

TEXTURAL AND OPTICAL PROPERTIES OF GELATIN GEL AND
APPLICATION OF KUBELKA-MUNK THEORY ON ITS MEASURED COLOR

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สมบัติทางเนื้อสัมผัสและการสะท้อนแสงของเจลาตินเจดและการประยุกต์ทฤษฎีบูลคา-มังก์
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งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาผลของความเข้มข้นเจลาตินและอัตราส่วนระหว่างน้ำเชื่อม
กลูโคสและน้ำตาลซูโครสต่อสมบัติทางเนื้อสัมผัสและสมบัติเชิงแสงของเจลาตินเจล โดยแปรระดับ
ความเข้มข้นของเจลาติน 3 ระดับ (ร้อยละ 7.75, 8.00 และ 8.25) และอัตราส่วนระหว่างน้ำเชื่อมกลูโคส
และน้ำตาลซูโครส 5 ระดับ (0:0, 0:100, 30:70, 70:30 และ 100:0) เตรียมเจลาตินเจลในแม่พิมพ์ที่ทำ
จากซิลิโคนขนาด ยาว 5 ซม. กว้าง 5 ซม. และสูง 2.5 ซม. การเติมน้ำตาลซูโครสส่งผลให้ค่าความ
แข็ง (hardness) และความเหนียว (gumminess) ของเจลาตินเจลที่ทุกภาวะแตกต่างกันอย่างมีนัยสำคัญ
($p \leq 0.05$) ค่าความยืดหยุ่น (springiness) และค่าการเกาะติด (cohesiveness) ของเจลาตินเจลในทุก
ภาวะมีค่าอยู่ในช่วง 0.95 – 0.99 และ 0.93 – 1.00 ตามลำดับ ซึ่งมีค่าแตกต่างกันเพียงเล็กน้อย เจลาติน
ที่ความเข้มข้นร้อยละ 8.25 มีค่าการกุดแตก (rupture force) สูงที่สุดอย่างมีนัยสำคัญ ($p \leq 0.05$) การ
เติมน้ำตาลทำให้ค่าการกุดแตกและระยะทางของการกุดจนแตกแตกต่างกันอย่างมีนัยสำคัญ ($p \leq$
0.05) การวิเคราะห์สมบัติเชิงแสงของเจลาตินเจลทุกภาวะบนพื้นหลังสีขาวมีแนวโน้มไปในทิศทาง
เดียวกัน โดยการเติมน้ำเชื่อมกลูโคสทำให้ค่าความสว่างของเจลาตินเจลเพิ่มขึ้น และการเติมน้ำตาลซูโครส
ส่งผลต่อการเพิ่มขึ้นของความสทึบของสีของเจลาตินเจล ผลการคำนวณค่า spectral distributions of
 K/S ที่ได้จากทฤษฎีคูเบลคา-มังก์ โดยใช้ Saunderson's correction และการตัดแปรทฤษฎีคูเบลคา-มังก์
เพื่อใช้กับผลิตภัณฑ์กึ่งโปร่งแสงมีความแม่นยำมากสำหรับการอธิบายลักษณะเชิงแสงของเจลาติน
เจล ความสัมพันธ์ระหว่างค่าสัมประสิทธิ์ของการดูดกลืนแสง (absorption coefficient) และความ
เข้มข้นของสีที่เติมลงในเจลาตินเจล มีลักษณะเชิงเส้นตรง ($r = 0.94$) ดังนั้นจึงสามารถใช้ความสัมพันธ์
ดังกล่าวทำนายรูปแบบการสะท้อนแสงของเจลาตินเจลที่เติมสีที่ระดับความเข้มข้นสีต่างกัน ได้.

ภาควิชา เทคโนโลยีทางอาหาร

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The effect of gelatin concentrations and glucose syrup to sucrose ratios on textural and optical properties were investigated using texture analyzer and spectrophotometer. Three gelatin concentrations (7.75%, 8.00% and 8.25%) and five glucose syrup to sucrose ratios (0:0, 0:100, 30:70, 70:30 and 100:0) were experimented. Gelatin gel samples were prepared using a 5 cm (L) x 5 cm (W) x 2.5 cm (H) silicone mold. The addition of sugar affected the hardness and gumminess of gelatin gel samples at all conditions ($p \leq 0.05$). Springiness and cohesiveness of gelatin gel samples at all conditions were slightly different ranging from 0.95–0.99 and 0.93–1.00, respectively. Puncture test indicated that gel with 8.25% gelatin concentration had the highest rupture force ($p \leq 0.05$). The addition of sugars increased the rupture force and distance of rupture point ($p \leq 0.05$). The reflectance spectra of all gelatin gel samples showed the same trend against white background. The addition of glucose syrup into the mixture resulted in an increase in lightness, and the addition of sucrose into the mixture resulted in an increase in chroma. Spectral distributions of K/S calculated from the Kubelka-Munk model with Saunderson's correction and modified Kubelka-Munk model for semitransparent products were more precise in describing the optical characteristics of gelatin gel samples. The relationship between absorption coefficient and concentration of colored gelatin gel showed a linear relationship ($r = 0.94$), which can be used to predict the reflectance at various concentrations of color.

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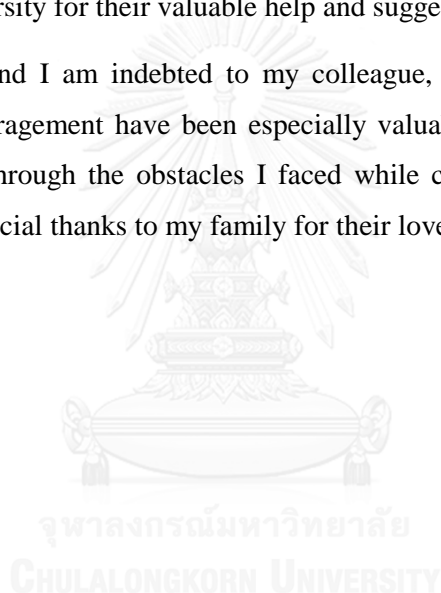
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CHAPTER I

INTRODUCTION

Food gel is easier to recognize than to define. Gelatin is considered to be one of the most commonly used gels in the food industry. Gelatin is a transparent substance derived from collagen of various animals. The temperature dependent form of gel state gives gelatin its unique properties. Color and texture are important aspects of gelatin products. The gel strength of gelatin gels depends on gelatin concentration and sweetener is often incorporated into gelatin products to improve textural properties, clarity, and flavor of gelatin (Burey *et al.*, 2009). Textural profile analysis is a common method used in the food industry to obtain textural properties of products. It has been well correlated with sensory evaluation (Lau *et al.*, 2000).

Defining the color of gelatin is difficult because the light transmission pattern for a translucent substance is very different from the liquid and solid. This is because for translucent product, like gelatin gel, light is absorbed, reflected, transmitted, and scattered. Measuring the color and reproducing the same exact color for these products is a serious problem due to the scattering particles within the layers of these products (Calvo & Salvador, 1997). Conventional methods like CIELAB show the overall color in food, but do not express how scattering occurs. Light scattering makes the color perception different from that CIELAB perceived.

By applying the Kubelka-Munk theory, light absorbed, reflected, transmitted and scattered are considered, giving more detailed results compared to the conventional method does. Researchers have modified the original Kubelka-Munk theory to fit various food types testing in experiments. Researchers found that the

application of Kubelka-Munk theory is helpful when applied to some foods such as rosemary honey (Negueruela & Perez-Arquillue, 2000), fruit gels (Calvo & Salvador, 1997), and tomatoes (Lana *et al.*, 2006).

The objectives of the study were to develop an understanding of textural and optical properties of gelatin gels and the application of Kubelka-Munk theory on gelatin gels.



CHAPTER II

LITERATURE REVIEW

2.1 Gelatin

Gelatin is a protein obtained by partial hydrolysis of collagen derived from skin, bones, and hides of animals such as pigs, cows and fish. It is considered to be one of the main ingredients for protein based gelled food. The term 'gelatin' originated from Latin word 'gelatus' which describes its characteristic properties. The thermally reversible gelation property of gelatin is very unique and found to be an unmatched selection not only in the food industry but in others like pharmaceutical, photography, and cosmetics (Keenan, 2008). The use of gelatin can be traced back to 4000 BC in ancient Egypt when gelatin was extracted by cooking pieces of hide in water and animal glue was formed. The first commercial gelatin manufacturing began in the seventeenth century. In the nineteenth century, commercial production has been improved to achieve the manufacture of good quality gelatin gels (Keenan, 2008). Today, gelatin has found its way into many industries, according to the Gelatin Manufacturers of Europe a total production for 2001 as 269,000 tonnes. Of this quantity, the use in food industry was counted about 70% of the gelatin production (Jones, 2004).

2.2 Composition and Structure

Gelatins are large and complex polypeptides, derived from collagen. Collagen exists as a triple helical rod shape made up of three parallel chains. Usually the typical triple amino acid sequence is composed of triplets in the form of glycine – X -Y, with glycine-proline-Y occurred more frequently and in particularly glycine-proline-

hydroxyproline (Jones, 2004), shown in Figure 2.1a. Hydrogen bonds between the nitrogens of glycines and the oxygens of prolines connect the three simple helices, which allow the collagen to twist and turn into a helical structure (Keenan, 2008), Figure 2.1b, and stabilize the triple helix.

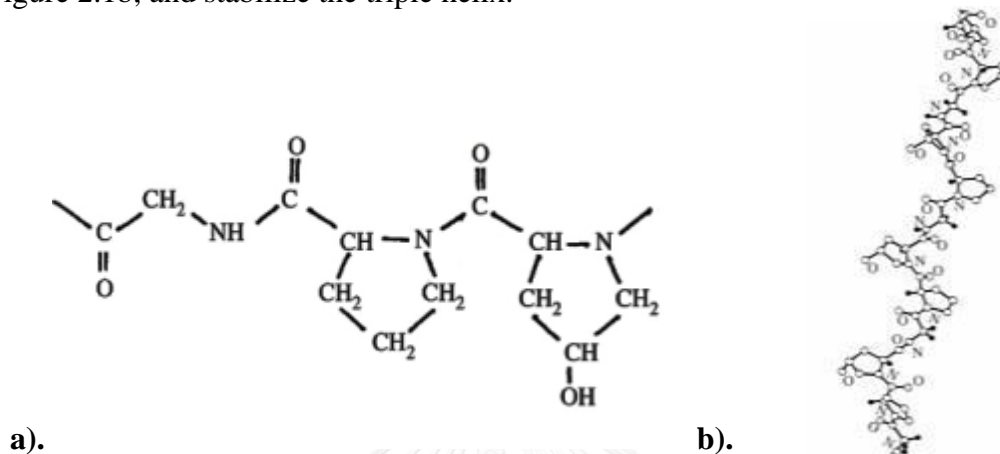


Figure 2.1 Collagens structure: (a) typical triple amino acid (b) triple helical structure (Charvolin & Sadoc, 2012)

2.3 Types of Gelatin

In the food industry, there are two types of gelatin: Type A and Type B. Figure 2.2a, shows that in year 2006, the materials which were most commonly used in gelatin production were pig skin, bovine hides, bones, and other counted as 44%, 28%, 27% and 1%, respectively. Figure 2.2b shows the geography of gelation production, which were Western Europe with 39%, other for 22%, North America for 20%, Latin America for 17%, and Eastern Europe for 2% (GME, 2012).

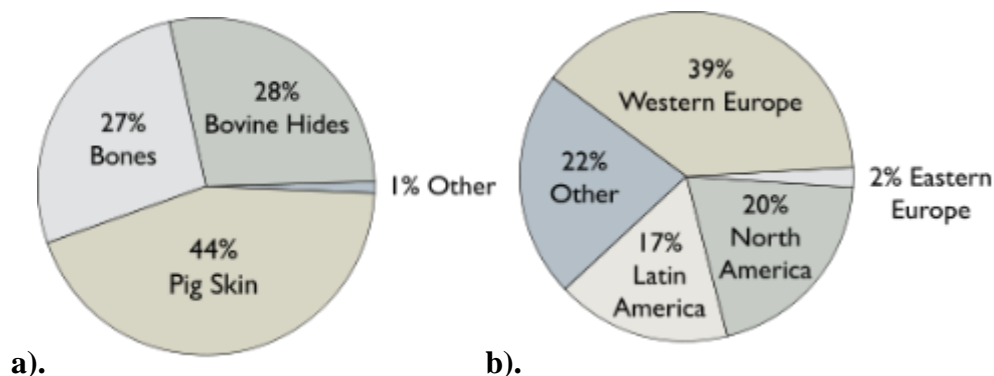


Figure 2.2 Production of gelatin in 2006: (a) material used in gelatin production (b) gelatin production by geography (GME, 2012)

The process that bones and hides undergo before becoming gelatin distinguishes the gelatin types. Type A gelatin undergoes acid treatment and type B gelatin undergoes alkaline treatment. Figure 2.3 shows a flow chart of typical commercial process of turning hide and bone into gelatin. Figure 2.3a shows the process of bones going through alkaline treatment and Figure 2.3b shows the process of hides going through acid treatment. Extraction materials and methods result in the different amount of amino acids in gelatin. Table 2.1 shows the composition of amino acids in gelatin using acid treatment and alkaline treatment. For both treatments, glycine accounts for about one-third of all the residues in gelatin. Type B gelatins, alkaline treatment, are lack of glutamine and asparagine because they are converted into their carboxyl form by alkali deamination (Haug & Draget, 2009).

