MICROWAVE GLYCATION OF SOY PROTEIN ISOLATE

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I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

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ABSTRACT

MICROWAVE GLYCATION OF SOY PROTEIN ISOLATE

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Soy protein is used as a functional ingredient in the food industry since it has good functional properties like gelling, emulsifying, water and oil holding capacity. It contains all the essential amino acids, lowers the cholesterol, and the risk of cardiovascular disease. Therefore, the improvement of the functional properties of soy protein is an important issue for food science and industry. Glycation is known as the non-enzymatic reaction between the carbonyl group of sugars and the free amino group of proteins. It is known to increase solubility, emulsifying activity, and stability of the proteins. In this study, microwave glycation of soy protein isolate in an aqueous medium with fructose, glucose, and D-allulose (rare sugar) were performed. Protein-sugar ratio (1.6 and 7.2) and the reaction pH (7 and 10) were the other factors examined. As the control, microwave glycation was compared to water bath glycation for soy protein isolate glycated with fructose at pH 10. The concentration of free amino groups was measured by OPA method. Reducing sugar concentration of glycated protein was quantified by HPLC experiments. The formation of the final Maillard products was examined by the UV-VIS at 420 nm and the effects of glycation on solubility were measured by the Lowry method. Structural changes of the soy protein isolate after glycation were investigated by Fourier Transform Infrared spectroscopy (FT-IR) and Time Domain Nuclear Magnetic Resonance (TD-NMR) relaxometry.

Alkaline pH was found to be more effective for microwave glycation of soy protein isolate. The reactivity of sugars for microwave glycation reaction was ordered as D-allulose > fructose > glucose. Microwave heating at alkaline conditions caused the isomerization reaction of fructose to D-allulose and glucose, D-allulose to fructose, and glucose to fructose. Microwave heating did not have an effect on increasing the browning degree of the protein upon heating and it was interpreted as microwave glycation did not form the final stage Maillard products. Moreover, microwave heating was found to decrease the solubility of protein at pH 10. FT-IR results showed that a higher degree of glycation reaction was provided by microwave heating. T_2 relaxation times obtained by TD-NMR provided valuable information about the structural modification of glycated soy protein isolate and the mobility of water in the system before and after glycation. According to results, microwave heating was found to be more effective for glycation of soy protein isolate than water bath heating.

Keywords: Microwave glycation, Soy protein isolate, D-allulose, Time Domain NMR (TD-NMR), Fourier Transform Infrared Spectroscopy (FT-IR)

ÖZ

SOYA PROTEİNİ İZOLATININ MİKRODALGA İLE GLİKASYONU

Namlı, Serap
Yüksek Lisans, Gıda Mühendisliği
Tez Danışmanı: Doç. Dr. Mecit Halil Öztop
Ortak Tez Danışmanı: Prof. Dr. Servet Gülüm Şumnu

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Soya proteini, jelleştirme, emülsifiye etme, su ve yağ tutma kapasitesi gibi iyi işleme kabiliyetine sahip olduğundan gıda endüstrisinde fonksiyonel bir bileşen olarak kullanılır. Tüm esansiyel amino asitleri içerir, kolesterolü düşürür ve kalp-damar hastalıkları riskini azaltır. Bu nedenle, soya proteininin fonksiyonel özelliklerinin geliştirilmesi, gıda bilimi ve endüstrisi için önemini korumaktadır. Glikasyon, şekerlerin karbonil grubu ile proteinlerin serbest amino grubu arasındaki enzimatik olmayan esmerleşme reaksiyonu olarak bilinir. Glikasyon, proteinlerin çözünürlüğünü, emülsifiye etme kabiliyetini ve stabilitesini arttırır. Bu çalışmada soya proteini izolatının fruktoz, glikoz ve D-allüloz (nadir şeker) içeren sulu bir ortamda mikrodalga glikasyonu yapılmıştır. Protein-şeker ağırlık oranı (1.6 ve 7.2) ve reaksiyon pH'ı (7 ve 10) incelenen diğer faktörlerdir. Mikrodalga glikasyonu, pH 10'da fruktoz ile glike edilmiş soya proteini izolatı için su banyosu glikasyonuyla karşılaştırılmıştır. Serbest amino gruplarının konsantrasyonu, OPA metodu ile ölçülmüştür. Glike edilmiş proteinin indirgen şeker konsantrasyonu HPLC deneyleriyle ölçülmüştür. İleri seviye Maillard ürünlerinin oluşumu, 420 nm'de spektrofotometre ile incelenmiştir. Glikasyonun çözünürlük üzerindeki etkileri Lowry metodu ile ölçülmüştür. Soya proteini izolatının glikasyon sonrası yapısal değişiklikleri, Fourier Transform Infrared spektroskopisi (FT-IR) ve Time Domain

Nükleer Manyetik Rezonans (TD-NMR) relaksometre ile incelenmiştir. Alkalin pH'ın soya proteini izolatının mikrodalga glikasyonu için daha etkili olduğu bulunmuştur. Şekerlerin mikrodalga glikasyonu için reaktivitesi, D-allüloz> fruktoz> glikoz olarak sıralanmıştır. Alkali şartlardaki mikrodalga ısıtmanın, fruktozun D-allüloz ve glukoza, D-allülozun fruktoza ve glukozun fruktoza izomerleşmesine neden olduğu tespit edilmiştir. Mikrodalganın, glikasyon soncucunda soya proteinin esmerleşme derecesini arttırma etkisine sahip olmadığı belirlenmiş ve ileri seviye Maillard ürünlerini oluşturmadığı şeklinde yorumlanmıştır. Ayrıca, mikrodalgada ısıtmanın, pH 10'da proteinin çözünürlüğünü azaltma etkisine sebep olduğu bulunmuştur. FT-IR sonuçları, mikrodalganın daha yüksek dereceli bir glikasyon reaksiyonuna sebep olduğunu göstermiştir. TD-NMR ile elde edilen T_2 relaksometre süreleri, glikasyona uğramış soya proteini izolatının yapısal modifikasyonu ve glikasyondan önce ve sonra sistemdeki suyun hareketliliği hakkında değerli bilgiler sağlamıştır. Sonuçlara bakıldığında, mikrodalga ile ısıtmanın soya protein izolatının glikasyonu için su banyosu ile ısıtmadan daha etkili olduğu bulunmuştur.

