out that gummy candy made from 9% of mixed Arabic gum-gelatin does not set at 80°C and 90°C hydration temperature. It is also observed that their gelling temperature were much lower than that of gummy candy made with gelatin only. There is no fracturability detected at all samples of Gummy Candy which relates to their high cohesiveness value. The effect of concentration and type of gelling agents were observed to be affecting the value of hardness, gumminess and adhesiveness. Springiness and cohesiveness is not affected by those two parameters. It showed that Gummy Candy made from extracted Tilapia Gelatin has lower value in hardness, gumminess and adhesiveness. Even so, they showed value with the Haribo Gummy Candy, except for adhesiveness as no beeswax as finishing of Gummy Candy were used in this study. Moreover, the hydration temperature has no significant effect on the rheological nor the textural properties.

The effects of increasing concentration on viscosity, gelling, melting and textural properties such as hardness, gumminess and adhesiveness comes from the increasing amount of junction for hydrogen bonding of the gelatin molecules with the sugar solution which caused a harder gel. Meanwhile the effects of different type of gelling agent can be seen clearly on the textural properties as they have different amino acid content such as hydroxyproline, which is responsible for the formation of the triple helix and also its stabilization. The effect of the gelatin hydration temperature had showed no significant

effect in rheological but at textural properties of gummy candy, the hardness and gumminess showed decrease in all type of gelling agent at 80 °C to 90 °C.

5.2 Recommendations for Future Work

The gummy candy formulations can be improved with the use of different type of sugar such as low calorie sugars (stevia) and vitamin c should be used to see the effects of formulation on the textural properties of the gummy candy. The other textural properties can also be used such as colour, clarity and opacity to study the effect of the gelatin type to that factor of gummy candy as that affects the preference of the customers.

Different type of gelatin can also be utilized from different type of source such as chicken, and characterization of them can be done by standards bloom strength test, viscosity, gelling and melting temperature, amino acid composition at 6.67% concentration. It would be easier to relate the properties of the gummy candy made from that gelatin once they have done the characterization. The bonds related to the network formation can be studied by using FT-IR and DSC can be used to study the thermal analysis.

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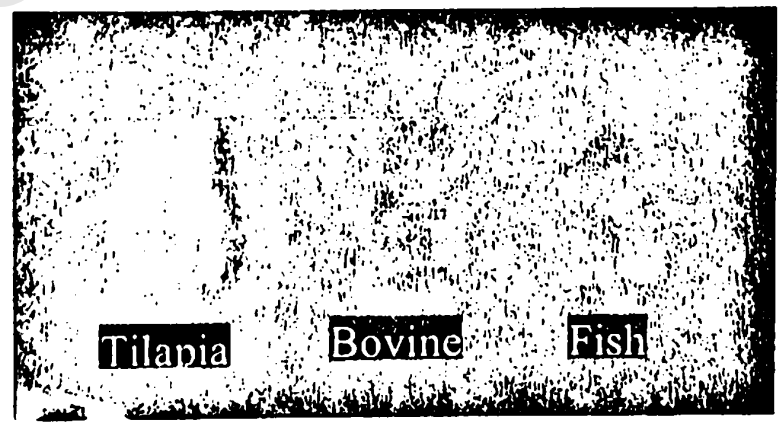
APPENDIX

Appendix 1: Tilapia scales

Appendix 2: Tilapia gelatin

Appendix 3: Gummy candy solution with tilapia gelatin

Appendix 4: Gummy candy solutions with bovine gelatin



Appendix 5: Gummy candy after molding

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	G'	G''	G'	G''
40	46.35	42.77	9.914	13.67
38	15.48	18	7.834	11.03
36	1.685	3.273	4.704	7.808
34	0.8133	1.814	1.962	4.265
32	0.5768	1.5	0.6754	2.463
30	0.5514	1.691	0.6991	2.933
28	0.9981	2.795	2.09	5.387
26	5.628	6.283	8.653	10.04
24	21.41	9.87	30.35	15.55
22	67.92	13.07	89.53	20.59
20	146.6	14.09	180.8	24.77
18	243.8	16.19	295.2	31.67
16	372.4	23.56	430.2	62.58
14	465.8	73.53	642.1	215.5
12	653.3	192.9	771.5	-18.66
10	939	175.6	1271	-146.9

Appendix 6: G'/G" bovine 9% 70 °C during gelling

	G'	G''	G'	G''
10	988.4	53.43	1160	-43.7
12	686.6	69.23	1023	-109.9
14	788.3	257.6	724	-659.6
16	742.2	-12.45	694.1	-230.6
18	845.1	250.9	864.5	137.6
20	629.3	-231.1	904.3	57.53
22	646.6	43.39	789.9	85.82
24	545.9	-5.848	646.1	34.82
26	442.4	25.72	521	47.44
28	306.2	10.45	384.1	10.38
30	162	13.16	220.4	8.393
32	74.25	9.944	101.1	15.49
34	11.77	6.269	25.04	10.47
36	0.7421	1.894	2.052	3.952
38	0.06218	0.6446	0.1745	1.299
40	0.06806	0.4551	0.3116	1.141