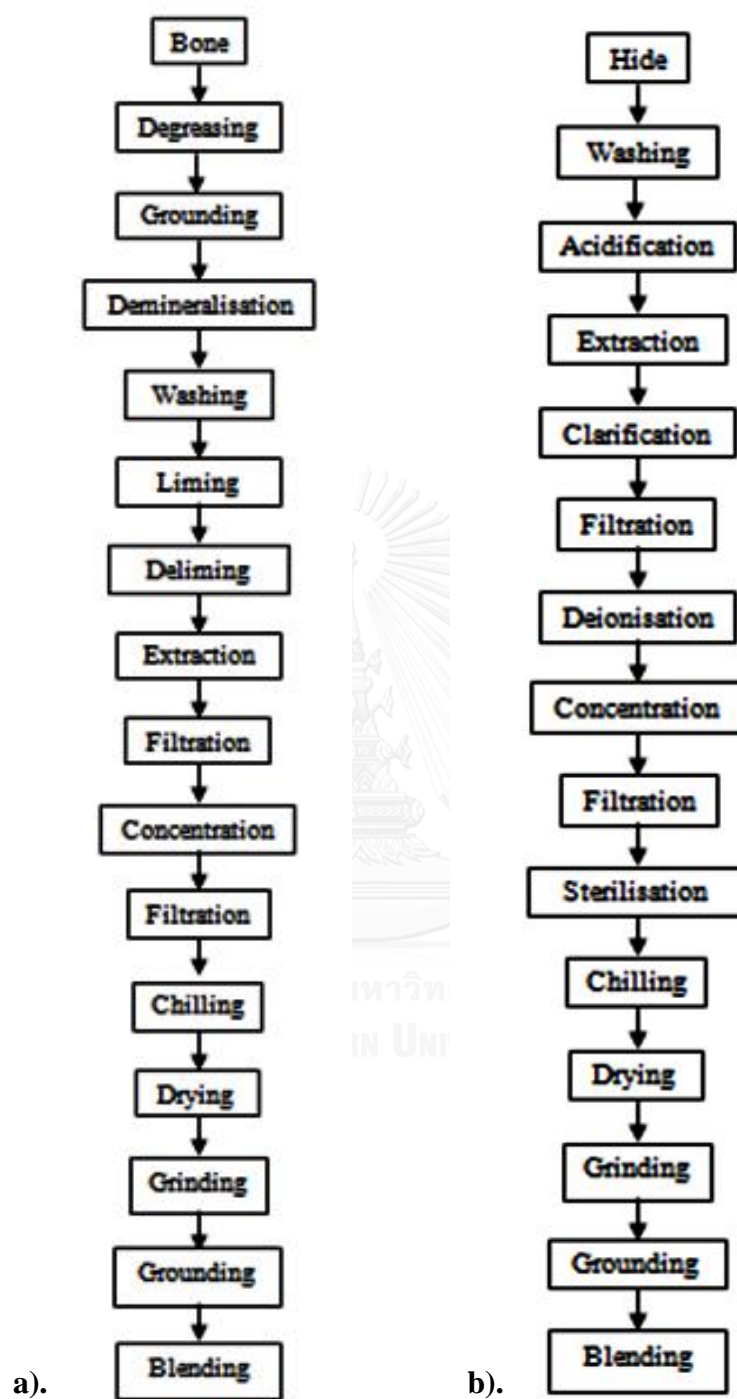


Figure 2.3 Flowchart of typical conversion of collagen into gelatin in commercial production (a) using alkaline treatment from bone (b) using acid treatment from hide (Jones, 2004)

Table 2.1 The composition of amino acids in gelatin derived from different extraction methods (values are given as numbers of amino acid residues per 1000 residues)

Amino Acids	Type A gelatin (hide)	Type B gelatin (bone)
Alanine	112	117
Arginine	49	48
Asparagine	16	
Aspartic Acid	29	46
Glutamine	48	
Glutamic acid	25	72
Glycine	330	335
Histidine	4	4
4-Hydroxyproline	91	93
Hydroxylysine	6	4
Isoleucine	11	10
Leucine	24	24
Lysine	28	27
Methionine	4	4
Phenylalanine	14	14
Proline	124	132
Serine	33	35
Threonine	18	18
Tyrosine	1	3
Valine	22	26

Source: Babel (1996)

2.3.1 Type A Gelatin

Type A gelatin undergoes an acid treatment, which is usually applied to skin of animals such as pigs, cows, and fish. Typical conversion of collagen into gelatin using acid treatment is as follows. Skins are macerated, washed, and swelled in acid. The swelling step can last up to 30 hours depending on the type of material and acid used in the process. There are three types of acid that can be used, which are hydrochloric acid, phosphoric acid, or sulfuric acid. Five steps of extraction are performed in the process and each extraction step is made at an increasing temperature from previous step. For the first extraction, temperature starts off at 55 °C and the last extraction usually ends at 100 °C. When the extractions are done, the grease is removed and the gelatin solution is filtered. Gelatin solution is concentrated using vacuum evaporation to about 20 to 40% of solid concentration, and gelatin solution is cooled. Then it is extruded as noodles and dried. The dry gelatin is then processed into the specification for their usage (Keenan, 2008).

Many researchers used type A gelatin in their experiments. Holm *et al.*, (2009) used type A gelatin from porcine skin to study the sweetness and texture perception in structures gelatin gel with embedded sugar rich domain. Kasapis *et al.* (2003) also used type A gelatin derived from young pig skin to study gelatin gel in mixture with sugar. They explained that gelatin extracting from young animals had relatively low density of cross-link material, which allowed the application of acid to be milder and more rapid.

2.3.2 Type B Gelatin

Type B is gelatin that undergoes alkaline treatment. Alkaline treatment is commonly applied to bones. It can also be used with bovine hides and pork skins as well. Typical process for type B gelatin starts off with crushing and degreasing the bones. The bones are then treated with cool hydrochloric acid to remove all the mineral contents. When all minerals are removed, bones are washed and stored in lime slurry for gentle daily agitation. To remove the lime slurry, the bones are washed for at least 15 to 30 hours for demineralized bone to acidify to pH of 5 to 7. Then the extraction process for type B continues similarly to that of type A (Keenan, 2008). Lau *et al.* (2000) used type B gelatin from bovine in the study on texture profile and turbidity of gellan/gelatin mixed gel.

2.3.2 Forms of Gelatin

There are two forms of gelatin present in the market: powder and leave as shown in Figure 2.4. Powder is more difficult to use, but very common in the industrial because it is easier to manage during the process and lower in the cost. Many researchers used gelatin in powder form to run their experiments. Muñoz *et al.* (1986a) used flavored gelatin powder from Kool-Aid™ in their experiment to study the effect of gelatin concentration on gel texture. Holm *et al.* (2009) also used gelatin, powder form, extracted from porcine skin to study the sweetness and texture perceptions in sugar rich gelatin gels.

Gelatin leaves are very easy to use and very common in household cooking and pastry making. However, the cost is a lot higher when comparing to powder form, and not commonly used in the food industry.



Figure 2.4 Forms of gelatin: gelatin leaves and gelatin powder (Meppem, 2011)

2.4 Factors Affecting Gel Formation

2.4.1 Types and Amount of Sugar

Gelatin gel may form in the absence of sugar, however it has been reported that the addition of sugar increased the gel strength (Holm *et al.*, 2009). In gelatin gel, sugars stabilize gel network and improve texture. The addition of sugar to gelatin gel system reduces the haziness, enhance thermal stability and support gel structure (Holm *et al.*, 2009; Kasapis *et al.*, 2003). Kasapis *et al.* (2003) studied gelatin vs polysaccharide in mixture with sugar. They found that the addition of sucrose, fructose, glucose or glucose syrup up to 40% created stronger, and more thermally stable structures for both gelatin and polysaccharide network than with lower level of sugar content. However, at the intermediate levels of co-solute the polysaccharide gels collapsed, but gelatin gels formed reinforced networks. For most gelatin products, sucrose is used as sweetener and is often used in combination with glucose syrup. This is because glucose syrup is used to prevent sucrose crystallization, and due to its high dissolved solid content, it helps preventing microbial growth by lowering water activities (Burey *et al.*, 2009). Therefore, no preservative is needed in high sugar content gelatin product.

2.4.2 Temperature

One of the most important properties of gelatin is the ability to form thermally reversible gels. Because of this property, gelatin has been widely utilized in the food industry. The transformation of protein from the “sol” state to a “gel-like” state is facilitated by heat under an appreciate condition (Fennema, 1996). Gelatin gel is prepared by heating and it is gradually weakened at the temperature above 40°C (GMIA, 2012). Heat induced gel formation is a two-stage process. The first stage is denaturation of native protein. At this point polymerization of protein occurred to some degree. The second stage is the formation of protein network resulted from protein-protein and protein-solvent interaction, which gives a three-dimensional protein network which forms the gels (Zayas, 1997).

Many studies related to the effect of temperature on gelatin have been published. Osorio *et al.* (2007) studied the effect of concentration, bloom degree, and pH on gelatin melting and gelling temperatures using small amplitude oscillatory rheology. Results from their study showed that melting and gelling temperature values increased with increasing bloom degrees and pH values. Both melting and gelling temperatures increased with increasing gelatin concentrations. Fonkwe *et al.* (2003) studied the effect of gelatin temperature on storage modulus during gelation of gelatin dessert, Strawberry Jell-O[®]. Results from their study indicated that gelatin gel samples that gelled at temperature higher than 8 °C had more vicious characteristics than gelatin gel samples gelled at 0 and 4 °C. When gelatin gel settled at a higher temperature, a few collagen-like junctions were formed and the remainders of each chain would be disordered which weak gels were generated (Ledward, 1986).

2.4.3 Gelatin Concentration

The strength of gelatin increased with increasing concentration of gelatin. Lau *et al.* (2000) reported that for gellan/gelatin mixed gel, the gel strength of gelatin gel depended on the gelatin concentration and the results were in agreement with previous studies that forces increased with increasing concentration in gelatin gels (A. M. Muñoz *et al.*, 1986a). Holm *et al.* (2009) studied on the sweetness and texture perception in structure gelatin gels with embedded sugar rich domain at different total gelatin concentration. Results showed that samples with lower total gelatin concentration perceived as softer when comparing to samples with higher total gelatin concentration. The addition of gelatin to other product like corn milk yogurt showed to have an effect on gel strength as well (Supavitpatana *et al.*, 2008). They reported that as the percentage of gelatin concentration increased gel strength of corn milk yogurt also increased. The increase in gelatin concentration resulted in greater gel strength because the higher gelatin concentrations gave more intense intermolecular contacts, stronger protein-protein and protein dissolvent interactions with greater number of bonds created (Osorio *et al.*, 2007; Zayas, 1997).

2.5 Properties of Gelatin Gel

2.5.1 Textural Properties

2.5.1.1 Instrumental Texture Profile Analysis

Instrumental Texture Profile Analysis has been used for many decades to measure the textural properties of food. Many experiments have been conducted using TPA to access the mechanical properties of foods such as date flesh (M. S. Rahman & Al-Farsi, 2005), candies (Figiel & Tajner-Czopek, 2006), sunflower-sesame kernel snack (Gupta *et al.*, 2007), and meat products (Martinez *et al.*, 2004). The parameters

received from the TPA force have been well correlated with the sensory evaluation of textural parameters (Bourne, 2002).

Muñoz *et al.* (1986b) compared and differentiated mechanical properties (hardness, springiness, and cohesiveness) of different gels formulated with gelatin, sodium-alginate, and κ -carrageenan. They reported that gelatin was the firmest and most elastic among the three gels.

The sensorial definitions of the four chosen characteristics from textural profile analysis are shown in Table 2.2.

Table 2.2 Textural parameters obtained from TPA and sensorial definitions

Parameters	Sensorial Definition
Hardness	Force required to compress the gelatin samples between plates. Hardness is the point of deepest compression.
Springiness	Rate at which a deformed gelatin gel spring back to its undeformed condition after the force is removed
Cohesiveness	The strength of internal bonds making up the body of the gelatin gels.
Gumminess	Only applies to semi-solid product like gelatin gel. It is the energy required to disintegrating gelatin gel to a state ready for swallowing.

Source: Brookfield Texture Analyzer (2014)

2.5.1.2 Puncture Test

According to the standard measurement, gel strength is used for the assessment of the grade and quality of gelatin gel (GMIA, 2012). Rupture test is another form of hardness test. Bloom value and strength of gelatin gel can be obtained from rupture test. Rupture test is a destructive test designed to measure the critical point of physical failure of the sample, which can be used to classify the quality of gelatin gel sample (Chaing, 2013). Muñoz *et al.* (1986a) applied puncture test to gelatin gel samples with different gelatin concentrations of 22, 24, 28, 35, and 45 g/L. The rupture forces of the gelatin samples were compared between oral testing (biting gelatin gel with front teeth) and manual testing (pressing the gelatin gel with index fingers) to mechanical measurements. The results showed that the maximum force increased with increasing concentration of gelatin. The correlation coefficients between oral and manual measurement, and mechanical measurement were well correlated.

However, texture is not only an important attribution to the gelatin gel products; color is very important to consumer acceptance of gelatin gel products.

2.5.2 Optical Properties

2.5.2.1 Color of Gelatin

Color of gelatin does not have any influence on functional properties of gelatin. The color of gelatin depends upon the type of raw material used and the extraction method. Gelatin produced from pig skin usually has lighter color than that extracted from bones because final extraction of bones contains large amount of pigmented materials than gelatin extracted from skin (Panda, 2011).

2.5.2.2 Turbidity of Gelatin

There are many factors that may cause turbidity in gelatin. Extraction process is an important factor that affects the turbidity of gelatin. Kittiphattanabawon *et al.* (2010) determined the turbidity of gelatin extracted from skin of blacktip shark, at different temperatures (45, 60, and 75 °C) and times (6 and 12 h). As the time and temperature of the extraction increased, the turbidity of gelatin also increased. This was because when protein was treated at high temperature for a long time the aggregation was induced, resulted in the increase in turbidity (Johnson & Zabik, 1981). Kittiphattanabawon *et al.* (2010) reported that the coagulum could be formed in blacktip shark gelatin solution; which could interfere with the development of ordered gel network, resulted in the formation of turbid gel.

The mixture of different types of gelatin may result in the gel turbidity depending on the ratios of the mixture and the exact pH value between 5 to 7 because the formation of gelatin coacervates between the oppositely charged gelatin micelles (Panda, 2011). Lau *et al.* (2000) studied the turbidity of gellan/gelatin mixed gels. The results showed that the increasing of gelatin concentration in the mixture increased gel turbidity because of the gellan-gelatin coacervate (Chilvers & Morris, 1987) and the incompatibility between protein and polysaccharide (Lau *et al.*, 2000).

2.5.2.3 Absorption and Scattering of Translucent Media

Absorption and scattering contain distinct information on the medium. Absorption is determined by the pigments and constitutes of the pulp that produce characteristic spectral features in the visible spectrum region. Conversely, scattering is due to the local variation of the dielectric constant inside the medium (Cubeddu *et al.*, 2003).

Translucent products have very unique characteristics. The pattern of the translucent color is characterized by selective absorption and at the same time as scattering. The scattering of translucent media depends upon the chemical structure of the surroundings, refractive index, and the structure of boundary surface (Klein, 2010). Negueruela and Perez-Arquillue (2000) distinguished two different colors of rosemary honey by using the absorption and scattering spectral, which in traditional method like CIELAB was not able to distinguish because of the translucent characteristic of honey made it difficult to see their true color. They mentioned that the differences in color of rosemary honey were due to light scattering in the interior of the sample, which is responsible for the small color differences observed in the reflection of rosemary honeys.

2.6 CIELAB

2.6.1 Introduction

Color attribute is the visual organization of color. Color attributes are chroma, hue, and lightness. Lightness shows how dark and light of color is. The lightest is white and the darkest is black. Hue is the actual color pigment such as red, pink and yellow. Lastly, chroma is the intensity of the color. Color space (Figure 2.5) shows lightness, hue, and chroma in a more uniform color difference in relation to human's perception (Ladanyia, 2010).

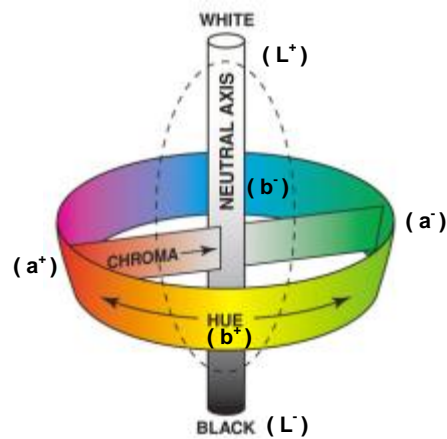


Figure 2.5 Color space (Heuer, 2000)

CIELAB is a popular color scale in use today and it is commonly used in the food industry. Results from CIELAB are presented in numerical values in color space that based on cube root transformation of color data as L^* , a^* , b^* (Kriss & Green, 2010). L^* represents lightness, a^* represents chromaticity on (-) green and (+) red; b^* represents chromaticity on (-) blue to (+) yellow. CIELAB is over expanded in the yellow. The numerical value from the CIE $L^*a^*b^*$ is very good for monitoring color trend during the processing because it gives numerical system like L25 and L26. However, when numerical CIE $L^*a^*b^*$ are translated into numerical CIE $L^*C^*h^*$, it becomes very useful in sensory analysis. This is because the CIE $L^*C^*h^*$ describes color the same way as human verbally communicates. It gives identifications to the color appearance like yellow, blue, and green (Ruiz *et al.*, 2007).