Anahtar Kelimeler: Mikrodalga ile glikasyon, Soya proteini izolatı, D-allüloz, Zamansal Alanda NMR (TD-NMR), Fourier Dönüşümlü Kızılötesi Spektroskopisi (FT-IR)

To my beloved family and my love…

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INTRODUCTION

1.1. Maillard Reaction

Maillard reaction is known as the non-enzymatic browning reaction and one of the most important reaction occurs during processing of food and contributes to aroma, color and flavor of food such as bread, roasted nut, toasted cereals, pizza, flamed chicken, meat, coffee, and beer (Martins, Martins, & Jongen, 2017; Nooshkam, Varidi, & Bashash, 2019; Perez-Locas & Yaylayan, 2010; Wei, Liu, & Sun, 2018). The reaction occurs between reducing sugars and proteins even at moderate temperatures. The naturally occurring reaction between the reducing end of sugar and the amino acid was first discovered by Louis-Camille Maillard in 1912 and since then, it has been gaining popularity in food science and industry (Akhtar & Ding, 2017; Xu, Huang, Xu, Liu, & Xiao, 2019). It produces desirable flavors when occurs at coffee beans, cocoa beans, and meat. The reaction also brings appetizing color to bread crust, cookies, and roasted nuts. Besides, Maillard reaction causes formation of antioxidant, antimutagenic and antibiotic compounds as well as improvement of functional properties of proteins (Nooshkam et al., 2019; Perez-Locas & Yaylayan, 2010). On the other hand, it causes off-flavors, nutritional loss of protein and production of toxic compounds (Nooshkam et al., 2019; O'Mahony, Drapala, Mulcahy, & Mulvihill, 2017). HMF (5-hydroxymethylfurfural) and acrylamide, which are known toxic compounds, could be produced during Maillard reaction. Therefore, thermally processed foods such as infant formulas, UHT milk, and fruit juices should be treated carefully. Also, nutritional loss occurs since the Maillard reaction causes loss of essential amino acids (particularly lysine). Moreover, Maillard reaction often causes color change on the surface of the food. It is caused by brown pigmented insoluble compounds called melanoidins. They have variable nitrogen contents and degree of

Maillard (browning) is generally assessed by the concentration of melanoidins. Unluckily, the color change is not always desirable in foods like dried fruits and egg powder.

1.1.1. Mechanism of Maillard Reaction

Maillard reaction is composed of a complex network of different reaction pathways as seen in Figure 1.1 (Martins et al., 2017). To have a better understanding, Hodge (1953) explains Maillard reaction in three stages; early, intermediate and advanced stages. At the early stage, a reversible reaction between the carbonyl group of reducing sugar and available amino group of a protein occurs with the release of the water molecule and Schiff base is formed (Oliver, Melton, & Stanley, 2006; Poulsen et al., 2013). Schiff base compound is known as thermodynamically unstable and it produces colorless Amadori products by irreversible Amadori rearrangement (O'Mahony et al., 2017; Poulsen et al., 2013).

The intermediate stage of the Maillard reaction consists of degradation of Amadori products into short-chain carbonyl compounds depending on the water activity, pH and temperature (O'Mahony et al., 2017). These reactive intermediate compounds undergo various reactions including enolization, fragmentation, dehydration, cyclization, and oxidation (Sedaghat Doost, Nikbakht Nasrabadi, Wu, A'yun, & Van der Meeren, 2019). Colorless or yellow intermediate products are also called as pre-melanoidins known as unsaturated and susceptible to polymerization (Poulsen et al., 2013).

The advanced stage consists of variable reaction pathways and also depends on reaction conditions. The temperature and pH of the environment play an important role at this stage. Pre-melanoidins causes the formation of low or high molecular weight brownish nitrogenous polymers known as melanoidins (Liu et al., 2012; O'Mahony et al., 2017). These produced polymers are the main causes of toxic compounds and off-flavors (Sedaghat Doost et al., 2019). Since most of the color and flavor compounds are formed at this stage, outputs assign the quality of food products.

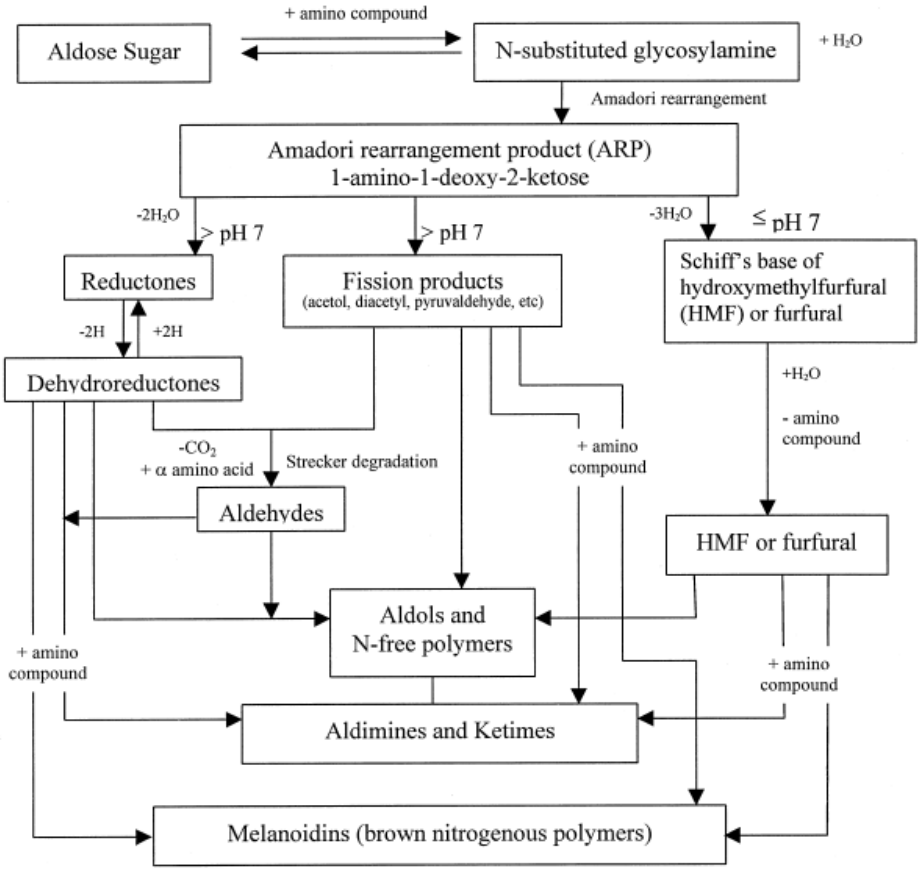

Figure 1.1. *Hodge's Maillard Scheme (Martins et al., 2017)*

1.1.2. Factors Affecting the Maillard Reaction

1.1.2.1. Time and Temperature

Maillard reaction is a spontaneous reaction and proceeds even at moderate temperatures. Yet, the reaction rate of Maillard increases with heating time and temperature (Abd El-Salam & El-Shibiny, 2018; Perez-Locas & Yaylayan, 2010; Wang & Zhong, 2014). However, high temperatures may cause denaturation of proteins or toxic compounds may be formed during processing. Also, the proportion of open-chain form reducing sugar increases with increasing temperature which leads to an increase in the reactivity of sugar molecules (O'Mahony et al., 2017). Therefore,

heating time and temperature could be set according to desired browning, flavor and texture of the foods (Perez-Locas & Yaylayan, 2010).