Appendix 7: G'/G" bovine 9% 70 °C during melting

	G'	G''	G'	G''
40	124	91.39	197.5	138.1
38	83.34	68.52	188.6	132.5
36	49.4	47	144.9	115.7
34	19.87	24.07	99.29	86.46
32	8.199	11.36	15.18	22.49
30	15.11	11.37	24.06	16.56
28	59.45	16.07	86.67	22.46
26	146.9	24.54	183.4	25.28
24	265.6	24.12	329.4	39.38
22	471.5	15.72	551.5	27.64
20	662.7	45.86	827.8	105.4
18	864.3	21.68	1207	-152.7
16	779.1	229.3	1315	265.6
14	1177	118.3	1319	-231.9
12	1648	-626.7	2366	526.2
10	1509	-587.4	1143	475.5

Appendix 8: G'/G" bovine 12% 70 °C during gelling

	G'	G''	G'	G''
10	1335	225.1	151.2	427.3
12	806.8	-457	-782.1	817.1
14	2346	-93.89	372.8	1449
16	1882	-182.4	561.3	891.2
18	2316	-68.78	228.1	2409
20	1768	554.7	453.6	1963
22	1461	394	-775.8	1747
24	1452	-8.295	128.5	1648
26	1241	20.69	679.4	1779
28	995.5	162	311	999
30	616	77.63	139.8	862.9
32	339.8	3.683	59.7	463.7
34	164.7	18.36	26.09	233.9
36	39.98	10.86	15.92	69.72
38	5.319	4.763	9.834	12.26
40	4.518	5.704	25.04	25.05

Appendix 9: G'/G" bovine 12% 70 °C during melting

	G'	G"	G'	G"
40	165	104.9	177.4	114.8
38	89.35	70.75	53.56	49.74
36	21.19	26.74	25.29	29.1
34	4.634	7.614	5.988	10.07
32	5.57	8.481	7.685	11.17
30	22.97	17.1	29.94	19.59
28	78.74	26.28	107.8	29.26
26	209.6	35.07	238.6	34.66
24	427.7	45.42	417.3	49.34
22	705.9	145.1	719.8	128.6
20	1062	77.24	1024	218.9
18	1250	241.2	1751	-230.4
16	1226	-1435	1394	207.3
14	2333	124.2	1520	266.3
12	2138	-1337	2334	-1205
10	1794	1315	2859	1822

Appendix 10: G'/G" bovine 15% 70 °C during gelling

	G'	G"	G'	G"
10	-250.6	-581.9	1494	52.32
12	1256	-2189	1535	158.1
14	3211	-1069	1181	-455
16	2822	1618	950.7	266.4
18	22610	-9442	2458	494.6
20	2177	-24.34	1830	293.3
22	1423	128.3	1934	-180.7
24	2107	605.6	1922	332.7
26	1852	366.2	1255	-57.41
28	2032	-495.8	1544	683.5
30	1103	170.8	841.3	-37.4
32	466.7	46.14	416.9	6.335
34	159.4	27.48	206.4	25.12
36	29.42	13.84	47.17	17.29
38	3.814	5.373	5.968	7.262
40	2.781	2.483	2.012	3.485

Appendix 11: G'/G" bovine 15% 70 °C during melting

	G'	G"	G'	G"
40	0.1293	24.36	24.36	17.85
38	0.1494	31.87	31.87	23.07
36	0.1815	32.11	32.11	23
34	0.248	32.68	32.68	23.86
32	0.378	14.58	14.58	13.92
30	0.8702	2.697	2.697	4.723
28	3.368	3.412	3.412	5.381
26	6.475	9.874	9.874	8.162
24	14.43	23.06	23.06	10.8
22	27.49	40.59	40.59	13.25
20	45.59	70.31	70.31	16.26
18	67.64	106.3	106.3	19.47
16	93.46	146.5	146.5	23.39
14	121.9	190.6	190.6	29.7
12	148.2	231.9	231.9	34.37
10	175.8	280	280	39.6

Appendix 12: G'/G" fish 9% 70 °C during gelling

	G'	G"	G'	G"
10	312.7	26.24	195.5	0.805
12	360.8	28.94	205.3	37.1
14	360.7	22.47	213.2	38.51
16	350.3	25.76	215.6	33.26
18	344.5	20.19	209.8	30.85
20	317.3	14.97	201.4	23.66
22	305.9	15.25	186.4	25.54
24	265	19.36	168.3	11.82
26	241.8	14.58	154.2	20.26
28	201.7	11.06	125.9	17.33
30	144.6	9.341	93.86	14.1
32	91.73	7.56	61.62	10.66
34	43.88	5.755	31.99	8.407
36	12.87	4.425	11.47	6.292
38	4.833	2.483	2.674	5.024
40	16.94	1.135	0.632	13.3