2.6.2 Color Difference

Color difference is very important in the food industry. It uses to indicate how different the color of the sample is from the standard color. The Eq. (2.1) shows the equation to calculate the color difference used in the food industry known as ΔE_{ab}^* 1976.

$$\Delta E_{ab}^* = [(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2]^{1/2} \quad (2.1)$$

For the CIE $L^*a^*b^*$, color difference can be calculated using the numerical value received from the sample subtract the numerical value of standard. If the ΔL^* is positive then the sample is lighter than the standard. If negative, it would be darker. If the Δa^* is positive then the sample is more red than the standard, and if negative then it would be more green. Lastly, if Δb^* is positive then the sample is more yellow than the standard and if negative it would be more blue. CIE ΔE_{ab}^* in the above equation shows that the total color difference is based on the $L^*a^*b^*$ color differences and was intended to be a single number measurement for pass/fail decision for the color of food products (HunterLAB, 2001). The value of “1” is normally the smallest different that human eyes can see, only if the two colors are next to each other for any material. Figure 2.6 is an example of calculated ΔE^* for different shade of green paint. Color different shows “how far apart” are the two colors and color tolerance shows the meaning of “how far apart” number (Upton, 2013).



Figure 2.6 An example of calculated ΔE^* for different shades of green paint (Monovector, 2011)

2.7 The Kubelka-Munk Theory

2.7.1 Introduction

In 1931, P. Kubelka and F. Munk came up with the Kubelka-Munk analysis for the prediction of the optical properties of any given materials (Winkler, 2003). It is a mathematical color model that used to describe the reflectance of opaque samples, Eq. (2.2).

$$K/S = \frac{(1 - R_\infty)^2}{2R_\infty} \quad (2.2)$$

$$R_\infty = a - b \quad (2.3)$$

$$a = 1/2 \left(R + \frac{R_0 - R + R_g}{R_0 R_g} \right) \quad (2.4)$$

$$b = (a^2 - 1)^{1/2} \quad (2.5)$$

In Eq. (2.2), R_∞ is the reflectance of an infinitely thick layer of material that can be determined through Eqs. (2.3) - (2.5) as for R is reflectance of sample against white background, R_0 is reflectance of sample against black background, and R_g is reflectance of white background. There are four factors that the reflectance of the sample at each wavelength depends on: an absorption spectrum, K ; a scattering spectrum, S ; the sample thickness, X ; and the reflectance spectrum of the substrate or backing, R .

This theory describes both light absorption and light scattering in colored samples using the measurement of reflectance. It is applied on a wavelength-by-wavelength basis throughout the visible region of the spectrum (Hoffman, 1998). Light can be scattered in any direction, the theory considers two net fluxes: upwards, j , and downwards, i , as shown in Figure 2.7.

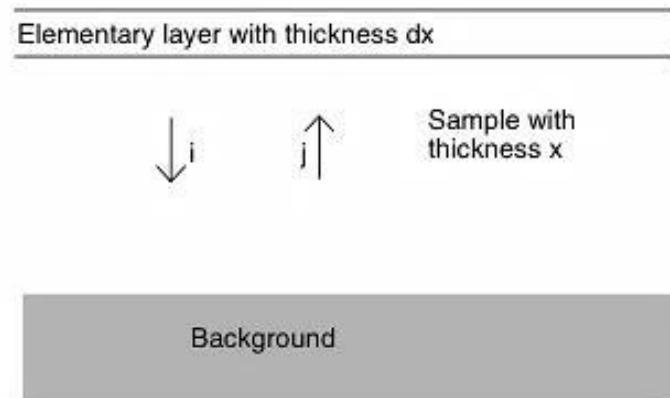


Figure 2.7 Light path of Kubelka-Munk model (Meléndez-Martínez *et al.*, 2011)

This theory has an extensive use in the industry of paint, ink, and textile. In food science, the Kubelka-Munk analysis has been applied in the study of optical properties of a wide range of different products such as translucency of tomatoes (Lana *et al.*, 2006), change in optical properties of osmodehydrofrozen of kiwi fruits (Talens *et al.*, 2002), and color of virgin olive oil in relationship to sample thickness of 5.0, 10.0, and 50.0 mm path length cells (Gómez-Robledo *et al.*, 2008).

Lana *et al.* (2006) used the Kubelka-Munk theory to measure the development of translucency in tomatoes from green to red. Results from the experiment showed that the changes in K/S ratios from the Kubelka-Munk analysis of tomatoes were well correlated with the translucency observed visually. Talens *et al.* (2002) also used the application of Kubelka-Munk theory to study the translucency of osmodehydrofrozen kiwi fruits. Results from vacuum pulse showed negative effects because color of kiwi fruits was darker and less vivid, and a high increase in translucency from K/S curve was observed. Unfortunately for Gómez-Robledo *et al.* (2008) the Kubelka-Munk theory did not provide a reliable result when applied to their experiment for color

predictions. The authors mentioned that the use of two-flux Kubelka-Munk theory applied in the experiment was inappropriate for the transparency of the oil samples.

2.7.2 Modified Kubelka-Munk Theory Applied in Food

Most research in food used the classic Kubelka-Munk model (Calvo & Salvador, 1997; Lana *et al.*, 2006; Negueruela & Perez-Arquillue, 2000; Talens *et al.*, 2002). There are many limitations to the application of Kubelka-Munk theory. The Kubelka-Munk theory does not take the partial reflection of light at the air to layer interface as the light enters and leaves the layer. Nobbs (1985 and 1997) defined this problem and applied the correction to the measured reflectance before they are used in Kubelka-Munk equations. The effects of boundary reflections were proposed by Saunderson and now it is known as the Saunderson's correction, Eq. (2.6). Saunderson's correction is used to calculate the true reflectance, R , of the samples. Where ρ is the measured reflectance, r_e and r_i are fix variables, and t_e and t_i are determined through the Eqs. (2.7) - (2.8).

$$R = \frac{\rho - r_e}{t_e - t_i + r_i(\rho - r_e)} \quad (2.6)$$

$$t_e = 1 - r_e \quad (2.7)$$

$$t_i = 1 - r_i \quad (2.8)$$

Saunderson added the boundary reflection coefficients into the account. First coefficient, r_e , describes the fractional reflectance when light entering the sample is partially reflected at the air-sample interface. Similarly, the second coefficient, r_i , describes the fractional reflectance when the light exiting the sample is partially

reflected back into the sample at the sample-air interface (Klein, 2010) as shown in Figure 2.8.

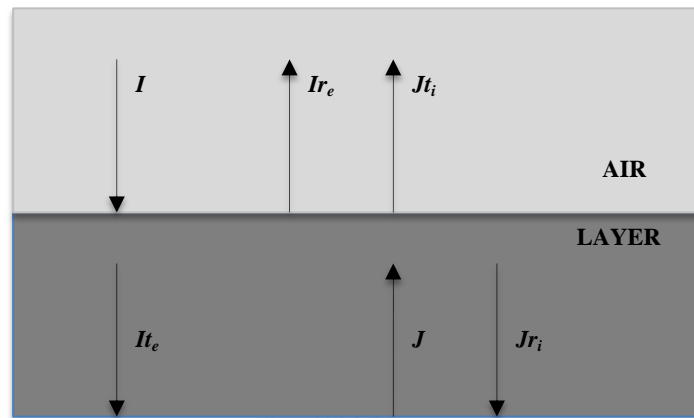


Figure 2.8 Partial reflection of the fluxes I and J at air to layer interface (Nobbs, 1997)

Generally, glass and many polymeric materials have refractive indices about 1.5, the correction coefficients when flux i is a collimated beam and flux j is a diffused beam, $r_e = 0.040$ and $r_i = 0.600$; r_i varies with thickness of the sample and with its opacity. The r_i is larger than r_e is due to the large fraction of diffuse flux j when reaches the air to layer boundary at the angel of incident greater than the critical angel. Then the light is completely reflected back in the layer. This effect only occurs in flux j . Figure 2.9 shows the relationship between measured reflectance and true reflectance. The partial reflection at the surface can have a substantial effect on measured reflectance; the effect for true reflectance is greatest at about 60%, which reduced the interface to the measure value of 40% (Nobbs, 1997).

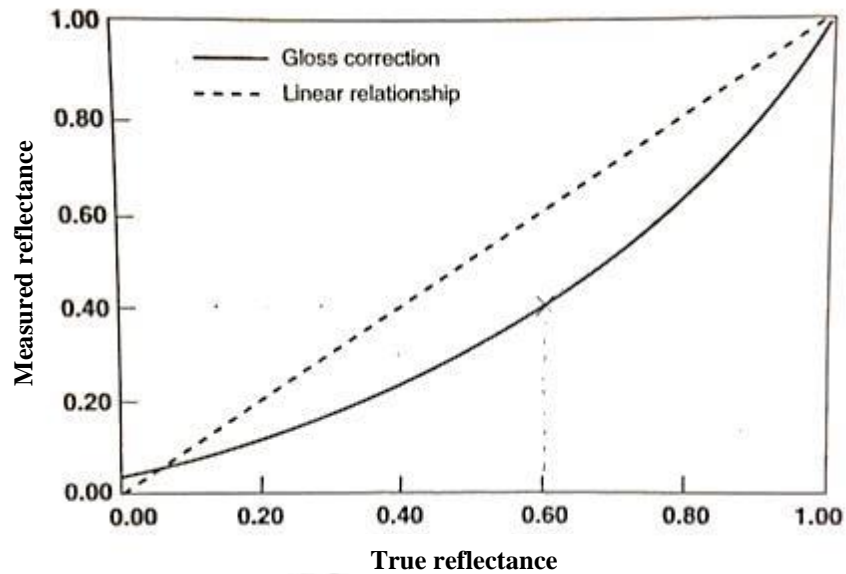


Figure 2.9 Relationship between measured and true reflectance for a coating of refractive index 1.5 (Nobbs, 1997)

For samples that are very translucent like gelatin gels, a correction has been made to the reflectance spectra that obtained from samples before the application for Kubelka-Munk theory may be required. Due to the greater complexity that added to the modification, the corrections by Saunderson using the true reflectance have not yet been applied in the food industry. This correction should be added because the Kubelka-Munk theory has many limitations, and with Saunderson's correction it would provide a more accurate result.

CHAPTER III

METHODOLOGY

3.1 Materials

All materials, except synthetic colorant, used in the experiment were purchased from local grocery stores in Bangkok, Thailand. Synthetic colorant was kindly provided by Brenntag Ingredients (Thailand) Public Company Limited.

3.1.1 Gelatin

- Gelatin leaves, a product of Gelita[®], Germany. (Appendix A.1)

3.1.2 Chemicals and Instruments

The following chemicals were used in this study:

- Refined sugar, Food grade (Mitr Phol Sugar Group, Bangkok, Thailand).
- Glucose syrup, Food grade (Charoenworrakit Co., Ltd., Samut Prakan, Thailand) (Appendix A.2).
- Synthetic colorants, Eurocent Ponceau 4R (E124). Food grade (Brenntag, Mülheim an der Ruhr, Germany) (Appendix A.3).

The following instruments were used in this study:

- Texture analyzer (Stable Micro System Model TA.XT2i, Godalming, UK)
- Spectrophotometer (X-Rite Model SP62, MI, USA)
- Balance (Mettler Toledo, Switzerland)

3.2 Methods

3.2.1 Gelatin Gel Preparation

Gelatin samples were prepared as follows: gelatin leaves were soaked in iced water (1 ± 1 °C) for 7 minutes. Gelatin leaves were taken out from the iced water bath,

excess water was squeezed out and soaked gelatin leaves were placed in a glass beaker. Gelatin leaves were shred into smaller pieces and carefully mixed with hot water, and/or glucose syrup and/or sucrose at the appropriate amount of the mixtures. The effect of gelatin concentrations (3 levels) and glucose syrup to sucrose ratios (5 levels) were studied (Table 3.1). Gelatin solutions prepared were poured into a 5 cm (L) x 5 cm (W) x 2.5 cm (H) silicone molding (Appendix A.4) through double layer filter cloth. The samples were then left to cool at room temperature (25 ± 1 °C). Once they began to set, they were placed in a refrigerator (4 ± 1 °C) to finish setting and held overnight before further analysis.

Table 3.1 Gelatin concentrations and glucose syrup to sucrose ratios used in the experiment

Gelatin Concentration (% w/w) (g gelatin/100 g of total)	Glucose Syrup to Sucrose Ratios (40 g sugar / 100 g of total)
7.75	0:0
	0:100
	30:70
	70:30
	100:0
8.00	0:0
	0:100
	30:70
	70:30
	100:0
8.25	0:0
	0:100
	30:70
	70:30
	100:0

3.2.2 Gelatin Gel Properties

Gelatin gel samples were taken out from the refrigerator, left to equilibrate to room temperature (25 ± 1 °C), and subjected to analysis.

3.2.2.1 Textural Properties

Textural properties of gelatin gels were evaluated through textural profile analysis and puncture test using Texture Analyzer (Stable Micro System Model TA-XT2i, Godalming, UK) interfaced with a computer program, The Exponent Lite Express.

3.2.2.1.1 Textural Profile Analysis

The gelatin gel samples were placed between parallel flat plate fixtures fitted to a texture analyzer interfaced with a computer. A deformation of 30% was applied to all samples using probe p/100, 100 mm diameter. Gelatin gel samples were compressed twice with pre-test speed of 1 mm/s, post-test speed of 1 mm/s with auto trigger force (Pons & Fiszman, 1996). Six samples from the batch were tested, three results within the same range were selected, and results were reported as the mean of triplicates.

A typical texture profile analysis graph is presented in Figure 3.1. Texture profile analysis parameters were calculated as follows: hardness is presented as the peak of the first compression in kg; springiness as the ratio of the time duration of force input during the second compression to the first compression in percent; cohesiveness as the ratio of the area of the positive force of the second compression to the first compress; and gumminess as the multiplication of hardness and cohesiveness in kg.

This experiment was done in triplicate. The completely randomized design (CRD) was used as the experimental design. Data were analyzed using analysis of variance (ANOVA). A Tukey's multiple range tests were used to determine the difference among sample means ($p = 0.05$).

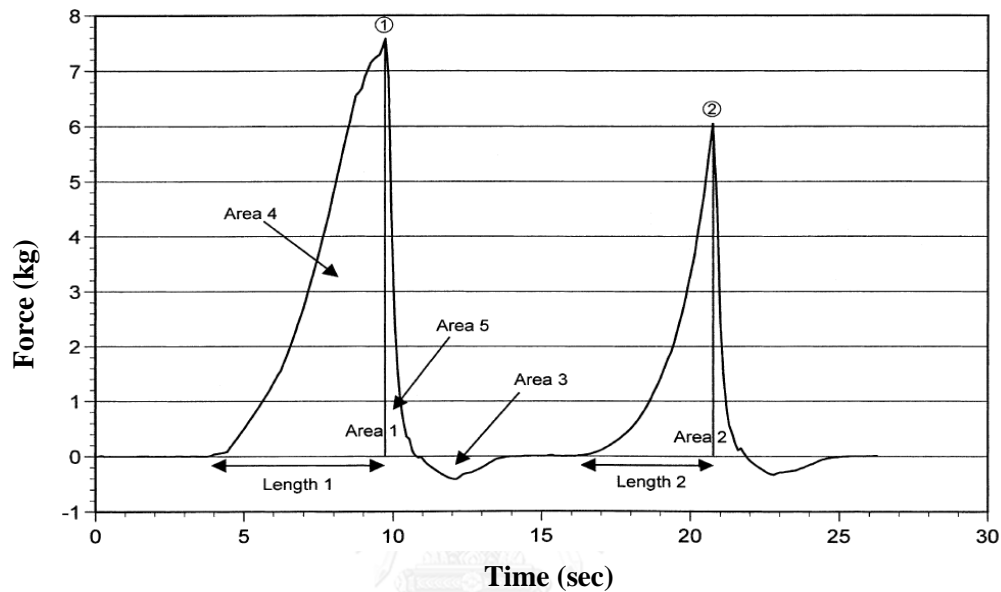


Figure 3.1 Typical graph of two cycles compression of the samples to determine texture profile analysis values (Caine *et al.*, 2003)

3.2.2.1.2 Puncture Test

Rupture force was determined by placing gelatin gel samples on the plate of texture analyzer centered with the probe p/0.05h, 5 mm in diameter. The samples were then subjected to the puncture test at 5 mm/sec to a depth of 18 mm with pre-test speed of 0.5 mm/s, post-test speed of 0.5 mm/s with auto trigger force of 5 gram (Muñoz *et al.*, 1986a; GMIA, 2012). A typical puncture force profile is shown in Figure 3.2. The first peak force in gram force presents the value of rupture force.