1.1.2.2. pH of Medium

The pH of the medium is also an important parameter for the Maillard reaction. The alkaline condition increases the reactivity of carbonyl and amine groups involved during the initial stage of the Maillard reaction (Perez-Locas & Yaylayan, 2010). At the pH above the isoelectric point of the amino acid, the amine group is negatively charged while the carbonyl group of the sugar is positively charged (Perez-Locas & Yaylayan, 2010). Therefore, the basic environment (e.g. pH 9) is known to increase the reactivity of the amine group (Abd El-Salam & El-Shibiny, 2018). Also, the open-chain form of hexose sugars increases with increasing pH values, which makes the carbonyl group more reactive at the initial condensation reaction (Perez-Locas & Yaylayan, 2010). During the Maillard reaction, a pH drop is observed. Sugar degradation causes acid formation and amino groups are converted into less basic forms (Nursten, 2005a). As pH decreases, protonated amino groups are dominated in equilibrium and they are considered as less reactive at the initial step of the Maillard reaction (Martins et al., 2017). Since pH has a strong effect on the reaction mechanism, it is better to work with alkaline buffer solutions. Also, some toxic compounds like HMF are formed at acidic conditions (Nursten, 2005b).

1.1.2.3. Water Activity

Since Maillard reaction initiates with condensation and dehydration steps, presence of water affects the reaction rates and pathways. Increasing the water activity enhances the diffusivity and mobility of the reactants. On the other hand, high water activity inhibits the reversible condensation reaction (O'Mahony et al., 2017). According to Labuza and Baiser (1992), browning rate increases from water activity of 0.2 and 0.3, reaches a maximum of around 0.5 to 0.8 and decreases after 0.8 (Figure 1.2.).

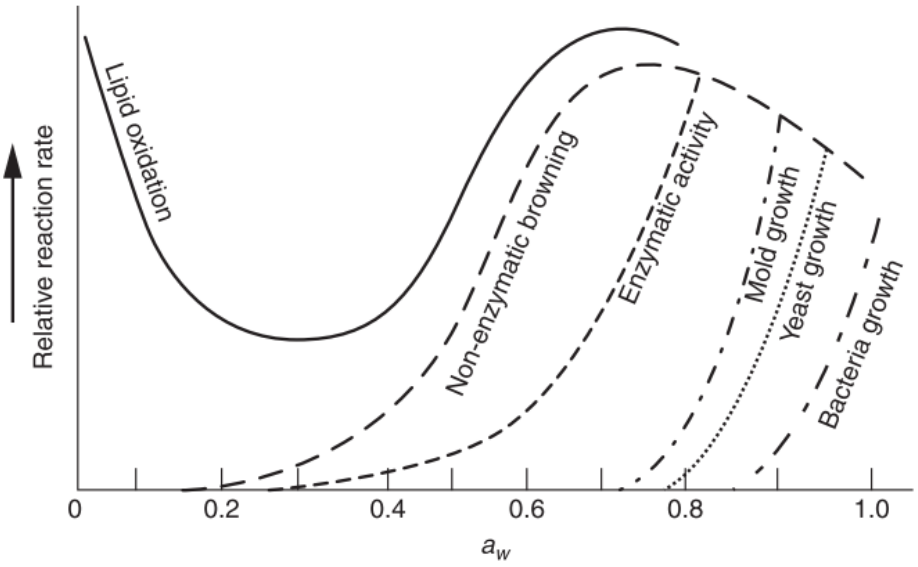

Figure 1.2. *Effects of water activity on relative reaction rate. Adapted from Hedegaard & Skibsted (2013)*

1.1.2.4. Type of Sugar and Protein

The sugar and protein types used in Maillard reaction affect the physicochemical properties, flavor and browning degree of the compounds produced (O'Mahony et al., 2017; Perez-Locas & Yaylayan, 2010). For example, sugars having rapid open ring form tend to increase the reaction rate and the degree of Maillard reaction (Abd El-Salam & El-Shibiny, 2018). The increasing molecular weight of the amino and carbonyl groups decreases the reactivity because of steric hindrance effects. As an example; monosaccharides are more reactive than disaccharides and polysaccharides (O'Mahony et al., 2017; Perez-Locas & Yaylayan, 2010). Therefore, sugar and protein type are one of the most important factors for Maillard reaction rate.

1.2. Non-Enzymatic Protein Glycation

Improving the functional properties of food proteins such as solubility, swelling, gelling capacity, and emulsifying ability is one of the major concerns in the food

science and food industry (Liu, Ru, & Ding, 2012). There are several methods to promote functional properties including physical, chemical and enzymatic methods such as hydrolysis, acetylation, acidification, esterification and enzymatic cross-linking (Spotti et al., 2013). However, most of the chemical methods are not advised since they include toxic components or they may change digestibility and allergenicity of food products (Nagasawa, Takahashi, & Hattori, 1996; Spotti et al., 2013). Therefore, controlled Maillard reaction has been gaining popularity over the last 15 years in terms of protein modification by conjugation of proteins with reducing sugars without using any chemical catalysts (Liu et al., 2012; Niu, Jiang, Pan, & Zhai, 2011; O'Mahony et al., 2017; Oliver, Melton, & Stanley, 2006; Sun et al., 2018). Since advanced Maillard products generally form toxic compounds, off-flavors and cause the loss of nutritional value, it is desired to finalize conjugation in the early stages of the Maillard reaction (Nooshkam et al., 2019; O'Mahony et al., 2017). Conjugation of proteins with sugars initiates with condensation reaction between the carbonyl group of reducing sugar and the amino group of protein which results in the production of Schiff bases and finally forms Amadori rearrangement (Bielikowicz et al., 2012; Zha, Dong, Rao, & Chen, 2019). These reactions are known as the initial step of the Maillard reaction and named as glycation reactions.

Glycation reaction is a spontaneous reaction basically accelerated by heating (Niu et al., 2011). Although histidine, tryptophan, and arginine are used to some extent, the primary amino source is often supplied by lysine (Liu et al., 2012). Since glycation originates from Maillard reaction, intrinsic and extrinsic factors including pH, temperature, time, water activity and protein and sugar type also affect the functional and physicochemical properties of glycated proteins (Carlos Arboleya, Javier Moreno, Sanmartin, & Villamiel, 2009; Liu et al., 2012). Therefore, glycation may cause both positive and negative changes in protein such as color, aroma and flavor formation, altered texture, solubility and emulsification properties (Oliver et al., 2006; Spotti et al., 2013; Zha et al., 2019).