Appendix 13: G'/G" fish 9% 70 °C during melting

	G'	G"	G'	G"
40	130.8	66.47	44.36	32.05
38	146.2	88.49	35.57	29.63
36	82.45	63.92	26.76	24.12
34	40.23	39.55	21.37	21.35
32	13.8	17.15	12.91	14.74
30	12.29	11.85	15.58	13.38
28	27.24	12.21	35.36	13.62
26	63.06	14.22	81.55	17.39
24	107	16.26	138.4	21.54
22	178.4	21.22	213.9	25.7
20	260.5	28.49	316.1	29.21
18	359	17.74	422.1	51.76
16	464	25.41	576	80.05
14	573.6	22.71	633.6	60.23
12	709.6	33.69	879.5	106.3
10	782.3	224.1	895.8	147.3

Appendix 14: G'/G" fish 12% 70 °C during gelling

	G'	G"	G'	G"
10	1007	25.55	1331	-126.8
12	892.7	122.5	1086	-126.6
14	834.9	-180	930.5	135.5
16	1020	-26.11	1091	2.562
18	890.3	154.1	1139	361.9
20	816.8	120.6	939.1	47.98
22	740.6	31.85	1063	176.9
24	649.7	22.73	727.4	424.6
26	561.3	62.88	734.5	1.706
28	477.8	25.22	535.5	44.32
30	343.4	18.45	401.5	49.16
32	221.4	16.08	292.8	22.48
34	117.6	12.07	151.6	17.39
36	42.56	8.673	56.74	10.81
38	15.14	12.504	13.05	7.173
40	33.46	23.5	11.5	9.779

Appendix 15: G'/G" fish 12% 70 °C during melting

	G'	G"	G'	G"
40	85.08	66.36	4.709	5.197
38	87.69	64.43	4.605	5.616
36	84.28	64.84	8.498	9.021
34	44.49	43.01	13.11	11.97
32	15.69	17.63	26.63	16.43
30	35.39	17.64	60.52	20.64
28	122.4	22.31	133.3	24.4
26	278.5	25.76	284.5	41.26
24	458.4	31.12	488.9	35.31
22	754.5	11.15	763.7	50
20	1043	44.08	1161	18.45
18	1375	59.66	1235	-74.7
16	1274	478.4	1788	47.65
14	1357	-273.7	2048	710
12	1856	2371	1923	-83.84
10	5804	2693	-961.9	1169

Appendix 16: G'/G" fish 15% 70 °C during gelling

	G'	G"	G'	G"
10	1754	284	879.5	-337.9
12	1719	87.26	1335	62.34
14	1898	251.9	1026	-72.27
16	2302	186.8	1092	73.82
18	1952	-97.22	1155	-90.57
20	1458	250.3	937.9	181.9
22	1439	283.4	921.1	-36.46
24	1522	-16.1	755.2	7.611
26	1451	-5.043	627.6	45.02
28	1145	117	488.9	49.45
30	915.1	65.91	326.2	22.34
32	560.1	46.08	185.1	13.29
34	262.2	21.41	82.94	8.536
36	70.29	14.02	23.27	5.549
38	9.656	6.826	3.907	2.613
40	1.487	2.636	0.7182	0.9523