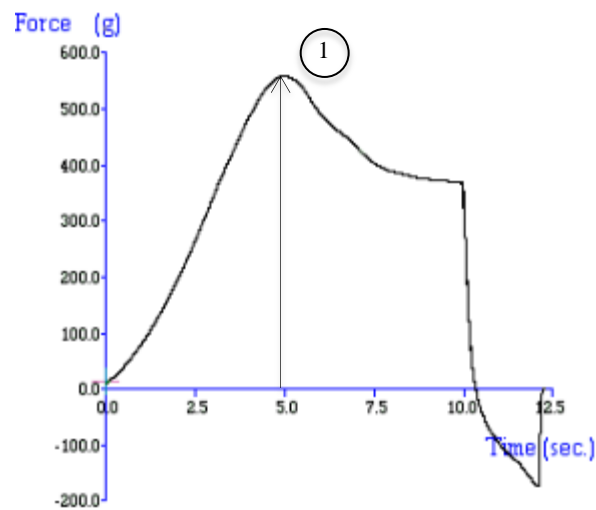


Figure 3.2 Typical graph of Bloom test for gelatin sample to determine rupture force (Stable Micro System Ltd., 2014)

The experiments were conducted in triplicate, and the average values of three replications were reported for which mean values and standard deviation were determined. A total of three replication were done.

The completely randomized design (CRD) was used as the experimental design. Data were analyzed using analysis of variance (ANOVA). A Tukey's multiple range tests were used to determine the difference among sample means ($p = 0.05$).

3.2.2.2 Optical Properties

3.2.2.2.1 Spectral Reflectance

Reflectance spectra and color measurements of gelatin gel samples prepared in Section 3.2.1 were studied using a spectrophotometer (X-Rite-SP62). Reflectance spectra were taken for all samples prepared against white and black backgrounds. Ceramic tiles were used as backgrounds. The reflectance of both white and black

background were measured (Appendix B.1). The reflectance measurements were taken in the visible light range from 400 to 700 nm at 10 nm intervals. Reflectance measured against white background were used for the study of the characteristics of gelatin gel samples and for the Kubelka-Munk analysis. However, the reflectance of samples measured against black background were used in Kubelka-Munk analysis only.

Gelatin gel sample is not completely opaque, so the infinite reflectance (R_{∞}) cannot be simple determined from the measured reflectance of sample but from the measured reflectance over white and over black backgrounds.

3.2.2.2.2 CIELAB

Using spectrophotometer, color of gelatin gel samples was also expressed in terms of CIE L^* , a^* , b^* , C^* with illuminant/observer: D65/10° for both black ($L^*=0.27$, $a^*=0.88$, $b^*=-1.01$, and $C^*=1.34$) and white backgrounds ($L^*=82.09$, $a^*=-0.44$, $b^*=0.28$, $C^*=0.52$). Where L^* represents lightness, a^* represents chromaticity on (-) green and (+) red; b^* represents chromaticity on a (-) blue to (+) yellow; and C^* is the intensity of color. Hue angle was calculated using Eq. (3.1). Hue angle expresses in degrees from 0°-360°, where 0° (red) located on $+a^*$, 90° (yellow) on $+b^*$, 180° (green) on $-a^*$, 270° (blue) on $-b^*$, and 360° = 0° (Duangmal *et al.*, 2008).

$$h_{ab} = \tan^{-1}(b^*/a^*) \quad (3.1)$$

The CIE total color difference (ΔE_{ab}^*) against white background was also calculated using Eq. (3.2).

$$\Delta E_{ab}^* = \sqrt{(L^* - L_0^*) + (a^* - a_0^*) + (b^* + b_0^*)} \quad (3.2)$$

In this analysis, three gelatin gel samples were measured; each sample was measured three times at the same point against each background, and then averaged. A total of three replications were done.

3.2.3 Application of Kubelka-Munk Theory

Four gelatin gel samples were selected based on their textural properties (Section 3.2.2.1). Reflectance obtained from white background and black background of these samples measured in Section 3.2.2.2.1 were subjected to Kubelka-Munk analysis.

3.2.3.1 The Kubelka-Munk Theory

The Kubelka-Munk theory that is commonly used in food industry (Lana *et al.*, 2006) was applied to the reflectance of four selected gelatin gel samples to obtain K/S ratio using Eq. (3.3).

$$K/S = \frac{(1 - R_\infty)^2}{2R_\infty} \quad (3.3)$$

Where R_∞ is the reflectance of an infinitely thick layer of material that can be determined through Eqs. (2.3)-(2.5).

3.2.3.2 Saunderson's Correction

The reflectance of four selected gelatin gel samples obtained from Section 3.2.2.2.1 were modified using the Saunderson's correction (Nobbs, 1997), Eq. (3.4), to obtain the true reflectance (R).

$$R = \frac{\rho - r_e}{t_e - t_i + r_i(\rho - r_e)} \quad (3.4)$$

Where ρ is the measured reflectance, r_e and r_i are fix variables, and t_e and t_i are determined through the Eqs. (2.7) - (2.8).

3.2.3.3 Modified Kubelka-Munk Theory

The modification of Kubelka-Munk theory for semitransparent products was applied for the characteristic of gelatin gel samples. When all the true reflectance spectra were calculated, the modified Kubelka-Munk theory (Nobbs, 1997) was applied to calculate the opaque reflectance (R_∞), absorbance coefficient (K), scattering coefficient (S), using equations Eq. (3.5) – (3.7)

$$K = \frac{Z}{D} \left(\frac{1 - R_\infty}{1 + R_\infty} \right) \quad (3.5)$$

$$S = \frac{Z}{D} \left(\frac{2R_\infty}{1 - R_\infty^2} \right) \quad (3.6)$$

$$Z = \frac{1}{2} \ln(\beta - 1) \quad (3.7)$$

The equations above include R_∞ , which can be determined from the true reflectance of sample over white and black background. R_w and $R_{g,w}$ are true reflectance of the sample over white background and of white background,

respectively. R_b and $R_{g,b}$ are the true reflectance of the sample over black background and of black background, respectively. The value of β is a function of K , S , and D via variable Z over black and white background, and can be obtained from Eq. (3.8).

$$\beta = \left(\frac{R_{g,w} - R_w}{R_w - R_\infty} \right) \left(\frac{1 - R_\infty^2}{1 - R_{g,w}R_\infty} \right) \quad (3.8)$$

$$\beta = \left(\frac{R_{g,b} - R_b}{R_b - R_\infty} \right) \left(\frac{1 - R_\infty^2}{1 - R_{g,b}R_\infty} \right)$$

Rearranging Eq. (3.8) produces a quadratic equation for R_∞ , Eq. (3.9).

$$R_\infty = B - (B^2 - 1)^{1/2} \quad (3.9)$$

where B is given by Eq. (3.10)

$$B = \frac{(1 + R_b R_w)(R_{g,w} - R_{g,b}) - (1 + R_{g,b} R_{g,w})(R_w R_b)}{2(R_b R_{g,w} - R_{g,b} R_w)} \quad (3.10)$$

The K/S can be calculated from Eq. (3.11)

$$K/S = B - 1 \quad (3.11)$$

For the application of Kubelka-Munk theory, data were presented in graphic format of spectral distributions of K/S coefficients.

3.2.4 Model Fitting Based on Kubelka-Munk Theory

For model-fitting based on Kubelka-Munk theory, one sample out of the four selected samples from Section 3.2.3 was randomly selected (7.75% at 70:30) and subjected to varying colorant concentrations using database system. Database system is the preparing of the sample with test colorant at a range of relative concentrations (Nobbs, 1997).

Gelatin gel sample with the selected condition was prepared with eight different levels of colorant concentrations varying from 0.0001% - 0.01% (w/w) shown in Table 3.2. The colorant used for this experiment was Eurocent Ponceau 4R.

Table 3.2 Colorant concentrations used in database system in the experiment

Treatment	Added Color (%)	Colorant Concentrations (% w/w) g colorant/ 100 g of total
1	1%	0.0001
2	3%	0.0003
3	6%	0.0006
4	12%	0.0012
5	25%	0.0025
6	50%	0.0050
7	75%	0.0075
8	100%	0.0100

The spectral reflectance, 400-700 nm, of all colored gelatin gel samples were measured over white background and back background. The opaque reflectance (R_{∞}) then, were calculated according to Figure 3.3. The absorption (K) and scattering (S) coefficients were determined according to the steps shown in Figure 3.4.

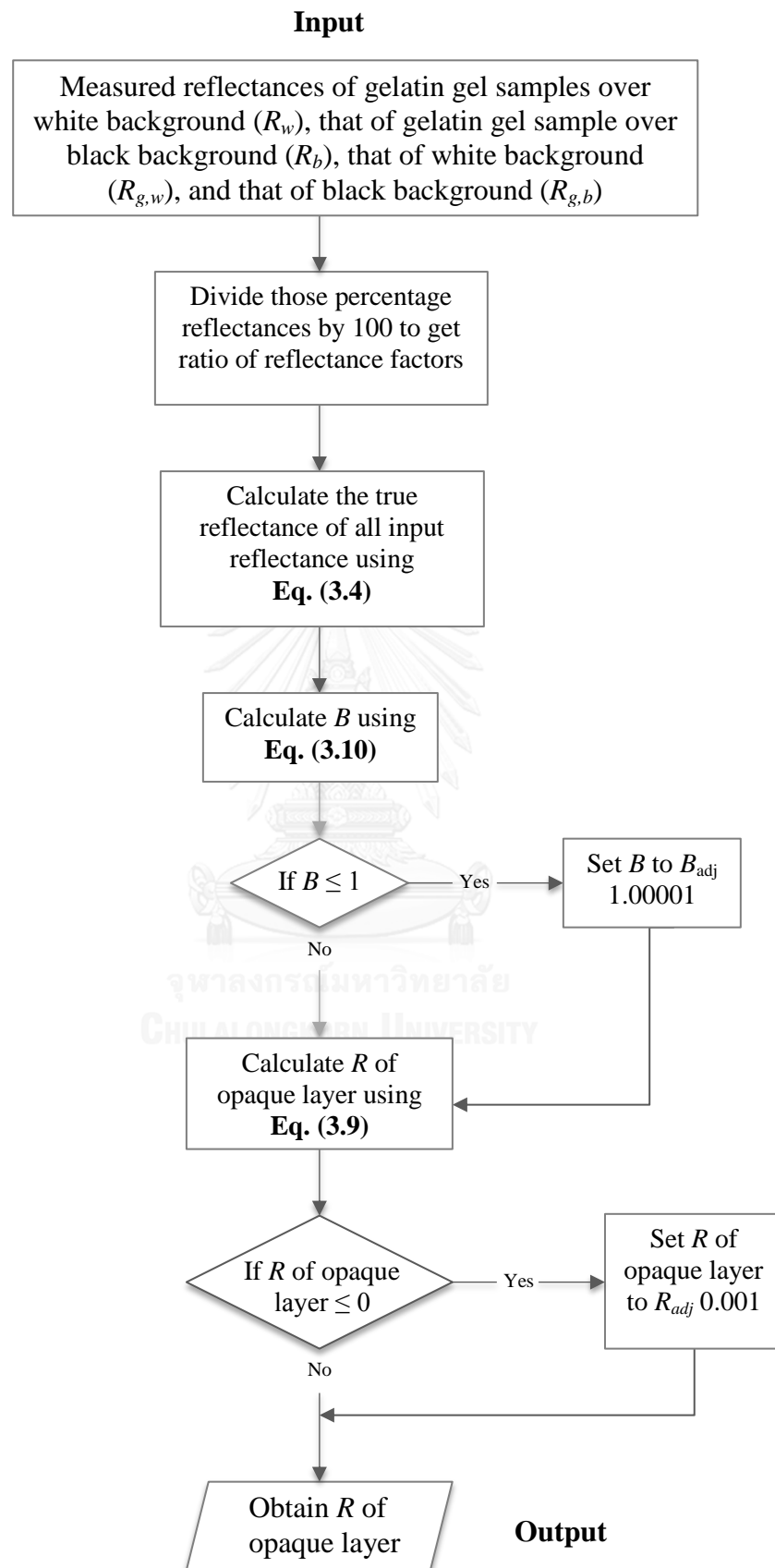


Figure 3.3 Determination of opaque reflectance flowchart

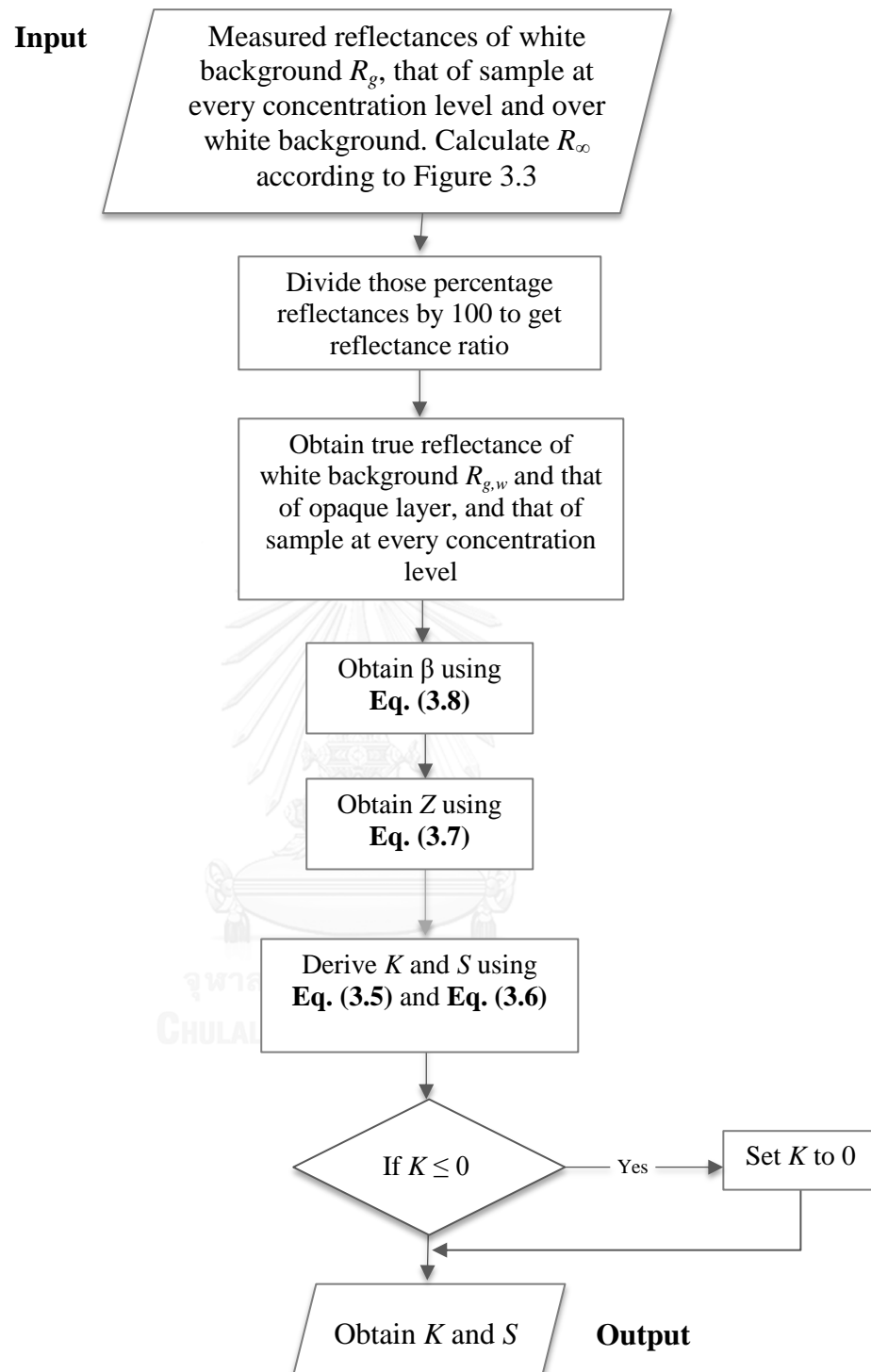


Figure 3.4 Determination of absorption and scattering coefficients flowchart

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Gelatin Gel Properties

4.1.1 Textural Properties

Texture is an important factor that describes quality and characteristic of gelatin gel. In this study texture of gelatin gel samples with different concentrations of gelatin (GC) and different ratios of glucose syrup to sucrose (GSR) were determined using two methods: (1) texture profile analysis (Supavitpatana *et al.*) evaluated using compressing plate, (2) puncture force evaluated using round tip puncture probe. In this study, TPA was presented in term of hardness, cohesiveness, springiness, and gumminess, while puncture test was presented as rupture force. The results of TPA and puncture test for gelatin gel samples with different GC and different GSR were discussed.