Since glycation improves the functional properties of proteins, it has been applied to different protein sources. It was found to increase solubility and emulsion activity of oat protein isolate when conjugated with β-glucan (L. Zhong et al., 2019a). Similarly, in another work, oat- β-glucan conjugates had the highest swelling property, emulsification activity and fat binding capacity (Sun et al., 2019). When peanut protein isolate was glycated with dextran and gum arabic, it was found that emulsion activity, stability, and solubility of proteins were highly improved (Li, Xue, Chen, Ding, & Wang, 2014). Dried egg white protein glycated with fructose, glucose, and allulose showed higher antioxidant activities with increased processing time (Sun, Hayakawa, & Izumori, 2004). Pea protein concentrate and gum arabic glycation increased the solubility and emulsification activity (Zha et al., 2019). Soybean protein isolate was glycated with chitosan oligosaccharide and glucose, consequently, it was reported that glycation increased emulsification activity of soybean isolate (Xu et al., 2019).

1.3. Glycation Methods

Since glycation is a useful and safe method for improving the functional properties of proteins, the development of industry-feasible methods is needed (Oliver et al., 2006). There are conventional and novel methods used for glycation. Conventional methods are basically wet glycation and dry glycation while novel methods include microwave heating (Guan, Qiu, Liu, Hua, & Ma, 2006; Guan et al., 2011; Kamboj, Singh, Tiwary, & Rana, 2015; Tsubokura, Fukuzaki, Noma, Igura, & Shimoda, 2009; Tu et al., 2015; Wang et al., 2013), ultrasonication (Chen et al., 2019; Fu et al., 2019; Li et al., 2014; Perusko, Al-Hanish, Velickovic, & Stanic-Vucinic, 2015), pulsed electric field (Guan et al., 2010; Sun, Yu, Zeng, Yang, & Jia, 2011; Wang, Wang, Guo, Ma, & Yu, 2013) and electrospinning (Kutzli, Gibis, Baier, & Weiss, 2018, 2019; Turan, Gibis, Gunes, Baier, & Weiss, 2018).

1.3.1. Conventional Glycation Methods

1.3.1.1. Dry Heating Glycation

Dry heating glycation is one of the most used conventional glycation methods in the field of food science. It is achieved at lower water activities compared to wet heating glycation. Preparing sugar and protein solutions at a particular pH and freeze-drying of sugar-protein solution is generally the first step of the process. After obtaining powder form, the dried mixture is kept under specific temperature and relative humidity for hours to weeks (Sedaghat Doost et al., 2019). Common temperature used in dry glycation is between 60-130 ºC while relative humidity is between 60-80% (O'Mahony et al., 2017). On the other hand, since the glycation process needs several hours to weeks and controlled circumstances, it cannot be used in industrial-scale extensively.

1.3.1.2. Wet Heating Glycation

Wet heating glycation is an alternative to dry heating since it has a shorter processing time. It is achieved by direct contact of sugar and protein solutions while heating. Sugar-protein solutions are prepared at a particular pH and heated at a specific temperature and time. Generally, solutions are cooled immediately after heating to stop the chain reaction of Maillard. Applied glycation temperatures are generally between 60-95 ºC (O'Mahony et al., 2017). However, since direct heating is applied to solutions, protein denaturation and polymerization occur in some conditions. These drawbacks may be reduced by macromolecular crowding method which is increasing the concentration of macromolecules (especially proteins) to modify the properties of these macromolecules. In that case, the native structure of macromolecules could be conserved during glycation (Sedaghat Doost et al., 2019).

1.3.2. Microwave Glycation

Microwaves are known as electromagnetic waves having a frequency between 0.3 and 300 GHz. For microwave heating, 0.915 and 2.45 GHz are mostly used. When

electromagnetic waves reach to a dielectric material, some energy is transmitted and some are reflected whereas part of the energy is absorbed and converted to heat (Meda, Orsat, & Raghavan, 2016). Simply, microwave heating is produced by the ability of a material to absorb and convert that electromagnetic waves into heat. Therefore, microwave heating supplies rapid and uniform heating (which is also called as volumetric heating). The heating mechanism of microwave is explained by dipolar rotation and ionic conduction mechanisms. The dipolar rotation mechanism is explained by the friction created by the realigning of polarized dipolar molecules to the direction of the electric field. Since the electric field has high frequency, rotation of dipoles occurs 2.45 billion times per second and heat is generated by the friction of rotating dipoles (Guo, Sun, Cheng, & Han, 2017). Ionic conduction mechanism is explained by the ionic nature of the food sample. High frequency oscillating electric field causes oscillatory displacement of ions and induces heating of the food material (Chandrasekaran, Ramanathan, & Basak, 2013). Microwave heating reduces cooking time with increased heating rate. Also, the microwave heating method is known as an energy-saving, space-saving, having lower maintenance requirements, and it improves yield and purity in chemical synthesis when compared to conventional heating methods (Guan et al., 2011). Besides, microwave heating preserves flavor and nutritional properties of food more than conventional methods (Chandrasekaran et al., 2013). Unlike conventional heating methods having convection, conduction, and radiation heat transfer mechanisms, microwave heating supplies volumetric heating to the food (Sumnu & Sahin, 2005). Therefore, the use of microwave heating has gained popularity in the food science and industry. Microwave heating has been used in drying (Sumnu, Turabi, & Oztop, 2005), roasting (Uysal, Sumnu, & Sahin, 2009), frying (Barutcu, Sahin, & Sumnu, 2009; Mecit Halil Oztop, Sahin, & Sumnu, 2007), baking (Ozge Keskin, Sumnu, & Sahin, 2004; Ozge, Sumnu, & Sahin, 2009), pasteurization and sterilization (Auksornsri, Bornhorst, Tang, & Tang, 2018; Stanley & Petersen, 2017), extraction (Cassol, Rodrigues, Pelayo, & Noreña, 2019; Setyaningsih, Palma, & Barroso, 2012) and for glycation processes (Bi et al., 2015; Zhang et al., 2012). Microwave also brings about selective heating which produces

non-uniform temperature distribution causing uneven heating, overheating or hot and cold spots (Ekezie, Sun, Han, & Cheng, 2017). Selective heating occurs due to selective absorption of microwave energy by polar molecules. To overcome non-uniform heating, adjusting heating conditions (time, power, etc.), using combined methods, altering food size and geometry, using special oven designs have been offered as alternatives (Sumnu & Sahin, 2005). Although conventional glycation methods give sufficient results for improving the functional properties of proteins, the process takes a long time from hours to weeks. Therefore, new glycation techniques are investigated. Microwave heating is an alternative to the conventional glycation process since it provides higher heating rates with shorter times. In the glycation process, the production of advanced Maillard products is not recommended because they include potential toxic and mutagenic compounds. Since microwave glycation takes a shorter time than conventional methods, there is a possibility of producing advanced Maillard products to a lesser extent.