Appendix 17: G'/G" fish 15% 70 °C during melting

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Test ID	Batch		Force 1	Area-FT 1:2	Time-diff. 1:2	Area-FT 1:3	Area-FT 2:3	Area-FT 4:6	Time-diff. 4:5	Hardness	Fracturabi	Adhesivene	Springiness	Cohesiver	Gummines	Chewiness	Resilience
			g	g.sec	sec	g.sec	g.sec	g.sec	sec	g	g	g.sec					
			Force 1	Area F-T 1:2	Time Difference 1:2	Area F-T 1:3	Area F-T 2:3	Area F-T 4:6	Time Difference 4:5	Force 2	Force 3	Variable	J#/F#	I#/G#	K#*O#	P#*N#	H#/E#
Start Batc	bovineb																
ovinec1	bovineb		1175.396	398.827	0.92	746.641	347.813	741.673	0.82	1181.607		285.3	0.911	0.993	1173.744	1046.163	0.872
ovinec2	bovineb		1330.602	411.354	0.86	756.177	344.823	722.065	0.8	1371.652		212.295	0.93	0.955	1309.774	1218.395	0.838
ovinec3	bovineb		1391.784	199.411	0.47	374.857	175.446	360.455	0.425	1436.975		243.802	0.934	0.962	1381.767	1249.47	0.88
End Batch	bovineb																
[redacted]	bovineb ((	AVERAGE	1299.261	336.531	0.750	625.892	289.361	608.064	0.682	1330.078	#DIV/0!	247.132	0.925	0.970	1288.428	1171.343	0.863
D.	bovineb ((	STDEV("B	111.547	118.914	0.244	217.455	98.664	214.660	0.223	132.663	#DIV/0!	36.616	0.012	0.020	105.641	109.517	0.022
oef. of V	bovineb ((	STDEV("B	8.585	35.335	32.578	34.743	34.097	35.302	32.641	9.974	#DIV/0!	14.816	1.328	2.085	8.199	9.350	2.583
Start Batc	bovinec																
ovineb1	bovinec		1610.529	574.64	0.89	1044.791	570.151	982.759	0.825	1688.489		14.946	0.927	0.941	1588.239	1472.244	0.992
ovineb2	bovinec		1470.315	407.092	0.71	711.662	389.569	672.862	3.285	1543.278			0.911	0.945	1459.137	6751.078	0.957
ovineb3	bovinec		1277.415	388.938	0.77	693.2	304.262	664.535	1.89	1320.036		0.879	0.924	0.959	1265.451	3106.106	0.782
End Batch	bovinec																
Average:	bovinec	AVERAGE	1452.753	456.89	0.79	816.551	421.3273333	773.3853333	2	1517.268	#DIV/0!	7.9125	0.9206667	0.948333	1437.609	3776.476	0.910
S.D.	bovinec	STDEV("B	167.250	102.378	0.092	197.877	135.760	181.371	1.234	185.599	#DIV/0!	9.947	0.009	0.009	162.467	2702.512	0.112
Coef. of V	bovinec	STDEV("B	11.513	22.408	11.601	24.233	32.222	23.452	61.684	12.232	#DIV/0!	125.711	0.924	0.997	11.301	71.562	12.346
Start Batc	bovinea																
ovinea1	bovinea		944.3	292.123	0.89	521.156	229.033	519.837	0.825	922.811		29.109	0.927	0.997	920.474	853.249	0.784
ovinea2	bovinea		911.103	297.241	0.86	549.784	252.543	524.743	0.81	938.589		58.953	0.942	0.954	895.839	843.755	0.85
ovinea3	bovinea		848.635	272.517	0.92	508.073	235.556	485.921	0.855	870.481		26.697	0.929	0.956	832.527	773.707	0.864
End Batch	bovinea																
Average:	bovinea ((	AVERAGE	901.346	287.294	0.890	526.338	239.044	510.167	0.830	910.627	#DIV/0!	38.253	0.933	0.969	882.947	823.570	0.833
S.D.	bovinea ((	STDEV("B	48.5731	13.0503	0.0300	21.3328	12.1369	21.1404	0.0229	35.6513	#DIV/0!	17.9672	0.0081	0.0243	45.3688	43.4430	0.0427
Coef. of V	bovinea ((	STDEV("B	5.389	4.543	3.371	4.053	5.077	4.144	2.761	3.915	#DIV/0!	46.970	0.873	2.505	5.138	5.275	5.131