4.1.1.1 Textural Profile Analysis

The effect of gelatin concentrations and glucose syrup to sucrose ratios on hardness, springiness, cohesiveness, and gumminess of gelatin gel samples are shown in Table 4.1 and analysis of variance are shown in C.1-C.4.

The effects of gelatin concentrations and glucose syrup to sucrose ratios on four textural parameters were obtained from TPA. Hardness is the strength of gelatin gel structure under compression. As gelatin concentration increased, the hardness of gelatin gel samples also increased. An increase in gelatin concentrations resulted in greater gel strength because the higher gelatin concentrations gave more intense intermolecular contacts and stronger protein-protein interaction (Zayas, 1997). Lau *et*

al. (2000) reported that in gellan/gelatin mixed gel, the gel strength of gelatin gel was dependent on gelatin concentration. These results were in agreement with previous studies showing that the gel force increased with increasing concentration of gelatin in gelatin gels (A. M. Muñoz *et al.*, 1986a).

The addition of sugars increased the hardness of gelatin gel samples compared to the samples with no sugar as shown in Table 4.1 ($p \leq 0.05$). Regardless of gelatin concentrations, the increasing of sucrose in the GSR increased both hardness and gumminess. This was probably due to the competition of the sugars for available water in the system creating stronger protein-protein interaction (Fennema, 1996). The increasing of glucose syrup in the GSR decreased both hardness and gumminess for all gelatin concentrations. In this experiment, glucose syrup contained 84.6° Brix (Appendix A.2), so the increase in glucose syrup also increased the amount of available water. This might result in the decrease in hardness and gumminess as glucose syrup ratio increased. The maximum hardness strength was found at GC of 8.25% with GSR of 70:30 ($p \leq 0.05$). At high concentration of sugars, sugars excessively compete with gelatin for water. Gelatin needs water for swelling, and unfolding. Sucrose also acts as plasticizer agent between gelatin molecules. Therefore, at higher concentration of sugars, the viscosity of gelatin sols decreases. Sugars stabilize protein by increasing the rigidity and strengthening protein-to-protein interaction in food protein gel (Semenova *et al.*, 2002). Sucrose helps gelatin dissolution and the combination of sucrose and glucose syrup establishes a continuous phase in gelatin, which strengthens gelatin gel (Burey *et al.*, 2009). The addition of sugar has been reported to increase the gel strength in gellan gum gels (Holm *et al.*,

2009), gelatin gels (Kasapis *et al.*, 2003), and corn starch gel (Sun *et al.*, 2014), which concurrent to our results.

At all levels of gelatin concentrations and sugar ratios used, a small difference in value of springiness, and cohesiveness was observed (Table 4.1). Springiness is the ratio of the time duration of force input during the second compression to first compression. The signature of gelatin gel is high springiness and high resilience (Grazela & Morrison, 2003). The obtained results on springiness showed that gelatin gels had high springiness, with values ranging from 0.95 to 0.99. When springiness was high, it requires more chewing energy in the mouth (M. Rahman & Al-Mahrouqi, 2009). These results agreed well with previous work done by Sun *et al.* (2014) on corn starch gels where all springiness values of glucose, fructose, and maltose syrups at the concentration of 0%, 1%, 3%, 13% and 25% were not different. They reported springiness ratio values for glucose syrup ranged from 0.97 to 1.00, and for fructose syrup and maltose syrup ranged from 0.95 to 0.99.

Table 4.1 Effect of gelatin concentrations and glucose syrup to sucrose ratios on textural profile analysis of gelatin gel samples

Gelatin Concentration (% w/w)	Glucose Syrup to Sucrose Ratios	Textural Profile Analysis			
		Hardness (gf)	Springiness	Cohesiveness	Gumminess (gf)
7.75	0:0	2742 ^a ± 280	0.96 ^{abcd} ± 0.01	0.94 ^a ± 0.00	2570 ^a ± 270
	0:100	6009 ^{ef} ± 380	0.95 ^a ± 0.01	1.00 ^d ± 0.01	5982 ^c ± 370
	30:70	5729 ^{cdef} ± 83	0.99 ^{cd} ± 0.01	0.98 ^{cd} ± 0.01	5619 ^{de} ± 80
	70:30	5166 ^{bcd} ± 31	0.97 ^{abcd} ± 0.01	0.98 ^{cd} ± 0.00	5040 ^{bcd} ± 30
8.00	100:0	4748 ^b ± 220	0.97 ^{abcd} ± 0.00	0.95 ^{abc} ± 0.01	4507 ^b ± 200
	0:0	3091 ^a ± 210	0.96 ^{abc} ± 0.01	0.93 ^a ± 0.01	2884 ^a ± 220
	0:100	6112 ^f ± 560	0.95 ^{ab} ± 0.02	0.98 ^{cd} ± 0.01	5909 ^{de} ± 690
	30:70	5973 ^{ef} ± 130	0.99 ^{cd} ± 0.01	0.98 ^{bcd} ± 0.01	5847 ^{de} ± 140
8.25	70:30	5825 ^{def} ± 460	0.98 ^{bcd} ± 0.01	0.95 ^{abc} ± 0.03	5446 ^{cde} ± 330
	100:0	4828 ^{bc} ± 18	0.98 ^{cd} ± 0.00	0.95 ^{abc} ± 0.01	4597 ^{bc} ± 20
	0:0	3264 ^a ± 200	0.96 ^{abc} ± 0.02	0.94 ^{ab} ± 0.02	3080 ^a ± 200
	0:100	7876 ^{gh} ± 430	0.97 ^{abcd} ± 0.00	1.01 ^d ± 0.01	7968 ^{fg} ± 480
8.25	30:70	7102 ^g ± 340	0.98 ^{cd} ± 0.00	1.00 ^d ± 0.01	7069 ^f ± 380
	70:30	8644 ^h ± 370	0.99 ^{cd} ± 0.00	0.93 ^a ± 0.01	8052 ^g ± 240
	100:0	4953 ^{abcd} ± 210	0.99 ^d ± 0.00	0.95 ^{abc} ± 0.01	4696 ^{bc} ± 150

Values are mean ± standard deviation of triplicate sample determinations

Means in the same column with different superscript letters are significantly different ($p \leq 0.05$)

All determinations were performed in triplicate

Cohesiveness is the measure of the resiliency of a product. It is the ratio of the area of the positive force of the second compression to the first compression. Obtained results on cohesiveness ranged from 0.93 to 1.01 (Table 4.1), which were similar to a study done by Muñoz *et al.* (1986a) on measurement of texture of gelatin gels at different gelatin concentrations that ranged from 0.91 to 0.98. Results also agreed with (Grazela & Morrison, 2003), who reported in their study of gelatin-free gummy confectionary using gellan gum and carrageenan, that typical gelatin gel showed cohesiveness and springiness in the 0.90 range ratio.

Gumminess is the multiplication of hardness and cohesiveness and it is the energy required to break down product into a ready to swallow state. Because of this correlation, the value of gumminess increased in the same trend as the hardness did. Since the springiness value was about 1, the value of gumminess was close to the hardness. The addition of sugars increased ($p \leq 0.05$) the gumminess compared to the samples with no sugar (Table 4.1). Among the different concentrations of gelatin gels at the same GSR (70:30) used, the highest gumminess observed was at 8.25% ($p \leq 0.05$).

4.1.1.2 Puncture Test

The effect of gelatin concentrations and glucose syrup to sucrose ratios on rupture force and rupture distance are shown in Table 4.2 and analysis of variance are shown in C.5-C.6.

Gel strength is an important measurement used to assess the grade and quality of gelatin gel (GMIA, 2012). Puncture test is another form of hardness test. For control samples, as the gelatin concentration increased the rupture force also increased ($p \leq 0.05$), as shown in Table 4.2. More intense intermolecular contacts and stronger

protein-proteins interaction were formed from an increase in gelatin concentration (Zayas, 1997), which resulted in an increase in the rupture force. Muñoz *et al.* (1986a) applied puncture test to gelatin gel samples with different gelatin concentrations of 22, 24, 28, 35, or 45 g/L and compared between oral and manual measurements to mechanical measurements in their study. The results showed that maximum forces increased with increasing concentration of gelatin, and the values from oral and manual measurement correlated well with the values mechanical measurement determined using correlation coefficient to calculate their relevance.

The depth at which ruptures were done on gelatin gel samples are shown in Table 4.2. As the depth of the puncture test increased, the rupture force also increased. For all levels of gelatin concentrations, highest rupture forces were found at GSR of 0:100 ($p \leq 0.05$), which paralleled to the depth of the puncture test. At the same gelatin concentration, the longest distance of rupture point was found at GSR of 0:100. The addition of sugars into gelatin gel samples increased rupture forces ($p \leq 0.05$), which showed a similar trend as results from hardness and gumminess in Table 4.1. Semenova *et al.* (2002) mentioned the addition of sugars in food protein gel increased the rigidity and stabilized the conformation by strengthening protein-to-protein interaction.

Table 4.2 Effect of gelatin concentrations and glucose syrup to sucrose ratios on rupture force of gelatin gel samples

Gelatin Concentration (% w/w)	Glucose Syrup to Sucrose Ratios	Puncture Test	
		Rupture Force (g)	Distance (mm)
7.75%	0:0	850.0 ^a ± 41	12.92 ^a ± 0.66
	0:100	2294 ^{ef} ± 140	16.96 ^c ± 0.57
	30:70	1844 ^d ± 9.0	16.31 ^{bc} ± 0.79
	70:30	1497 ^c ± 91	16.01 ^c ± 0.34
	100:0	1474 ^c ± 82	15.47 ^{bc} ± 1.4
8.00%	0:0	954.0 ^{ab} ± 50	12.78 ^a ± 0.11
	0:100	2717 ^g ± 32	16.99 ^c ± 0.35
	30:70	1889 ^d ± 39	16.93 ^c ± 0.63
	70:30	1649 ^c ± 26	16.14 ^c ± 1.82
	100:0	1898 ^d ± 31	16.40 ^c ± 0.31
8.25%	0:0	1099 ^b ± 75	13.52 ^{ab} ± 0.78
	0:100	2960 ^h ± 62	17.29 ^c ± 0.85
	30:70	2658 ^g ± 96	16.52 ^c ± 1.5
	70:30	2439 ^f ± 29	16.21 ^c ± 0.34
	100:0	2167 ^e ± 21	16.27 ^c ± 0.49

Values are mean ± standard deviation of triplicate sample determinations
Means in the same column with different superscript letters are significantly different ($p \leq 0.05$)
All determinations were performed in triplicate

4.1.2 Optical Properties

4.1.2.1 Spectral Reflectance

The reflectance spectra of gelatin gels against white background were studied. The graphs of reflectance spectra at different GC with different GSR are shown in Figure 4.1: (a) 7.75%, (b) 8.00%, and (c) 8.25% gelatin concentration. The reflectance spectra showed the increasing trend with two dips at blue zone (420 and 440 nm) and

the highest reflectance was observed at yellow-orange zone (600-700 nm). Gelatin gel samples appeared to be clearly light yellow. The result of the absorption of light in the blue zone and highly reflected at yellow zone gave the object its light yellow appearance. This fact can explain the dip in the blue zone of the reflectance spectra of gelatin gels because the result of this absorption causes the reflectance at 420 nm and 440 nm to drop (Holtzschue, 2011). However, a color is not a result of a reflectance of a single wavelength, it reflects light in a range of wavelength with some wavelengths stronger than the others. From this experiment, the strongest reflected wavelength was in the yellow to orange zone (600-700 nm), which correlated well with the perceived color of gelatin gel sample as light yellow. Similar trends of reflectance spectra were presented for all treatments shown. However, the reflectance curves of GC of 8.00% and 8.25% at GSR of 0:100 were above the others. These conditions have the highest reflectance in the 450-700 nm regions.

The reflectance spectra of gelatin gel sample measured against black background are shown in Appendix B.3. Black absorbs most of the color, and when black was used as background to measure the color of gelatin gel samples, the reflectance spectra of gelatin gel samples were all leveled with two dips in the blue zone (420 nm and 440 nm). More absorption occurred in the blue zone due to the natural color of gelatin gel samples, which resulted in the dips.