There are few studies about microwave glycation of proteins in the literature and existing studies mostly focused on glycation of ovalbumin protein. Tsubokura et al. (2009) studied microwave glycation of ovalbumin with glucose using the dry heating method. They found out that dry glycation was not an effective way for microwave glycation. Also, Wang et al. (2013) compared ovalbumin glycation treated by conventional and microwave methods by high resolution mass spectrometry and found out parallel results with previous research. In another research, microwave glycation of ovalbumin (with the dry heating method) was studied by changing microwave power and treatment time (Tu et al., 2015). It was concluded that the glycation rate and extent increased with increasing reaction time and microwave power. Moreover, they suggested microwave glycation as a novel approach to glycation of ovalbumin protein.

Bi et al. (2015) studied microwave glycation of casein with β-cyclodextrin. They experimented with the wet glycation method and compared conventional and microwave glycation of casein protein. It was reported that casein glycation was

accelerated with microwave treatment and improved functional properties were obtained.

Microwave glycation of bovine serum albumin with maltodextrin was examined by Nasrollahzadeh et al. with wet glycation method (2017) and they showed that microwave glycation was more effective than conventional glycation and it improved solubility, emulsion activity and foaming capacity of the protein.

To our knowledge, there are only two studies conducted about microwave glycation of soy protein. The first research was carried out by Guan et al. (2006). They studied microwave glycation of soy protein isolate with lactose, maltose, dextran, and soluble starch. Since microwave irradiation has a heating effect on polar molecules including water, they preferred wet glycation method. To examine the glycation extent; determination of free amino groups, browning index, amino acid analysis, Fourier Transform Infrared Spectroscopy (FTIR), and reaction rate calculation experiments were conducted. It was reported that microwave enhanced the mobility of sugar molecules, therefore, increased the reaction rate. When the free amino group concentrations reached the same level at microwave and conventionally glycated samples, the reaction time needed for conventional glycation was found to much longer than microwave glycation. The other research mostly focused on the mechanism of microwave glycation of soy protein isolate with lactose and soluble starch (Guan et al., 2011). They reported that the microwave irradiation could be the reason for the increase of water exposure to hydrophobic core residues of protein and the breaking of the disulfide bonds. Therefore, the average hydrodynamic radius of microwave glycated protein was found to be smaller than native soy protein isolate and conventionally glycated ones. Also, it was found out that microwave heating reduced the activation energy of soy protein isolate glycation.

1.4. Soy Protein Isolate

Soy protein is one of the most popular plant-based protein which has been used in the food industry as a nutritional and functional food ingredient since the 1960s (Singh,

Kumar, Sabapathy, & Bawa, 2008). It has been used in the production of bars, bread, dairy products, and beverages as a functional ingredient as well as in capsules and tablets in the supplement industry (Singh et al., 2008). Soy protein is easily digested in the body and amino acid composition and patterns simulate good quality animal protein sources like milk, meat, and eggs (Singh et al., 2008; Wolf, 1970). Providing 9 essential amino acids, soy protein provides lots of benefits such as proper amino acid composition balance, physiologically healthful constituents which reduces cholesterol and the risk of cardiovascular diseases (Nishinari, Fang, Guo, & Phillips, 2014; Singh et al., 2008).

Soy protein is made up of about 10% of albumins and 90% of globulins (Kunte, Gennadios, Cuppett, Hanna, & 0Weller, 1997). Globulins are related to hydrophobic, hydrogen bonding and disulfide bond subunits (Shan et al., 2015). Soy proteins are composed of four globulin fractions marked according to sedimentation rates, which are 2S, 7S, 11S and 15S in Svedberg units (Ciannamea, Stefani, & Ruseckaite, 2014). Among these fractions, nearly 37% of the total protein is 7S (β-conglycinin) while 31% is 11S (glycinin) (Kunte et al., 1997).

Soy flour, soy protein concentrate, and soy protein isolate are products processed from soybean. Soy flour is obtained by grinding of soybean into powder and contains 40-60% of protein (Ciannamea et al., 2014; Kalman, 2014; Koshy, Mary, Thomas, & Pothan, 2015). Soy protein concentrate is processed by eluting soluble components from defatted soy flour and it contains more than 65% protein (Koshy et al., 2015). On the other hand, soy protein isolate is known as the purest form of soy protein since it consists of more than 90% of protein (Singh et al., 2008). Soy protein isolate is produced by removing most of the non-protein components, fats, and carbohydrates (Singh et al., 2008; Wolf, 1970).

Soy protein isolates are known as the most functional form of soy proteins since it has good nutritional quality and excellent processing abilities like gelling and water and oil holding capacity (Nishinari et al., 2014; Singh et al., 2008). Also, soy proteins are

amphiphilic components, therefore, they are used to stabilize emulsions and foams (Dickinson, 2010).

On the other hand, since soy protein has larger molecular weight and globular structure, it has poor surface activity than lower molecular weight proteins like β-casein (W. Li et al., 2016). Poor functional properties of soy proteins has drawn attention to structural modification over the past decades (Boostani, Aminlari, Moosavi-nasab, Niakosari, & Mesbahi, 2017; de Oliveira, Coimbra, de Oliveira, Zuñiga, & Rojas, 2016; Li, Bao, Xu, & Chi, 2014; Xue, Li, Zhu, Wang, & Pan, 2013). Soy protein has 18 amino acids which include polar functional groups (carboxyl, amine, and hydroxyl groups) making it chemically reactive for modification (Tian et al., 2018).

1.5. Monosaccharides

Monosaccharides are simple carbohydrate molecules that cannot be hydrolyzed into smaller molecules. Therefore, they are also called simple sugars. Monosaccharides have an important role in the food industry since they are primary energy sources and valuable food ingredients. Also, they are abundant in nature, accessible, hence cheap. In formulation with other components like water, proteins and other carbohydrates, monosaccharides supply enhancing properties to structure and flavor. Therefore, simple sugars are substantial ingredients used in almost all food products including beverages, cakes, candies, etc. Moreover, monosaccharides are required components of Maillard reactions and reaction progression is influenced by molecular structure (Cheetangdee & Fukada, 2014).