Appendix 18: TPA Bovine at 70 °C

Test ID	Batch		Force 1	Area-FT 1:	Time-diff. 1:2	Area-FT 1:	Area-FT 2:	Area-FT 4:	Time-diff.	Hardness	Fracturabi	Adhesives	Springines	Cohesiver	Gummines	Chewines	Resilience
			g	g.sec	sec	g.sec	g.sec	g.sec	sec	g	g	g.sec					
			Force 1	Area F-T 1	Time Differe	Area F-T 1	Area F-T 2	Area F-T 4	Time Diffe	Force 2	Force 3	Variable	J#/F#	I#/G#	K#*O#	P#*N#	H#/E#
Start Batc	bovinec																
ovinec1	bovinec		2326.233	1146.423	0.7	2064.702	918.279	2020.767	0.695	2353.312		15.92	0.993	0.979	2303.236	3667.452	0.801
ovinec2	bovinec		2973.858	1004.498	0.74	1864.224	859.725	1782.101	0.67	2908.063		12.254	0.905	0.956	2779.957	3564.267	0.856
ovinec3	bovinec																
End Batch	bovinec																
Average:	bovinec (	AVERAGE	2650.046	1075.461	0.720	1964.463	889.002	1901.434	0.683	2630.688	#DIV/0!	14.087	0.949	0.967	2541.596	3615.860	0.828
S.D.	bovinec (	STDEV("B	457.940	100.356	0.028	141.759	41.404	168.762	0.018	392.268	#DIV/0!	2.592	0.062	0.016	337.093	72.963	0.039
Coef. of V	bovinec (	STDEV("B	17.280	9.331	3.928	7.216	4.657	8.876	2.590	14.911	#DIV/0!	18.402	6.515	1.665	13.263	2.018	4.684
Start Batc	bovineb																
ovineb1	bovineb		2385.528	508.35	0.74	956.643	448.293	909.478	0.695	2495.824		8.048	0.939	0.951	2372.774	2228.484	0.882
ovineb2	bovineb		2231.971	528.686	0.74	1000.463	471.777	958.644	0.675	2308.857		9.498	0.912	0.958	2212.346	2018.018	0.892
ovineb3	bovineb																
End Batch	bovineb																
Average:	bovineb ((	AVERAGE	2308.75	518.518	0.74	978.553	460.035	934.061	0.685	2402.341	#DIV/0!	8.773	0.9255	0.9545	2292.56	2123.251	0.887
S.D.	bovineb ((	STDEV("B	108.581	14.380	0.000	30.985	16.606	34.766	0.014	132.206	#DIV/0!	1.025	0.019	0.005	113.440	148.822	0.007
Coef. of V	bovineb ((	STDEV("B	4.703	2.773	0.000	3.166	3.610	3.722	2.065	5.503	#DIV/0!	11.687	2.063	0.519	4.948	7.009	0.797
Start Batc	bovinea																
ovinea1	bovinea																
ovinea2	bovinea		1306.201	247.956	0.56	464.074	216.119	426.649	0.55	1389.226		91.175	0.982	0.919	1277.192	1254.385	0.872
ovinea3	bovinea		1235.812	278.24	0.68	525.893	247.653	483.332	0.625	1324.833		106.608	0.919	0.919	1217.615	1119.131	0.89
End Batch	bovinea																
Average:	bovinea ((	AVERAGE	1271.007	263.098	0.620	494.984	231.886	454.991	0.588	1357.030	#DIV/0!	98.892	0.951	0.919	1247.404	1186.758	0.881
S.D.	bovinea ((	STDEV("B	49.773	21.414	0.085	43.713	22.298	40.081	0.053	45.533	#DIV/0!	10.913	0.045	0.000	42.127	95.639	0.013
Coef. of V	bovinea ((	STDEV("B	3.916	8.139	13.686	8.831	9.616	8.809	9.027	3.355	#DIV/0!	11.035	4.687	0.000	3.377	8.059	1.445
End of Test Data																	

Appendix 19: TPA Bovine at 80 °C

ID	Batch		Force 1	Area-FT 1:2	Time-diff. 1	Area-FT 1:3	Area-FT 2:3	Area-FT 4:6	Time-diff. 4	Hardness	Fracturabl	Adhesiveness	Springiness	Cohesivene	Gumminess	Chewiness	Resilience
			g	g.sec	sec	g.sec	g.sec	g.sec	sec	g	g	g.sec					
			Force 1	Area F-T 1:2	Time Differ	Area F-T 1:3	Area F-T 2:3	Area F-T 4:6	Time Differ	Force 2	Force 3	Variable	J#/F#	I#/G#	K#*O#	P#*N#	H#/E#
Batc	beefc																
1	beefc																
2	beefc		811.795	191.988	0.68	350.715	158.727	349.38	0.605	814.651		113.918	0.89	0.996	811.55	722.04	0.827
3	beefc		740.183	193.066	0.68	359.083	166.017	353.518	0.62	743.61		125.115	0.912	0.985	732.085	667.489	0.86
tch	beefc																
	(F)	AVERAGE	775.9890	192.5270	0.6800	354.8990	162.3720	351.4490	0.6125	779.1305	#DIV/0!	119.5165	0.9010	0.9905	771.8175	694.7645	0.8435
	(F)	STDEV("B	50.637	0.762	0.000	5.917	5.155	2.926	0.011	50.234	#DIV/0!	7.917	0.016	0.008	56.190	38.573	0.023
of V	beefc (F)	STDEV("B	6.526	0.396	0.000	1.667	3.175	0.833	1.732	6.447	#DIV/0!	6.625	1.727	0.785	7.280	5.552	2.766
Batc	beefb																
1	beefb		911.038	273.781	0.92	498.91	225.129	487.894	0.82	928.388		146.885	0.891	0.978	907.889	809.205	0.822
2	beefb		810.51	199.297	0.74	347.322	148.026	347.59	0.645	821.148		104.317	0.872	1.001	821.782	716.283	0.743
3	beefb																
tch	beefb																
	beefb (F)	AVERAGE	860.774	236.539	0.830	423.116	186.578	417.742	0.733	874.768	#DIV/0!	125.601	0.882	0.990	864.836	762.744	0.783
	beefb (F)	STDEV("B	71.084	52.668	0.127	107.189	54.520	99.210	0.124	75.830	#DIV/0!	30.100	0.013	0.016	60.887	65.706	0.056
of V	beefb (F)	STDEV("B	8.258	22.266	15.335	25.333	29.221	23.749	16.893	8.669	#DIV/0!	23.965	1.524	1.644	7.040	8.614	7.139
Batc	beefa																
a1	beefa		587.248	150.041	0.74	263.808	113.767	258.005	0.68	590.961		97.171	0.919	0.978	577.961	531.099	0.758
a2	beefa		690.633	168.333	0.74	291.822	123.49	283.044	0.665	706.483		90.105	0.899	0.97	685.232	615.783	0.734
a3	beefa		531.201	134.952	0.74	236.525	101.573	234.423	0.665	533.7		92.55	0.899	0.991	528.957	475.346	0.753
Batch	beefa																
age:	beefa (F)	AVERAGE	603.027	151.109	0.740	264.052	112.943	258.491	0.670	610.381	#DIV/0!	93.275	0.906	0.980	597.383	540.743	0.748
	beefa (F)	STDEV("B	80.879	16.716	0.000	27.649	10.982	24.314	0.009	88.013	#DIV/0!	3.588	0.012	0.011	79.927	70.713	0.013
of V	beefa (F)	STDEV("B	13.412	11.062	0.000	10.471	9.723	9.406	1.293	14.419	#DIV/0!	3.847	1.275	1.082	13.380	13.077	1.692