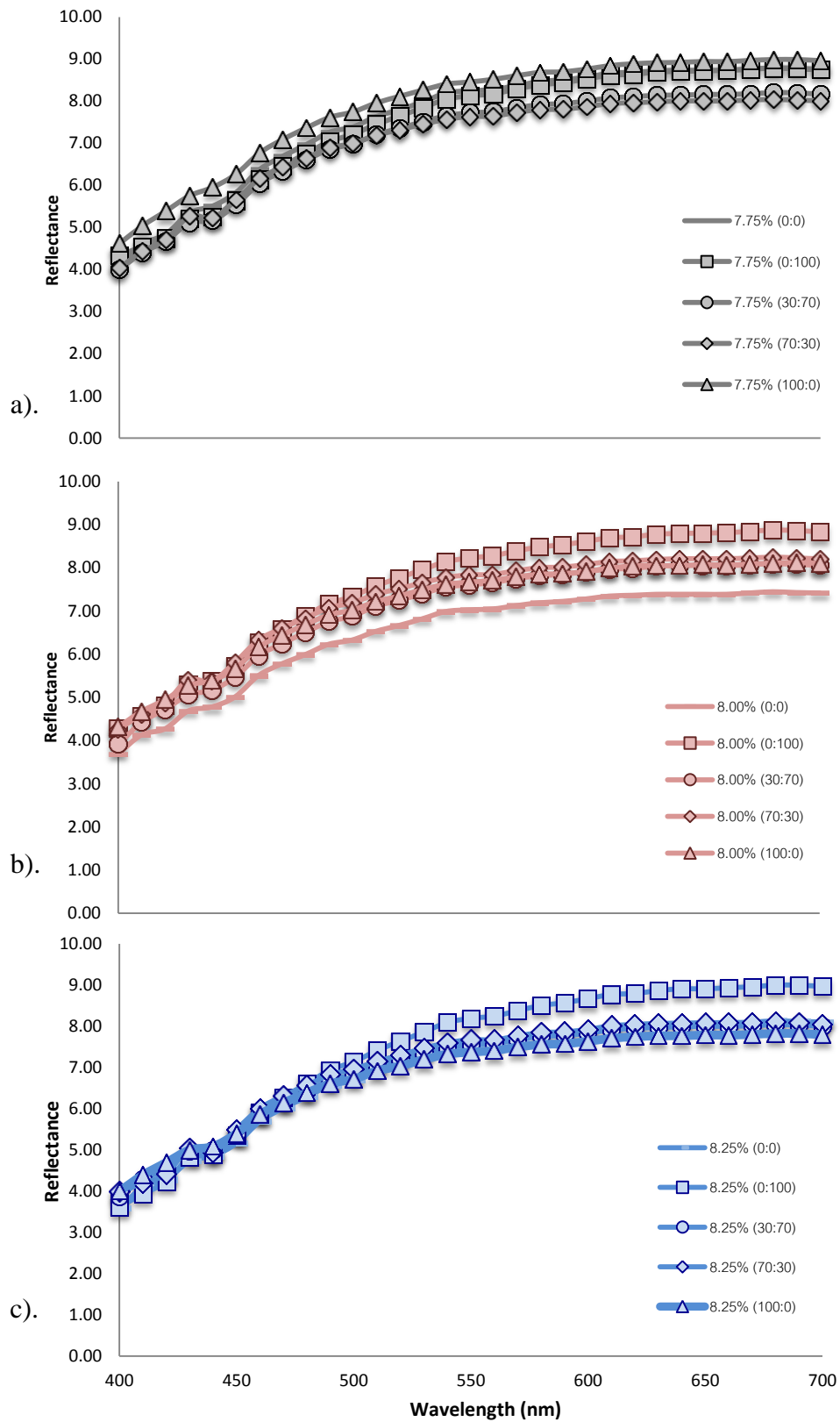


Figure 4.1 Reflectance spectra of (a) 7.75%, (b) 8.00%, and (c) 8.25% gelatin gel concentration against white background

4.1.2.2 CIELAB

CIE L^* is an important parameter when measuring the color of gelatin gel samples. Results showed that when glucose syrup was added into the mixture, gelatin gel samples contained higher L^* , as shown in Table 4.3. Gelatin gel samples containing glucose syrup were more transparent compared to gelatin gel samples containing sucrose because the refractive index of glucose syrup [12% ($n=1.3478$), 28% ($n=1.3635$), and 40% ($n=1.3937$)] was relatively close to refractive index of gelatin ($n=1.3471$) compared to that of sucrose. The refractive index of sucrose at 12%, 28%, and 40% were 1.3513, 1.3728, and 1.4441, respectively. As refractive indices of mixtures in the sample are close to each other, less scattering occurs causing a higher transparency in samples resulting in higher L^* when measured against white background. The difference of the refractive index between sucrose and gelatin was a little higher than the difference of refractive index between glucose syrup and gelatin resulting in a little more scattering and less transparency in the layer of gelatin.

The appearance of gelatin gel samples were yellow because calculated hue angle of gelatin gel samples were ranging from 82° - 87° (Table 4.3). An increase in sucrose concentrations led to an increase in chroma (Table 4.3). For all gelatin concentrations, the highest b^* was found at GSR of 70:30 and the lowest $-a^*$ was found at GSR of 70:30.

The calculation for total color difference, ΔE_{ab}^* , are shown in Appendix B.2.

Table 4.3 CIE L^* a^* b^* , CIE C^* and hue angle values of gelatin gel samples at different gelatin concentration and glucose syrup to sucrose ratios

GC (% w/w)	GSR	L^*	a^*	b^*	C^*	Hue Angle (degrees)
7.75	0:0	34.11 ± 0.17	-0.75 ± 0.03	8.08 ± 0.11	8.11 ± 0.11	84.72 ± 0.15
	0:100	32.34 ± 0.36	-0.54 ± 0.04	8.53 ± 0.87	8.54 ± 0.86	86.37 ± 0.11
	30:70	33.05 ± 0.27	-0.83 ± 0.06	7.64 ± 0.42	7.68 ± 0.41	83.80 ± 0.71
	70:30	32.89 ± 0.54	-0.90 ± 0.02	6.96 ± 0.03	7.25 ± 0.04	82.64 ± 0.12
	100:0	34.72 ± 0.41	-0.79 ± 0.06	7.21 ± 0.57	7.02 ± 0.55	83.72 ± 0.91
8.00	0:0	32.48 ± 0.68	-0.75 ± 0.10	7.29 ± 0.30	7.33 ± 0.31	84.14 ± 0.59
	0:100	32.98 ± 1.0	-0.69 ± 0.14	8.75 ± 1.75	8.78 ± 1.73	85.21 ± 1.9
	30:70	32.81 ± 1.1	-0.74 ± 0.08	7.51 ± 0.45	7.54 ± 0.44	84.34 ± 0.89
	70:30	33.32 ± 0.57	-0.92 ± 0.02	7.00 ± 0.54	7.07 ± 0.54	82.45 ± 0.77
	100:0	32.63 ± 0.89	-0.79 ± 0.07	6.88 ± 0.06	6.92 ± 0.06	83.45 ± 0.57
8.25	0:0	31.51 ± 0.44	-0.75 ± 0.09	8.94 ± 0.61	8.97 ± 0.62	85.17 ± 0.23
	0:100	32.69 ± 0.10	-0.57 ± 0.03	9.99 ± 0.77	10.01 ± 0.77	86.73 ± 0.13
	30:70	32.92 ± 0.25	-0.83 ± 0.06	7.71 ± 0.24	7.89 ± 0.21	83.87 ± 0.55
	70:30	33.87 ± 0.36	-0.98 ± 0.04	7.82 ± 0.22	7.76 ± 0.23	82.85 ± 0.41
	100:0	33.95 ± 0.33	-0.75 ± 0.05	7.05 ± 0.01	7.09 ± 0.01	83.89 ± 0.38

Values are mean ± standard deviation of triplicate sample determinations
All determinations were performed in triplicate

4.2 Application of Kubelka-Munk Theory

The measured reflectance were corrected by Saunderson's equation to obtain true reflectance of gelatin gel samples as discussed in the section of modified Kubelka-Munk theory applied in food (Section 2.7.2). The partial reflection at the surface had a considerable effect on measured reflectance in gelatin gel samples, the effect being about the same for all wavelength of true reflectance, which reduced by 31.5% by the interfaced to measured value, as shown in Figure 4.2.

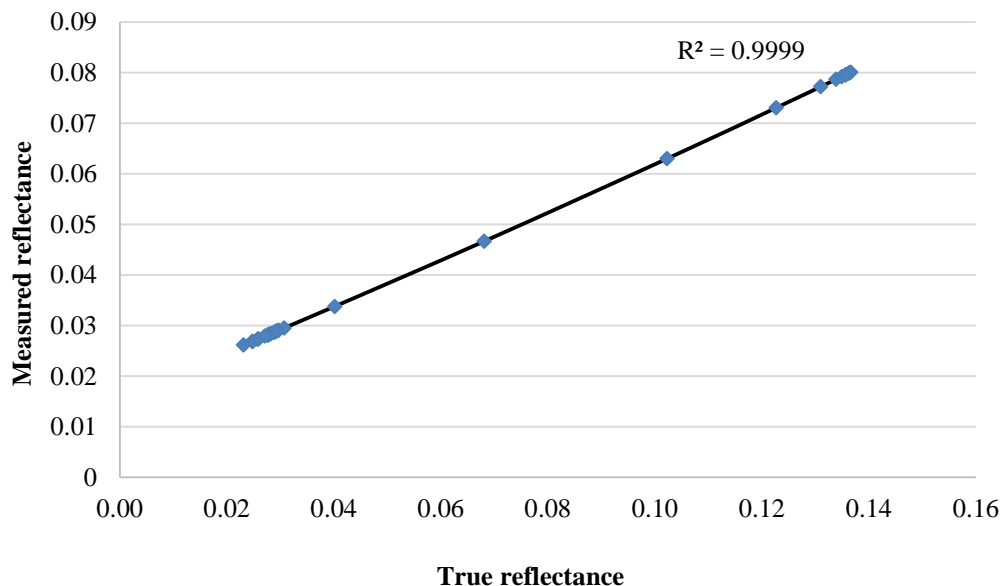


Figure 4.2 The relationship between true reflectance and measured reflectance

The fact that the prepared gels are translucent products suggests that the application of the Kubelka-Munk theory is applicable (Nobbs, 1997). Four gelatin gel samples were selected based on their textural properties: GC of 7.75% and 8.00% at GSR of 70:30 and 30:70. We applied the Kubelka-Munk equation, Eq. (2.2)-(2.5) with and without Saunderson's correction, Eq. (2.6), and modified Kubelka-Munk theory for semitransparent products, Eqs. (3.5) and (3.6), to the reflectance spectral of those gelatin gel samples obtained from Section 4.1.2.1. Results of the ratios of K/S ,

calculated from the three Kubelka-Munk models mentioned above are shown in Figure 4.3-4.5. Spectral distributions of the K/S show a slight difference for all samples using Kubelka-Munk model with Saunderson's correction (Figure 4.3) and Kubelka-Munk theory without Saunderson's correction (Figure 4.4). The K/S values of the Kubelka-Munk theory with and without Saunderson's correction showed the lowest curves at the condition of 7.75% GC and GSR at 30:70. Gelatin gel samples at this condition contained higher scattering within the layer resulted in a lower K/S values than other conditions. This is because of the differences in the refractive index between gelatin and sucrose, and gelatin and glucose syrup. Results from the modified Kubelka-Munk theory for semitransparent products (Figure 4.5) showed higher K/S in the blue zone (400-450 nm), falling rapidly until reaching the orange zone (600 nm). This phenomenon could be seen clearly only when the modified Kubelka-Munk theory for semitransparent layer was applied to gelatin gel samples (Figure 4.5). While results from the Kubelka-Munk theory used in the food industry with and without Saunderson's correction showed two peaks of absorption at 420 nm and 440 nm only. The K/S values in other wavelengths were leveled.

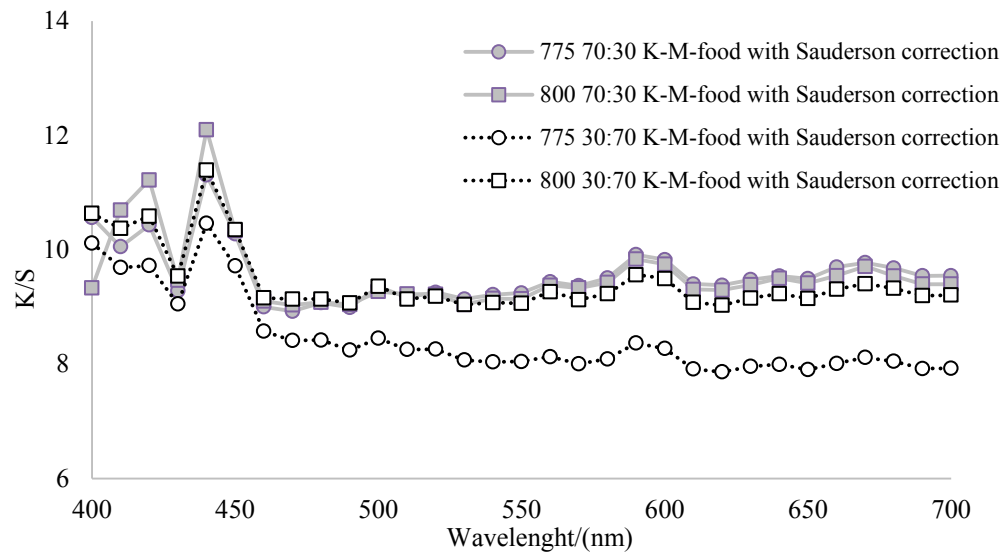


Figure 4.3 Spectral distributions of the K/S coefficients of selected gelatin gel samples calculated from Kubelka-Munk theory with Saunderson's equation

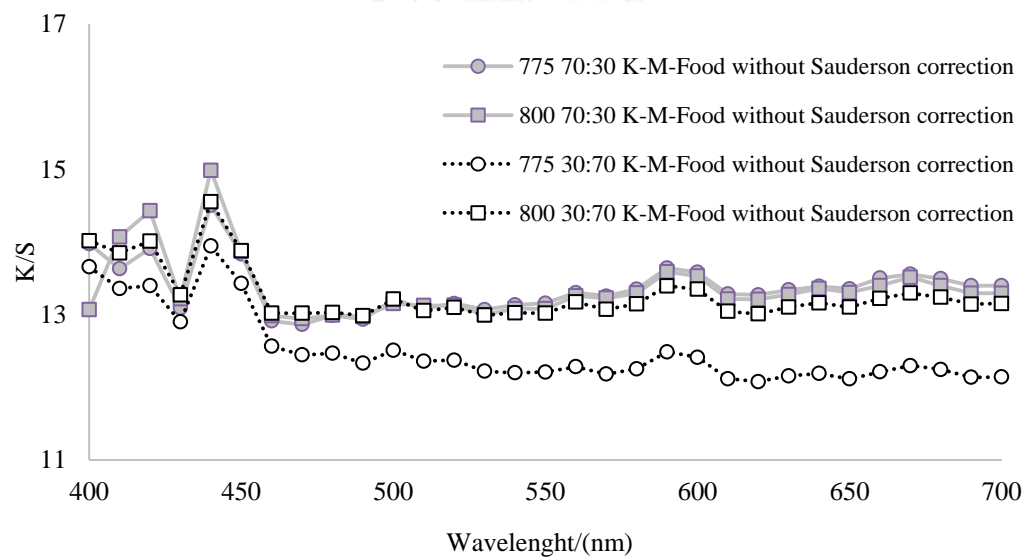


Figure 4.4 Spectral distributions of the K/S coefficients of selected gelatin gel samples calculated from Kubelka-Munk theory without Saunderson's equation

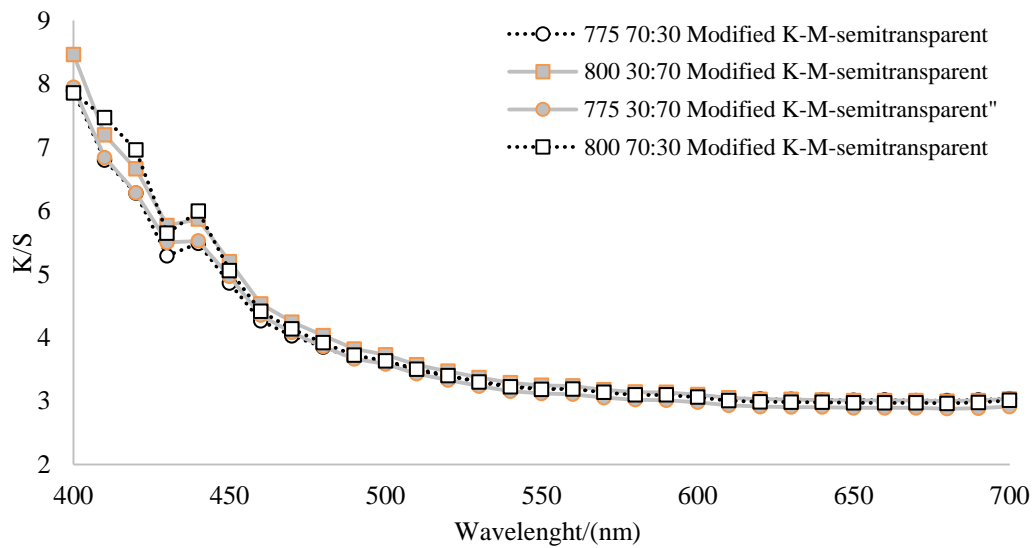


Figure 4.5 Spectral distributions of the K/S coefficients of selected gelatin gel samples calculated from modified Kubelka-Munk theory for semitransparent product.