Monosaccharides are formed by carbon atom chains in different lengths and named consistent with the number of carbon atoms (e.g. tetroses, pentoses, hexoses, etc.). They are mainly categorized as aldoses and ketoses according to carbonyl groups. When the highest numbered chiral carbon has a hydroxyl group on the right-hand side, the monosaccharide is called D sugars, otherwise called as L sugars (e.g. D-glucose, L-arabinose).

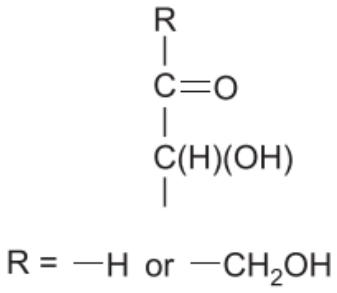

Figure 1.3. *A monosaccharide is called as Aldose when R is a hydrogen atom or Ketose when R is a -CH2OH group (BeMiller, 2019b)*

1.5.1. Glucose

Glucose is the most plentiful monosaccharide found in nature and it is used by cells as an energy source. It is also named as dextrose and found only as D-glucose in nature (BeMiller, 2019b). Since it has six carbon atom and being an aldehyde, known as the typical aldohexose.

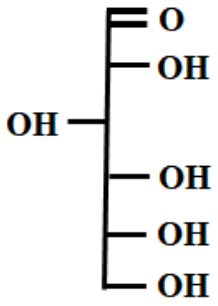

Figure 1.4. *Representation of D-Glucose*

The sweetness of glucose is about 70% of sucrose and it is used as an ingredient including baking, brewing, canning, and confectionary products (BeMiller, 2019a). It is also found in honey, fruits, and vegetables. Glucose also forms disaccharides and polysaccharides, generates sucrose, lactose, starch, cellulose, and glycogen.

Glucose can undergo Maillard reactions with proteins and it improves the functional properties. It was found to be effective in improving the functional properties of soy protein isolate (Tian, Chen, & Small, 2011). It was found that solubility and emulsification activity of soy protein isolate increased by glycation with glucose. Similar results were obtained in the glycation of β-lactoglobulin and it was concluded that the best candidate for glycation of β-lactoglobulin was glucose among fructose and allulose (Cheetangdee & Fukada, 2014).

1.5.2. Fructose

Fructose, also known as levulose or fruit sugar, is a typical example of ketohexoses. It is abundant in nature within fruits, plants, and honey. Since fructose has a higher ability to form hydrogen bonds with water, it is a better humectant than glucose. In other words, fructose absorbs more water than glucose (BeMiller, 2019b). The sweetness of fructose is about 1.2-1.7 times that of sucrose, thus higher than glucose (BeMiller, 2019a). The higher sweetness of fructose makes it useful in the production of confectionaries, bakery products, and drinks. Thus, fructose also exists as syrups such as high fructose corn syrup (HFCS), invert sugar syrup, and isoglucose syrup. Fructose is commercially produced by maize starch hydrolysis to glucose and transforming to fructose by enzymatic isomerization.

Sugars with the same number of carbon atoms are called isomers of each other. Carbonyl group and the corresponding hydroxyl group of sugar may undergo isomerization reaction. Isomerization reaction is a reversible reaction that converts aldose to another aldose or corresponding ketose or vice versa.

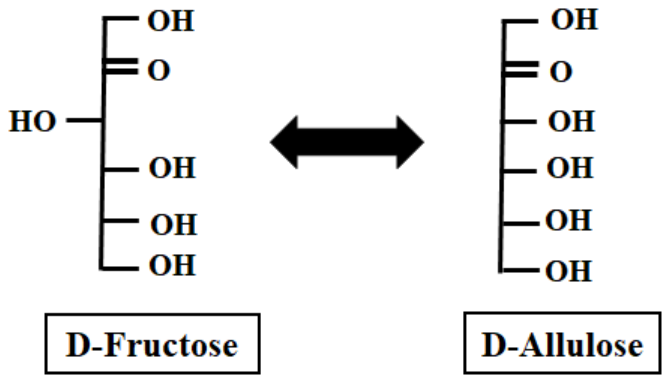

Figure 1.5. *Isomerization of D-fructose to D-Allulose*

The reaction could occur by an enzyme, or by itself in alkaline conditions, e.g. Lobry de Bruyn-Alberda van Ekenstein transformation (Yu et al., 2011). Immobilized isomerase enzyme is generally used for producing HFCS. Non-enzymatic isomerization reaction mostly occurs at high pH and temperature (Akram & Hamid, 2013; Oshima, Kimura, Kitakubo, Hayakawa, & Izumori, 2014). Therefore, Maillard reaction may cause the isomerization of sugars, and the molecular structure of sugars has effects on the progress of the reaction (Cheetangdee & Fukada, 2014). It was found that the heating of casein and sugar solutions caused the isomerization of fructose to glucose and vice versa (van Boekel & Brands, 1998). In a study, allulose formation in heated food products was examined (Oshima et al., 2014). It was concluded that allulose formation from fructose isomerization increased at a higher concentration of fructose, temperature, pH, and processing time.

1.5.3. D-Allulose

Rare sugars are defined as monosaccharides and their derivatives rarely observed in nature. There are almost 50 kinds of rare sugar discovered so far (Hossain, Yamaguchi, Matsuo, Tsukamoto, & Toyoda, 2015). Most popular rare sugars are D-allulose, D-tagatose, D-allose, and D-sorbose.

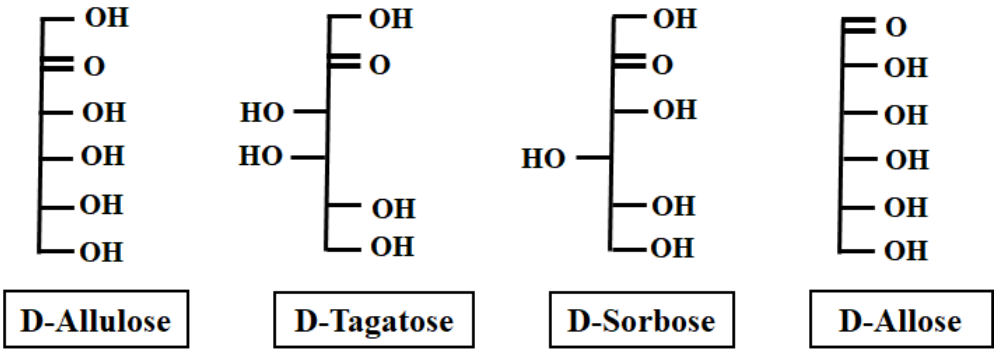

Figure 1.6. *Representation of some rare sugars*

D-Allulose (also known as D-psicose) is the C-3 epimer of fructose. It is not commonly found in nature and exists in small amounts in the leaves of Itea plant (Zuina), wheat, and some bacteria (Hossain et al., 2015). Also, a trace amount of D-allulose is found in heat-applied commercial products including D-glucose and D-fructose mixtures, steam-treated coffee, fruit juices, caramel, maple syrup, and molasses (BeMiller, 2019a; Hossain et al., 2015; Oshima, Kimura, & Izumori, 2006). The amount of D-allulose in these products varies according to sugar concentration, heating time and temperature (Zhang, Yu, Zhang, Jiang, & Mu, 2016).