Appendix 20: TPA Bovine at 90 °C

ID	Batch		Force 1	Area-FT 1:2	Time-diff. 1	Area-FT 1:3	Area-FT 2:3	Area-FT 4:6	Time-diff.	Hardness	Fracturabl	Adhesivene	Springiness	Cohesivene	Gumminess	Chewiness	Resilience
			g	g.sec	sec	g.sec	g.sec	g.sec	sec	g	g	g.sec					
			Force 1	Area F-T 1:2	Time Differ	Area F-T 1:3	Area F-T 2:3	Area F-T 4:6	Time Diffe	Force 2	Force 3	Variable	J#/F#	I#/G#	K#*O#	P#*N#	H#/E#
Batc	tilapiab																
lab1	tilapiab		1115.426	260.344	0.68	488.095	227.751	461.085	0.63	1169.827		117.119	0.926	0.945	1105.09	1023.833	0.875
lab2	tilapiab		920.384	211.865	0.68	392.166	180.301	370.662	0.635	964.861		85.724	0.934	0.945	911.955	851.605	0.851
lab3	tilapiab		1105.003	258.496	0.68	482.835	224.339	453.358	0.63	1163.045		150.288	0.926	0.939	1092.043	1011.746	0.868
Batch	tilapiab																
age:	tilapiab (F	AVERAGE	1046.938	243.568	0.680	454.365	210.797	428.368	0.632	1099.244	#DIV/0!	117.710	0.929	0.943	1036.363	962.395	0.865
	tilapiab (F	STDEV("B	109.723	27.471	0.000	53.930	26.465	50.124	0.003	116.429	#DIV/0!	32.286	0.004	0.003	107.938	96.137	0.012
of V	tilapiab (F	STDEV("B	10.480	11.279	0.000	11.869	12.555	11.701	0.457	10.592	#DIV/0!	27.428	0.457	0.366	10.415	9.989	1.415
of Test Data																	
Batc	tilapiaa																
lab1	tilapiaa		412.5560	81.4180	0.4400	149.5660	68.1480	134.7100	0.4050	458.3160		88.2340	0.9205	0.9007	412.7927	379.9569	0.8370
lab2	tilapiaa		402.4190	72.1410	0.4100	130.7820	58.6420	121.0340	0.3700	431.4030		97.2120	0.9024	0.9255	399.2478	360.2968	0.8129
lab3	tilapiaa		364.7970	87.7950	0.5000	154.9950	67.2000	142.1790	0.4900	394.2800		50.0770	0.9800	0.9173	361.6784	354.4448	0.7654
Batch	tilapiaa																
age:	tilapiaa (F	AVERAGE	393.2573	80.4513	0.4500	145.1143	64.6633	132.6410	0.4217	427.9997	#DIV/0!	78.508	0.934	0.914	391.240	364.900	0.805
	tilapiaa (F	STDEV("B	25.1631	7.8716	0.0458	12.7055	5.2361	10.7233	0.0617	32.1534	#DIV/0!	25.028	0.041	0.013	26.481	13.364	0.036
of V	tilapiaa (F	STDEV("B	6.3986	9.7844	10.1835	8.7555	8.0975	8.0844	14.6352	7.5125	#DIV/0!	31.879	4.345	1.382	6.769	3.662	4.524
Batc	tilapiac																
lab1	tilapiac		1195.396	398.827	0.92	746.641	347.813	741.673	0.82	1307.516		285.300	0.891			1046.163	0.872
lab2	tilapiac		1230.112	411.354	0.86	756.177	344.823	722.065	0.8	1333.432		192.295	0.930	0.955	1273.279	1218.395	0.838
lab3	tilapiac		1301.184	382.64862	0.47	382.857	175.446	360.455	0.425	1413.174		243.802	0.904	0.941	1330.485	1249.470	
Batch	tilapiac																
age:	tilapiac (F	AVERAGE	1242.231	397.610	0.750	628.558	289.361	608.064	0.682	1351.374	#DIV/0!	240.466	0.909	0.948	1301.882	1171.343	0.855
	tilapiac (F	STDEV("B	53.925	14.391	0.244	212.837	98.664	214.660	0.223	55.067	#DIV/0!	46.592	0.020	0.009	40.451	109.517	0.024
of V	tilapiac (F	STDEV("B	4.341	3.619	32.578	33.861	34.097	35.302	32.641	4.075	#DIV/0!	19.376	2.182	0.999	3.107	9.350	2.797