The application of Kubelka-Munk theory with and without the Saunderson's correction was applied to one of the four selected samples (7.75% at 70:30) with 0.01% colorant concentration. As mentioned earlier, Saunderson's correction is used to obtain true reflectance. Figure 4.6 shows the comparison between K/S coefficient obtained from true reflectance and measured reflectance of red gelatin gel samples. The K/S coefficients of true reflectance are equally high in blue and green regions with peak at 440 nm and lower at the orange-red zone (600-700 nm). This trend is the same for K/S obtained from true reflectance and measured reflectance. The only difference is that with the true reflectance the maximum and minimum of K/S are very distinct, according to the visual perception.

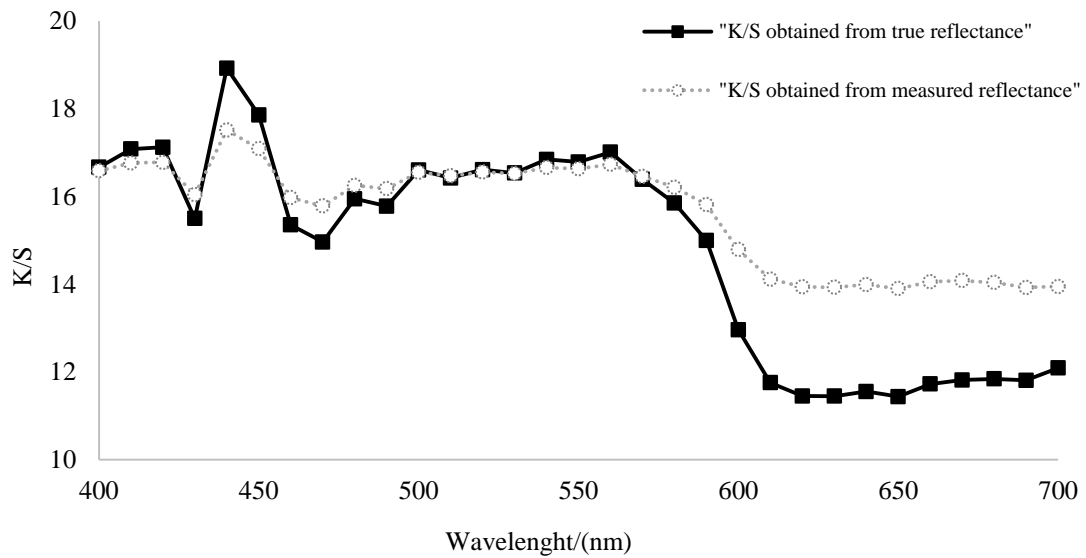


Figure 4.6 The comparison between the K/S coefficients obtained from true and measured reflectance.

4.3 Model-Fitting

Using the database system, eight gelatin gel samples of selected condition (7.75% at 70:30) were gradually varied in colorant concentration ranging from 0.01%-0.0001% (w/w) according to Table. 3.2. Figure 4.7 shows the relationship between the absorption coefficients, K , of gelatin gel samples and colorant concentrations at three wavelengths: 560 nm, 570 nm and 580 nm.

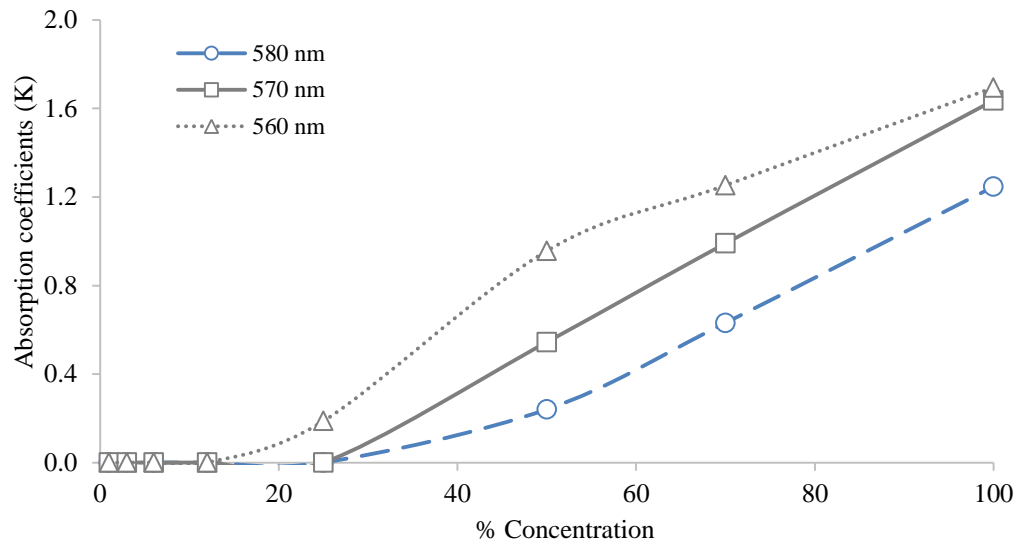


Figure 4.7 Relationship between absorption coefficients and colorant concentrations of three wavelengths

From this relationship, Eq. (4.1) was used to derive coefficients a_1 , a_2 , and a_3 for all wavelengths using the least square method.

$$K = a_1c + a_2c^2 + a_3c^3 \quad (4.1)$$

The absorption coefficients, K , obtained from calculation and from model-fitting using Eq. (4.1) were compared in Figure 4.8. Results showed that K from model-fitting and K from calculation were slightly different. It implies that this equation can be used for further prediction of reflectance at various concentration and possible color match predictions for gelatin gel samples.

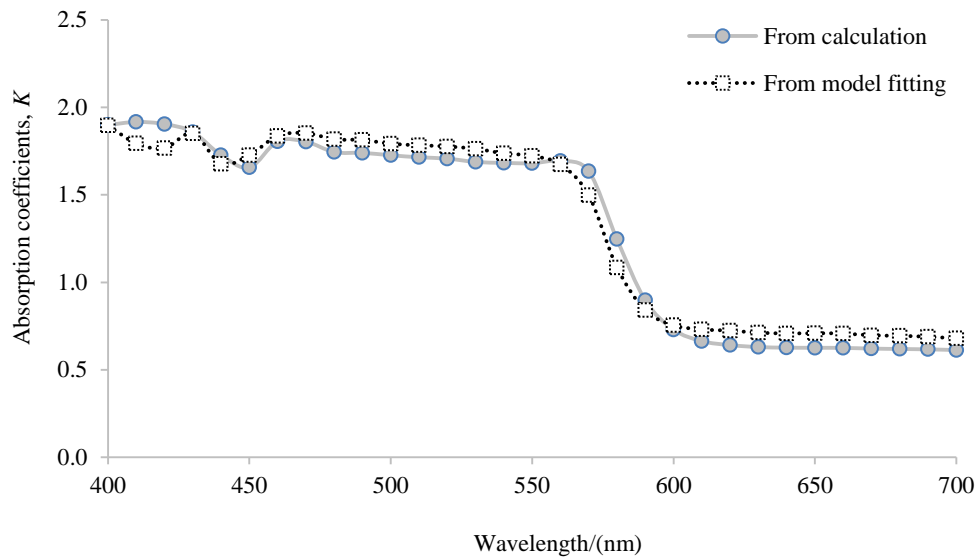


Figure 4.8 Comparison of K coefficients from calculation and K coefficients from model-fitting

The comparison between true reflectance (calculated from measured reflectance of prepared colored gelatin gel samples) and true reflectance calculated from model-fitting shows similar trend (Figure 4.9). However, the reflectance curve of true reflectance obtained from model-fitting was approximately 0.15 unit higher than reflectance curve of true reflectance calculated from measured reflectance of gelatin gel sample. This was probably because gelatin gel samples were measured without white opaque cover container causing light leakage around the periphery of semi-transparent gelatin gel during the measurement.

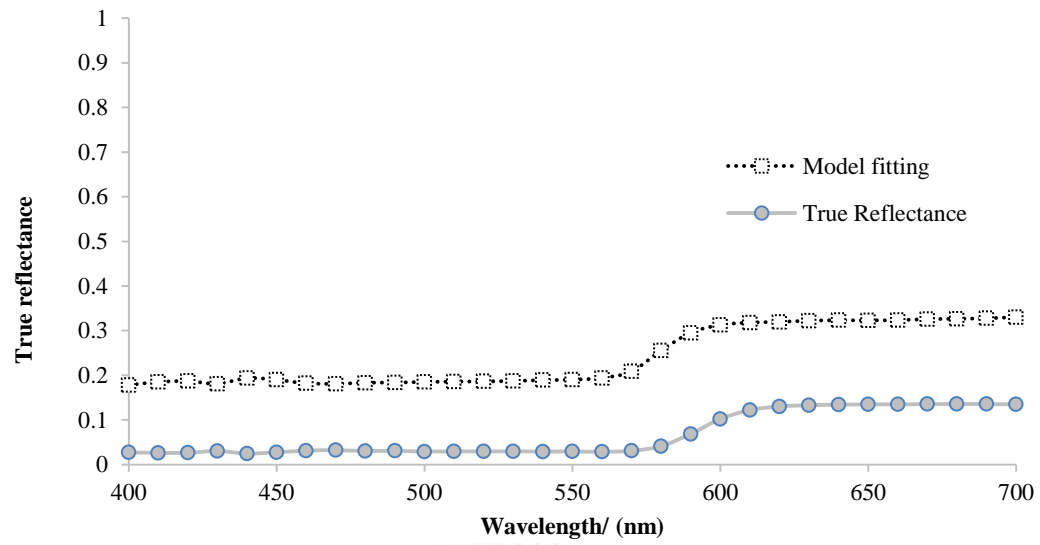
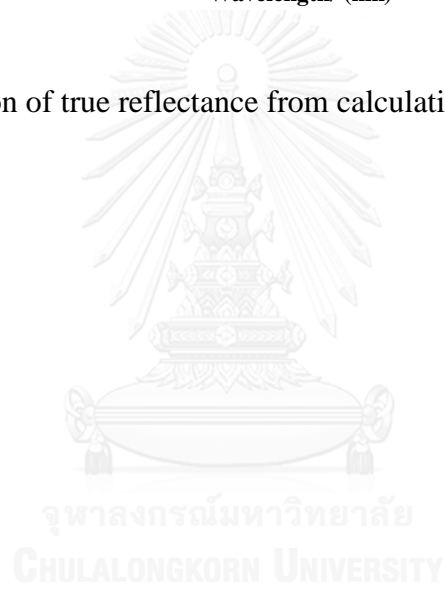


Figure 4.9 Comparison of true reflectance from calculation and true reflectance from model-fitting



CHAPTER V

CONCLUSIONS AND SUGGESSTIONS

Conclusions

The texture of gelatin gel was affected by glucose syrup to sucrose ratios and gelatin concentration levels. Hardness and gumminess were strongly affected by the addition of sugars. Rupture forces increased with increase in gelatin concentration and the addition of sugar.

The lightness (L^*) of gelatin gel samples were more affected by glucose syrup than sucrose. The increase in sucrose ratios was paralleled to the increasing of the chroma (C^*) of gelatin gel samples. The reflectance properties can be used to explain the natural color of gelatin gel samples. Reflectance spectra for all conditions showed the same trend. The strongest reflected wavelengths were in yellow to orange zone (600-700 nm), which correlated well with the perceived color of gelatin gel sample as yellow.

The Kubelka-Munk models were successfully applied to gelatin gel samples. Results from the experiment suggested that Saunderson's correction should apply when using the Kubelka-Munk theory on gelatin gel in order to obtain a more accurate K/S value that related to visual perception.

Suggestions

In order to see a distinguishable effect of gelatin concentration, a higher gap range is more suitable. However, food industry uses the gelatin at the range of 8-10%. Measuring the reflectance using spectrophotometer should be in an opaque container to avoid the leaking of light around the periphery of gelatin gel samples.

REFERENCES

- Babel, W. (1996). Gelatine—ein vielseitiges Biopolymer. *Chemie in unserer Zeit*, 30(2), 86-95.
- Bourne, M. (2002). *Food Texture and Viscosity: Concept and Measurement*. New York: Academic Press.
- Brookfield Texture Analyzer. . (2014). *What is texture analyzer?* Retrieved December, 2013, from www.brookfieldengineering.com
- Burey, P., Bhandari, B. R., Rutgers, R. P. G., Halley, P. J., & Torley, P. J. (2009). Confectionery gels: a review on formulation, rheological and structural aspects. *International Journal of Food Properties*, 12(1), 176-210.
- Caine, W., Aalhus, J., Best, D., Dugan, M., & Jeremiah, L. (2003). Relationship of texture profile analysis and Warner-Bratzler shear force with sensory characteristics of beef rib steaks. *Meat Science*, 64(4), 333-339.
- Calvo, C., & Salvador, A. (1997). Measurement of the colour and transparency of gels: application to fruit gels. *Food Hydrocolloids*, 11(4), 443-447.
- Chaing, E. (2013). *Rupture Test (Vol. 2014)*: Middle: Lab Unlimited Carl Stuart Group.
- Charvolin, J., & Sadoc, J.-F. (2012). About collagen, a tribute to Yves Bouligand. *Royal Society Publishing*, 2(5), 567-574.
- Chilvers, G., & Morris, V. (1987). Coacervation of gelatin-gellan gum mixtures and their use in microencapsulation. *Carbohydrate Polymers*, 7(2), 111-120.

- Cubeddu, R., Pifferi, A., Taroni, P., & Torricelli, A. (2003). Spectroscopic techniques for analyzing raw material quality. In I. Tothill (Ed.), *Rapid and On-Line Instrumentation for Food Quality Assurance* (pp. 270-289). Cambridge: Woodhead Publishing Limited.
- Duangmal, K., Saicheua, B., & Sueeprasan, S. (2008). Colour evaluation of freeze-dried roselle extract as a natural food colorant in a model system of a drink. *LWT-Food Science and Technology*, 41(8), 1437-1445.
- Fennema, O. R. (1996). *Food Chemistry* (3rd Ed.). New York: Marcel Dekker, Inc. .
- Figiel, A., & Tajner-Czopek, A. (2006). The effect of candy moisture content on texture. *Journal of Foodservice*, 17(4), 189-195.
- Fonkwe, L. G., Narsimhan, G., & Cha, A. S. (2003). Characterization of gelation time and texture of gelatin and gelatin-polysaccharide mixed gels. *Food Hydrocolloids*, 17(6), 871-883.
- GME. (2012). Official website of GME- Gelatin Manufactures of Europe.
- GMIA. (2012). Gelatin Handbook.
- Gómez-Robledo, L., Melgosa, M., Huertas, R., Roa, R., Moyano, M., & Heredia, F. (2008). Virgin-olive-oil color in relation to sample thickness and the measurement method. *Journal of the American Oil Chemists' Society*, 85(11), 1063-1071.
- Grazela, A. J., & Morrison, N. A. (2003). Washington DC Patent No. WO 2002030214A.

- Gupta, R., Sharma, A., & Sharma, R. (2007). Textural profile analysis of sunflower–sesame kernel confection (chikki). *Journal of Texture Studies*, 38(1), 153-165.
- Haug, I. J., & Draget, K. I. (2009). Gelatin. In G. O. Phillips & P. A. Williams (Eds.), *Handbook of Hydrocolloids*. Cambridge, UK: Woodhead Publishing Ltd.
- Heuer, C. (2000). *World Art*. University of Wisconsin.
- Hoffman, K. (1998). *Application of the Kubelka-Munk color model to xerographic images*. (Senior Research), Rochester Institute of Technology, New York
- Holm, K., Wendin, K., & Hermansson, A. (2009). Sweetness and texture perceptions in structured gelatin gels with embedded sugar rich domains. *Food Hydrocolloids*, 23(8), 2388-2393.
- Holtzschue, L. (2011). *Understanding Color: An Introduction for Designers*. New Jersey: John Wiley & Sons.
- HunterLAB. (2001). *The Basic of Color Perception and Measurement*. HunterLab Associates. Almere, Netherland: Nederland B.V.
- Johnson, T. M., & Zabik, M. E. (1981). Egg albumen proteins interactions in an angel food cake system. *Journal of Food Science*, 46(4), 1231-1236.
- Jones, J. T. (2004). Gelatin: Manufacture and physico-chemical properties. In F. Podczeck & B. E. Jones (Eds.), *Pharmaceutical Capsules* (3rd ed., pp. 23-56). London: Pharmaceutical Press.
- Kasapis, S., Al-Marhoobi, I. M., Deszczynski, M., Mitchell, J. R., & Abeysekera, R. (2003). Gelatin vs polysaccharide in mixture with sugar. *Biomacromolecules*, 4(5), 1142-1149.