Although D-allulose is rarely found in nature, it is chemically synthesized by a process called *Hexose Izumoring* strategy. According to Professor Ken Izumori, from Kagawa University Rare Sugar Research Centre, Japan, all ketohexoses and aldohexoses could be synthesized from cheap D-hexoses such as D-glucose or D-fructose. Thereby, D-fructose is converted to D-allulose by the ketose 3-epimerase enzyme called D-psicose 3-epimerase (DPEase) (Izumori, 2006). DPEase enzyme is mostly obtained from a specific microorganism, *Agrobacterium tumefaciens* (Zhang, Yu, Zhang, Jiang, & Mu, 2016).

Studies have demonstrated many health benefits of D-allulose including reducing glucose level, enhancing insulin resistance, and suppressing fat accumulation in the body (Hossain et al., 2015; Zhang et al., 2016). Furthermore, it does not have any

glycemic effect, considered as prebiotic, and promotes loss of body weight (Putten, Waal, Jong, & Heeres, 2017; Zeng, Zhang, Guan, Zhang, & Sun, 2013). The sweetness of D-allulose is almost 70% of sucrose (Zhang et al., 2016). Yet, the caloric value of D-allulose is about 0.39 kcal/g while that of sucrose is 4 kcal/g (O'Charoen, Hayakawa, Matsumoto, & Ogawa, 2014). Besides, a smaller amount of D-allulose is absorbed by the small intestine and metabolized into energy, and the rest is excreted by the urine. Hence, it could be considered as a low-calorie sweetener and used for the replacement of sucrose.

Established benefits and cost-effective producing methods of D-allulose have created an opportunity to use it in the food industry. D-Allulose was accepted as Generally Recognized as Safe (GRAS) by the US Food and Drug Administration (FDA), in June 2014. Since D-allulose has GRAS status, it is appropriate to use it as an ingredient in food products. Several food products with D-allulose have been marketed in Japan and the United States. It has also been launched as a natural sweetener by a couple of companies such as Tate & Lyla, Matsutani Chemical Industry Company, Bonumose LLC, and Anderson Global Group. Furthermore, in 2019, FDA declared that D-allulose will no longer be categorized under the sugars category in food labels. However, the European Food Safety Authority (EFSA) has not approved D-allulose yet.

Since D-allulose is also a reducing sugar, it undergoes Maillard reaction when heated with proteins and produces enhanced functional properties such as better anti-oxidative activity and gelling properties (Hossain et al., 2015). When compared to fructose, tagatose, and sorbose, allulose showed the best improvement in antioxidant activities, gelling, emulsifying, and foaming properties in glycated egg white proteins (Charoen, Hayakawa, & Ogawa, 2015). In another study, glycation of α-lactalbumin with allulose, fructose, and glucose was investigated. It was found that Maillard reaction rate, antioxidant activity, and the browning degree was the highest at allulose modified proteins (Sun, Hayakawa, Ogawa, Fukada, & Izumori, 2008; Sun, Hayakawa, Puangmanee, & Izumori, 2006). Besides, whey protein isolate glycated

with D-allulose showed higher emulsion properties (Puangmanee, Hayakawa, Sun, & Ogawa, 2008).

1.6. Objective of The Study

The objective of the present study is improving the functional properties of soy protein isolate by using D-allulose with the help of microwave heating. Different protein-sugar weight ratio (1.6 and 7.2) were applied at pH values of 7 and 10. In the first part of the study, microwave glycation of soy protein with glucose, fructose, and D-allulose was achieved. Properties of glycated proteins were examined by chemical and instrumental methods such as HPLC, NMR Relaxometry, and FTIR. In the second part of the study, effects of microwave glycation and water bath glycation were compared for the same conditions.

In the literature, D-allulose was found to be very effective in terms of improving the functional properties of proteins (Charoen et al., 2015; Hossain et al., 2015; Puangmanee et al., 2008). On the other hand, microwave heating was reported as a useful method since it accelerated Maillard reaction and produced more enhanced proteins than conventional methods (Bi et al., 2015; Guan, Qiu, Liu, Hua, & Ma, 2006; Nasrollahzadeh, Varidi, Koocheki, & Hadizadeh, 2017; Tu et al., 2015). However, there is no study about soy protein isolate glycation with D-allulose in the literature. Moreover, microwave glycation of soy protein isolate with D-allulose has not been studied yet. Therefore, the hypothesis of this study could be described as microwave heating will accelerate the glycation of soy protein isolate, and D-allulose will improve the properties of protein more than other sugars.

CHAPTER 2

MATERIALS AND METHODS

2.1. Materials

Soy protein isolate was purchased from Alfasol, Turkey. For glycation of soy protein isolate D(-)-fructose (Merck KGaA, Darmstadt, Germany), glucose (Dextrose, Tito, Turkey), and D-allulose (Santiva Inc, Downers Grove, IL, USA) were used as sugar sources. Sodium hydroxide (NaOH), sodium carbonate (Na_2CO_3), sodium bicarbonate ($NaHCO_3$), potassium sodium tartrate tetra-hydrate ($C_4H_4KNaO_6 \cdot 4H_2O$), zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$), o-phthaldialdehyde (OPA), sodium dodecyl sulfate (SDS), glycine, bovine serum albumin (BSA), acetonitrile, β-mercaptoethanol (2-mercaptoethanol) were purchased from Sigma-Aldrich Chemical Co. (Saint Louis, MO, USA). Sodium dihydrogen phosphate dihydrate ($NaH_2PO_4 \cdot 2H_2O$), di-sodium hydrogen phosphate dihydrate ($Na_2HPO_4 \cdot 2H_2O$), di-sodium tetraborate decahydrate ($Na_2B_4O_7 \cdot 10\ H_2O$), potassium hexacyanoferrate (II) trihydrate ($K_4[Fe(CN)_6] \cdot 3H_2O$), ethanol, Folin-Ciocalteu's phenol reagent were supplied from Merck KGaA (Darmstadt, Germany).