Appendix 21: TPA Tilapia at 70 °C

...t ID	Batch		Force 1	Area-FT 1:	Time-diff.	Area-FT 1:	Area-FT 2:	Area-FT 4:	Time-diff.	Hardness	Fracturabl	Adhesiven	Springines	Cohesiver	Gumminess	Chewiness	Resilience
			g	g.sec	sec	g.sec	g.sec	g.sec	sec	g	g	g.sec					
			Force 1	Area F-T 1	Time Diffe	Area F-T 1	Area F-T 2	Area F-T 4	Time Diffe	Force 2	Force 3	Variable	J#/F#	I#/G#	K#*O#	P#*N#	H#/E#
...rt Batc	tilapiac																
...apiab1	tilapiac		1115.426	260.344	0.68	488.095	227.751	461.085	0.63	1169.827		75.119	0.926	0.945	1105.09	1023.833	0.875
...apiab2	tilapiac		899.384	211.865	0.68	392.166	180.301	370.662	0.635	964.861		85.724	0.934	0.945	911.955	851.605	0.851
...apiab3	tilapiac		1005.003	258.496	0.68	482.835	224.339	453.358	0.63	1163.045		90.288	0.926	0.939	1092.043	1011.746	0.868
...d Batch	tilapiac																
		AVERAGE(	1006.604	243.568	0.680	454.365	210.797	428.368	0.632	1099.244	#DIV/0!	83.710	0.929	0.943	1036.363	962.395	0.865
		STDEV("B/	108.030	27.471	0.000	53.930	26.465	50.124	0.003	116.429	#DIV/0!	7.782	0.004	0.003	107.938	96.137	0.012
...oef. of V	tilapiac	STDEV("B/	10.732	11.279	0.000	11.869	12.555	11.701	0.457	10.592	#DIV/0!	9.297	0.457	0.366	10.415	9.989	1.415
...d of Test Data																	
...art Batc	tilapiab																
...apiab1	tilapiab																
tilapiab2	tilapiab		500.007	78.711	0.44	147.639	68.928	146.363	0.385	499.079		141.702	0.875	0.991	494.766	432.920	0.876
tilapiab3	tilapiab		574.751	117.486	0.62	214.492	97.006	205.936	0.515	586.245		126.018	0.831	0.960	562.860	467.537	0.826
End Batch	tilapiab																
Average:	tilapiab	AVERAGE(	537.379	98.099	0.530	181.066	82.967	176.150	0.450	542.662	#DIV/0!	133.860	0.853	0.976	528.813	450.228	0.851
S.D.	tilapiab	STDEV("B/	52.852	27.418	0.127	47.272	19.854	42.124	0.092	61.636	#DIV/0!	11.090	0.031	0.022	48.150	24.478	0.035
Coef. of V	tilapiab	STDEV("B/	9.835	27.950	24.015	26.108	23.930	23.914	20.428	11.358	#DIV/0!	8.285	3.678	2.264	9.105	5.437	4.158
Start Batc	tilapiaa																
tilapiab1	tilapiaa		294.05	71.861	0.65	124.943	53.083	123.211	0.535	296.264		63.476	0.823	0.986	292.157	240.468	0.739
tilapiab2	tilapiaa																
tilapiab3	tilapiaa		458.673	98.409	0.61	182.704	84.295	181.194	0.49	456.174		135.393	0.803	0.992	452.404	363.406	0.857
End Batch	tilapiaa		441.468	110.12	0.68	205.01	94.89	204.412	0.555	436.471		183.398	0.816	0.997	435.198	355.198	0.862
Average:	tilapiaa	AVERAGE(	398.064	93.463	0.630	153.824	68.689	152.203	0.513	376.219	#DIV/0!	159.396	0.814	0.989	372.280	301.937	0.798
S.D.	tilapiaa	STDEV("B/	90.488	19.603	0.028	40.843	22.070	41.000	0.032	113.073	#DIV/0!	33.945	0.010	0.004	113.312	86.931	0.004
Coef. of V	tilapiaa	STDEV("B/	22.732	20.974	4.490	26.552	32.131	26.938	6.209	30.055	#DIV/0!	21.296	1.234	0.400	30.437	28.791	0.454