- Keenan, T. R. (2008). Gelatin. In R. E. Kirk, D. F. Othmer & A. Seidel (Eds.), *Kirk-Othmer Food and Feed Technology* (Vol. 1, pp. 856-865). Hoboken: Wiley-Interscience.
- Kittiphattanabawon, P., Benjakul, S., Visessanguan, W., & Shahidi, F. (2010). Comparative study on characteristics of gelatin from the skins of brownbanded bamboo shark and blacktip shark as affected by extraction conditions. *Food Hydrocolloids*, 24(2), 164-171.
- Klein, G. (2010). *Industrial Color Physics*. New York: Springer.
- Kriss, M., & Green, P. (2010). *Color Management: Understanding and Using ICC Profiles* (Vol. 17). West Sussex, UK: John Wiley & Sons.
- Ladanyia, M. (2010). *Citrus Fruit: Biology, Technology and Evaluation*. Burlington: Academic press.
- Lana, M. M., Hogenkamp, M., & Koehorst, R. B. M. (2006). Application of Kubelka-Munk analysis to the study of translucency in fresh-cut tomato. *Innovative Food Science & Emerging Technologies*, 7(4), 302-308.
- Lau, M. H., Tang, J., & Paulson, A. T. (2000). Texture profile and turbidity of gellan/gelatin mixed gels. *Food Research International*, 33(8), 665-671.
- Ledward, D. A. (1986). Gelatin of gelatin. In V. J. Morris, J. R. Mitchell & D. A. Ledward (Eds.), *Functional Properties of Food Macromolecules* (pp. 171-201). London: Elsevier.

- Martinez, O., Salmeron, J., Guillen, M. D., & Casas, C. (2004). Texture profile analysis of meat products treated with commercial liquid smoke flavourings. *Food Control*, 15(6), 457-461.
- Meléndez-Martínez, A. J., Gómez-Robledo, L., Melgosa, M., Vicario, I. M., & Heredia, F. J. (2011). Color of orange juices in relation to their carotenoid contents as assessed from different spectroscopic data. *Journal of Food Composition and Analysis*, 24(6), 837-844.
- Meppem, W. (2011). Gelatine. Retrieved December, 2013, from <http://www.taste.com.au/how+to/articles/267/gelatin>
- Monovector, H. (2011). Color. Retrieved September, 2014, from <http://monovector.com/2011/07/colorcolorcolorcolorcolor/>
- Muñoz, A. M., Pangborn, R., & Noble, A. C. (1986a). Sensory and mechanical attributes of gel texture. I. Effect of gelatin concentration. *Journal of Texture Studies*, 17(1), 1-16.
- Muñoz, A. M., Pangborn, R., & Noble, A. C. (1986b). Sensory and mechanical attributes of gel texture. II. Effect of gelatin, sodium alginate, and kappa-carrageenan gel. *Journal of Texture Studies*, 17(1), 17-36.
- Negueruela, A. I., & Perez-Arquillue, C. (2000). Color measurement of rosemary honey in the solid state by reflectance spectroscopy with black background. *Journal of AOAC International*, 83(3), 669-674.
- Nobbs, J. H. (1985). *Kubelka—Munk theory and the prediction of reflectance* (Vol. 15).

- Nobbs, J. H. (1997). Colour-match prediction for pigmented materials. In M. R. (Ed.), *Colour Physics for Industry* (2nd ed., pp. 292-372). West Yorkshire: Society of Dyes and Colourists.
- Osorio, F. A., Bilbao, E., Bustos, R., & Alvarez, F. (2007). Effects of concentration, bloom degree, and pH on gelatin melting and gelling temperatures using small amplitude oscillatory rheology. *International Journal of Food Properties*, *10*(4), 841-851.
- Panda, H. (2011). *The Complete Book on Gums and Stabilizers for Food Industry*. Delhi, India: Asia Pacific Business Press Inc.
- Pons, M., & Fiszman, S. M. (1996). Instrumental texture profile analysis with particular reference to gelled systems. *Journal of Texture Studies*, *27*(6), 597-624.
- Rahman, M., & Al-Mahrouqi, A. I. (2009). Instrumental texture profile analysis of gelatin gel extracted from grouper skin and commercial (bovine and porcine) gelatin gels. *International Journal of Food Sciences and Nutrition*, *60*(s7), 229-242.
- Rahman, M. S., & Al-Farsi, S. A. (2005). Instrumental texture profile analysis (TPA) of date flesh as a function of moisture content. *Journal of Food Engineering*, *66*(4), 505-511.
- Ruiz, F., Angulo, C., & Agell, N. (2007). Support vector machines for color adjustment in automotive basecoat. In C. Angulo & L. Godo (Eds.), *Frontiers in Artificial Intelligence and Applications* (Vol. 146, pp. 19-27). Amsterdam, Netherlands: ISO Press.

- Semenova, M. G., Antipova, A. S., & Belyakova, L. E. (2002). Food protein interactions in sugar solutions. *Current Opinion in Colloid and Interface Science*, 7(5), 438-444.
- Stable Micro System Ltd. . (2014). Survey, UK: The Exponent Lite Express Program.
- Sun, Q., Xing, Y., Qiu, C., & Xiong, L. (2014). The pasting and gel textural properties of corn starch in glucose, fructose and maltose syrup. *PloS one*, 9(4), e95862.
- Supavitpatana, P., Wirjantoro, T. I., Apichartsrangkoon, A., & Raviyan, P. (2008). Addition of gelatin enhanced gelation of corn–milk yogurt. *Food Chemistry*, 106(1), 211-216.
- Talens, P., Martinez-Navarrete, N., Fito, P., & Chiralt, A. (2002). Changes in optical and mechanical properties during osmodehydrofreezing of kiwi fruit. *Innovative Food Science & Emerging Technologies*, 3(2), 191-199.
- Upton, S. (2013). Delta E: The Color Difference. Retrieved January, 2013, from <http://colorwiki.com/Delta E: The Color Difference>
- Winkler, J. (2003). *Titanium Dioxide*. Hannover, UK: Vincentz-Verlag.
- Zayas, J. F. (1997). *Functionality of Proteins in Food*. Berlin: Springer-Verleg.

APPENDIX



จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

APPENDIX A
DETAILED INFORMATION ON MATERIALS USED IN THE
EXPERIMENT

A.1 Detailed information of gelatin

Material	Brand	Lot #	Manufacture Date
Gelatin leaves	Gelita®	LB45405/02	13/01/2014

A.2 Detailed information of glucose syrup



ห้างหุ้นส่วนจำกัด เจริญวารกิจ

376 ม.7 อ.วิเศษ อ.บึงสามพัน จ.พิษณุโลก ต.บ้านใหม่ อ.เมือง จ.สมุทรปราการ 10280
 โทร. 0-2323-1072 , 0-2709-0618-9 แฟกซ์. 0-2323-1072

ANALYSIS	STANDARD	RESULTS
Dextrose Equivalent (%)	38.0 - 43.0	41.3
pH	4.8 - 5.2	4.9
SO ₂ (ppm)	150 - 300	213
Degree (% Brix)	84.0 - 86.0	84.6

A.3 Detailed information of synthetic colorant

Colorant Name	Code	Batch Number
Eurocert Ponceau 4R	730100M	21313

A.4 Silicone mold used in the experiment



APPENDIX B
ADDITIONAL DATA

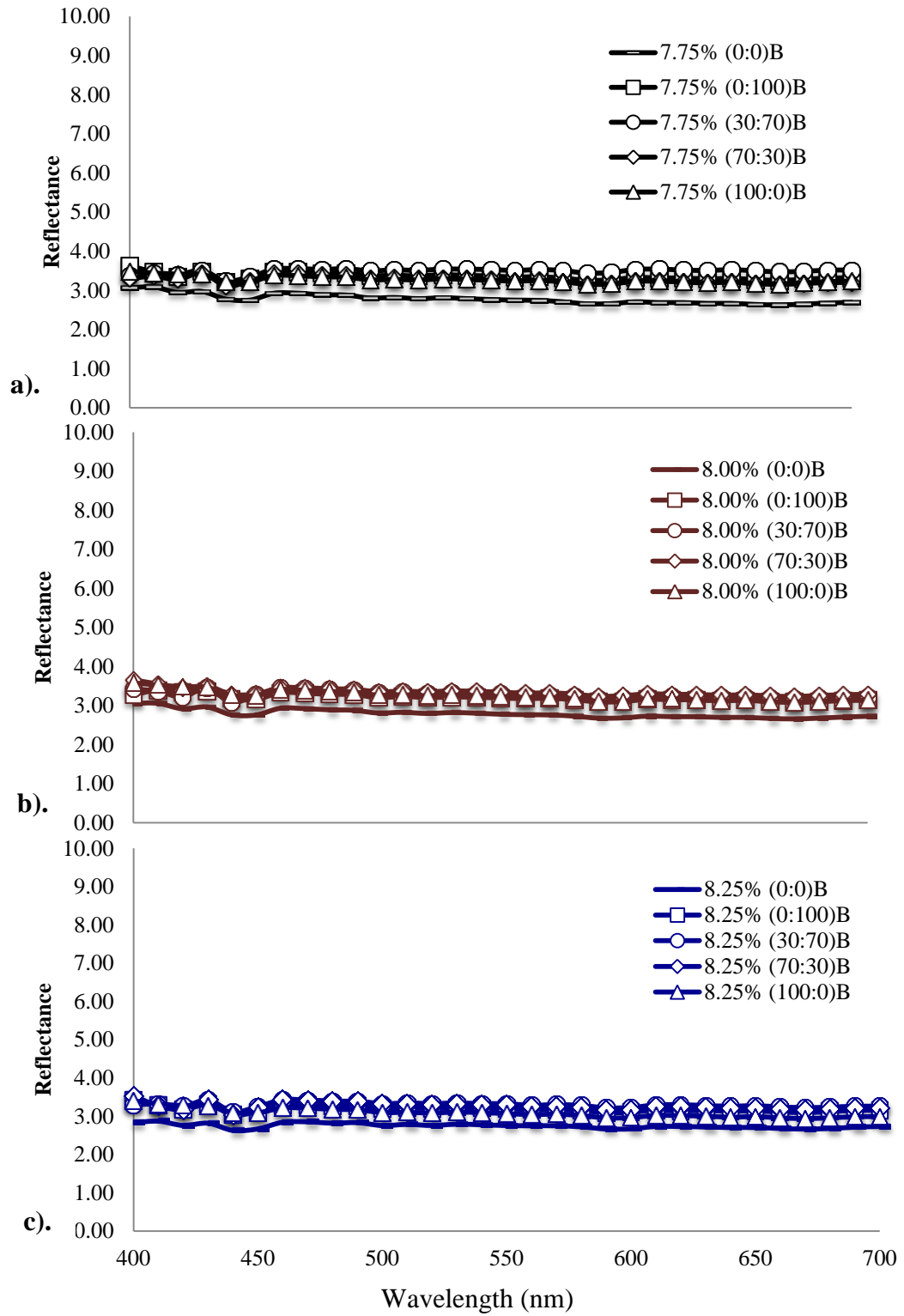
B.1 Reflectance of white ceramic tile and black ceramic tile used as backgrounds

Wavelength (nm)	BLACK CEREMIC	WHITE CEREMIC
400	5.803	63.717
410	5.490	65.156
420	5.456	65.365
430	5.401	65.834
440	5.284	65.699
450	5.274	65.689
460	5.245	66.429
470	5.248	66.48
480	5.191	66.415
490	5.176	66.582
500	5.132	66.416
510	5.095	66.296
520	5.091	66.283
530	5.076	66.387
540	5.091	66.322
550	5.112	66.059
560	5.093	65.933
570	5.057	65.981
580	5.039	66.185
590	5.004	66.122
600	5.000	66.205
610	5.020	66.208
620	5.057	65.972
630	5.066	65.966
640	5.076	65.887
650	5.100	65.770
660	5.142	65.632
670	5.194	65.352
680	5.332	65.156
690	5.624	64.965
700	5.986	64.632

B.2 Calculated ΔE of gelatin gel samples

Gelatin Concentration (%)	glu:su	ΔE 1976	ΔE 2000
7.75%	0:0		
	0:100	1.84	1.49
	30:70	1.15	0.93
	70:30	1.66	1.32
	100:0	1.06	0.82
8.00%	0:0		
	0:100	1.54	1.15
	30:70	0.40	0.31
	70:30	0.90	0.75
	100:0	0.44	0.34
8.25%	0:0		
	0:100	1.59	1.22
	30:70	1.87	1.44
	70:30	2.62	2.08
	100:0	3.09	2.39

B.3 Reflectance spectra of (a) 7.75 %, (b) 8.00% and (c) 8.25% gelatin gel concentration against black background



APPENDIX C
ANALYSIS OF VARIANCE

C.1 Effect of different gelatin concentrations and glucose syrup to sucrose ratios on hardness force of gelatin gel samples

Source	Sum of Squares	df	Mean Square	F	Sig.
Treatment	1.168 x 10 ⁸	14	8343670.856	89.880	.000
Error	2784950.878	30	92831.696		
Total	1.466 x 10 ⁹	45			

C.2 Effect of different gelatin concentrations and glucose syrup to sucrose ratios on springiness ratio of gelatin gel samples

Source	Sum of Squares	df	Mean Square	F	Sig.
Treatment	.009	14	.001	5.344	.000
Error	.004	30	.000		
Total	42.547	45			

C.3 Effect of different gelatin concentrations and glucose syrup to sucrose ratios on cohesiveness ratio gelatin gel samples

Source	Sum of Squares	df	Mean Square	F	Sig.
Treatment	.028	14	.002	12.639	.000
Error	.005	30	.000		
Total	41.890	45			

C.4 Effect of different gelatin concentrations and glucose syrup to sucrose ratios on gumminess force of gelatin gel samples

Source	Sum of Squares	df	Mean Square	F	Sig.
Treatment	1.165×10^8	14	8324681.098	88.598	.000
Error	2818814.209	30	93960.474		
Total	1.376×10^9	45			

C.5 Effect of different gelatin concentrations and glucose syrup to sucrose ratios on rupture force of gelatin gel samples

Source	Sum of Squares	df	Mean Square	F	Sig.
Treatment	17.785	14	1.270	305.41	.000
Error	0.125	30	0.004		
Total	179.089	45			

C.6 Effect of different gelatin concentrations and glucose syrup to sucrose ratios of distance of rupture point of gelatin gel samples

Source	Sum of Squares	df	Mean Square	F	Sig.
Treatment	90.504	14	6.465	9.181	.000
Error	21.123	30	0.704		
Total	1.127 x 10 ⁴	45			

VITA

Malynn Porayanee was born on October 20th, 1987 in Glendale, United States. In 2006, she entered California Polytechnic State University of Pomona, where she received her Bachelor of Science in Food Science and Technology in 2011.

She entered the department of Food Technology, Chulalongkorn University in 2012 for Master degree in Food Science and Technology. She presented some parts of her research at the 2nd International Conference on Agriculture and Agro-Industry organized by Mae Fah Luang University, in Chaing Rai on November 2014.