Appendix 22: TPA Tilapia at 80 °C

...st ID	Batch		Force 1	Area-FT 1:	Time-diff.	Area-FT 1:	Area-FT 2:	Area-FT 4:	Time-diff.	Hardness	Fracturabl	Adhesiven	Springines	Cohesiven	Gumminess	Chewiness	Resilience
			g	g.sec	sec	g.sec	g.sec	g.sec	sec	g	g	g.sec					
			Force 1	Area F-T 1	Time Diffe	Area F-T 1	Area F-T 2	Area F-T 4	Time Diffe	Force 2	Force 3	Variable	J#/F#	I#/G#	K#*O#	P#*N#	H#/E#
...art Batc	tilapiab																
...apiab1	tilapiab		469.239	142.115	0.74	266.164	104.048	259.992	0.695	475.021		79.03	0.939	0.977	464.005875	435.7893	0.732
...apiab2	tilapiab		621.582	129.078	0.56	238.118	98.04	221.531	0.515	625.295		102.047	0.920	0.930	581.737738	534.99096	0.760
...apiab3	tilapiab																
...d Batch	tilapiab																
...erage:	tilapiab (F	AVERAGE(	545.411	135.597	0.650	252.141	101.044	240.762	0.605	550.158	#DIV/0!	90.539	0.929	0.954	522.872	485.390	0.746
...D.	tilapiab (F	STDEV("B/	107.723	9.219	0.127	19.832	4.248	27.196	0.127	106.260	#DIV/0!	16.275	0.014	0.033	83.249	70.146	0.019
...ef. of V	tilapiab (F	STDEV("B/	19.751	6.799	19.581	7.865	4.204	11.296	21.038	19.314	#DIV/0!	17.976	1.487	3.446	15.921	14.452	2.598
...d of Test Data																	
...art Batc	tilapiaa																
...apiab1	tilapiaa		412.556	81.418	0.44	149.566	58.111	134.71	0.405	458.316		88.234	0.920	0.901	412.793	379.957	0.714
...apiab2	tilapiaa		402.419	72.141	0.41	130.782	56.142	121.034	0.37	431.403		97.212	0.902	0.925	399.248	360.297	0.778
...apiab3	tilapiaa		364.797	87.795	0.5	154.995	63.2	142.179	0.49	394.28		50.077	0.980	0.917	361.678	354.445	0.720
...d Batch	tilapiaa																
...erage:	tilapiaa (F	AVERAGE(	393.25733	80.45133	0.45	145.1143	59.151	132.641	0.421667	427.99967	#DIV/0!	78.508	0.934	0.914	391.240	364.900	0.737
...D.	tilapiaa (F	STDEV("B/	25.16312	7.871643	0.045826	12.70552	3.64212	10.72326	0.061712	32.153372	#DIV/0!	25.028	0.013	0.013	26.481	13.364	0.036
...ef. of V	tilapiaa (F	STDEV("B/	6.3986398	9.784354	10.1835	8.755527	6.157327	8.084424	14 63518	7.5124759	#DIV/0!	31.879	1.363	1.382	6.769	3.662	4.828
...art Batc	tilapiac																
...apiab1	tilapiac		725.261	81.418	0.62	198.648	68.148	188.067	0.585	740.528			0.944	0.947	701.084	751.572	0.837
...apiab2	tilapiac		829.966	72.141	0.56	274.185	58.642	265.698	0.53	868.945		123.882	0.946	0.969	842.048	796.936	0.813
...apiab3	tilapiac		652.621	87.795	0.54	106.114	67.2	106.352	0.525	672.324		109.281	0.972	1.002	673.832	528.795	0.765
...d Batch	tilapiac																
...erage:	tilapiac (F	AVERAGE(	735.94933	80.45133	0.573333	192.9823	64.66333	186.7057	0.546667	760.599	#DIV/0!	116.5815	0.954	0.973	738.988	692.434	0.805
...D.	tilapiac (F	STDEV("B/	89.154319	7.871643	0.041633	84.17862	5.236126	79.68172	0.033292	99.835305	#DIV/0!	10.324466	0.016	0.028	90.287	143.519	0.036
...ef. of V	tilapiac (F	STDEV("B/	12.114193	9.784354	7 261626	43.61986	8.09752	42.67772	6.089934	13.125879	#DIV/0!	8.8560073	1.655	2.872	12.218	20.727	4.524

Appendix 23: TPA Tilapia at 90 °C