2.2. Methods

2.2.1. Sample Preparation

For microwave and water bath glycation, wet glycation method was used. Therefore, soy protein and sugars (fructose, D-psicose, dextrose) were mixed in 0.1 M phosphate buffer (pH 7 and pH 10) at a total solid content of 10% (w/v). Weight ratios of protein-sugar were determined by preliminary studies as 1.6:1 and 7.2:1. The prepared solution was mixed with UltraTurrax (WiseTis Homogenizer, Witeg Labortechnik GmbH, Germany) at 7,200 rpm for 2 minutes to hydrate the soy proteins. Distilled

water was obtained from 0.2 µs/cm purity mpMinipure Dest system (mpMinipure Ultrapure Water Systems, Ankara, Turkey).

Preliminary studies were conducted to find the optimum protein-sugar weight ratio to work with. As being a ketose and a reactive sugar, fructose was used in the preliminary tests. Protein-sugar weight ratios of 1.6, 4, 5.6 and 7.2 were tested. The effects of increased protein concentration on glycation process were taken into consideration. According to the remaining free amino group concentration, reducing sugar concentration and Fourier Transform infrared spectrometry (FT-IR) measurements, two extreme values were selected (1.6 and 7.2).

2.2.2. Glycation of Soy Protein Isolate

2.2.2.1. Microwave Glycation

Soy protein-sugar solutions were heated at microwave oven (Advantium ovenTM, General Electric Company, Louisville, KY, USA). The maximum microwave power of the oven was found to be 700 W by using IMPI-2L test (Buffler, 1993). Protein-sugar solutions were heated at a power level of %30 for 4 minutes to bring the solution temperature to 90 ºC as this was the desired temperature for glycation considering the references (Nasrollahzadeh et al., 2017; Guan et al., 2011; Guan et al., 2006). Solutions were heated in 100 ml glass bottle with a volume of 35 mL and they were cooled in ice-bath immediately after heating to stop chain reactions. Microwave power and heating time were selected by preliminary studies. Main effects for the choices were reaching the optimum Maillard reaction temperature and avoiding overflow of the samples upon heating.

2.2.2.2. Water Bath Glycation

To understand the effect of microwave heating, as the conventional glycation method; glycation was performed in a water bath. Prepared protein-sugar solutions were heated in 100 mL glass bottle with a volume of 35 mL at a water bath (90 ºC) for 4 minutes. Solutions were cooled in ice-bath immediately after heating to stop the reactions.

Water bath glycation was only applied for glycation of soy protein isolate with fructose at pH 10 since preliminary results showed that fructose was the most active sugar at microwave glycation and glycation rate was the highest at pH 10. Therefore, water bath glycation results were compared with microwave glycated soy protein isolate with fructose at pH 10.

2.2.3. Lyophilization of Glycated Proteins

Samples were frozen at -18 ºC in the freezer (Arçelik, Turkey) and lyophilized (Beijing Songyuan Huaxing Technology Development Co., Ltd., China) for 48 hours. Finally, the dried samples were ground to powder form for further use. For the non-glycated soy protein isolates, the same procedures were applied except microwave or water bath heating. Each sample was prepared as triplicate and stored at 4 ºC for further analyses.

2.2.4. Quantification of Free Amino Groups

For the quantification of available amino groups, 100 mg of glycated samples were dissolved in 20 mL of corresponding buffer solutions (pH 7 or pH 10). Solutions were stirred in an orbital shaker (Daihan Scientific Co., Ltd., Korea) for overnight to obtain complete hydration. Mixed solutions were then centrifuged (MF-80, Hanil Science Industrial Co. Ltd., South Korea) at 4,000 rpm for 5 minutes. The supernatants were used as samples for the OPA experiment. Control samples consisted of native soy protein isolate only and the same procedures were applied. The measurements were performed as triplicates.

Available amino groups were quantified by the OPA method with some modifications (Guan et al., 2006). OPA solution was prepared daily by dissolving 40 mg OPA in 1 mL of ethanol (%95 v/v) followed by adding 25 mL of 100 mM sodium tetraborate (pH 9.7), 2.5 mL SDS (%20 w/w) and 100 μL β-mercaptoethanol. The solution was diluted to 50 mL with distilled water. OPA reagent of 1.5 mL was added to 0.5 mL sample and incubated at dark for 2 minutes before reading absorbance at 340 nm in UV/VIS Spectrophotometer Optizen Pop Nano Bio (Mecasys Co. LTD, Korea) The

calibration curve was obtained by daily with glycine for the concentrations of 0.01, 0.02, 0.03, 0.04, 0.05 g/L. Example of a calibration curve is given in the appendix B (Figure B.1).

2.2.5. Quantification of Reducing Sugar Content by High Pressure Liquid Chromatography (HPLC)

Glycated proteins of 0.25 g were dissolved in 9 mL of HPLC grade water (Milli-Q Water System, Millipore S.A., France). Solutions were stirred overnight at orbital shaker (Daihan Scientific Co., Ltd., Korea) at 180 rpm for 24 hours to ensure complete hydration. After hydration, 0.5 mL Carrez I and 0.5 mL Carrez II solutions were added and vortexed for 3 minutes to precipitate the proteins (Carrez I solution was obtained by dissolving 15 g potassium hexacyanoferrate (II) trihydrate in 100 mL HPLC grade water. Carrez II solution was obtained by dissolving 30 g zinc sulfate heptahydrate in 100 mL HPLC grade water). Solutions were centrifuged (MF-80, Hanil Science Industrial Co. Ltd., South Korea) at 4,000 rpm for 7 minutes and the supernatant was filtered with 0.45 μm nylon filters before HPLC-RID (Shimadzu Scientific Instruments, Japan) analysis. The measurements were performed as triplicates.

The HPLC system consisted of a degasser (DGU-20A_5), pump (LC-20AD), auto-sampler (SIL-20A HT), column oven (CTO-20A), and refractive index detector (RID-20A). The inertsil NH_2 column (dimensions of 250x4.6, 5μm) was purchased from Shimadzu Scientific Instruments (Japan). The mobile phase consisted of acetonitrile and water (80:20 v/v). Vacuum filtration by 0.2 μm membrane was applied to the mobile phase before the operation. Flow rate, injection volume, and oven temperature were 1 mL/min, 20 μl, and 40 ºC, respectively.

The calibration curve was obtained by sugar solutions for the concentrations of 5, 8, 10, 15, 20 g/L. Equation of calibration curves and retention times of the sugars are given in Table 2.1. An example of a chromatogram is given in Figure 2.1. for the concentration of 10 g/L. Calibration curves are given in the Appendix B (Figure B.2., 3., 4.).

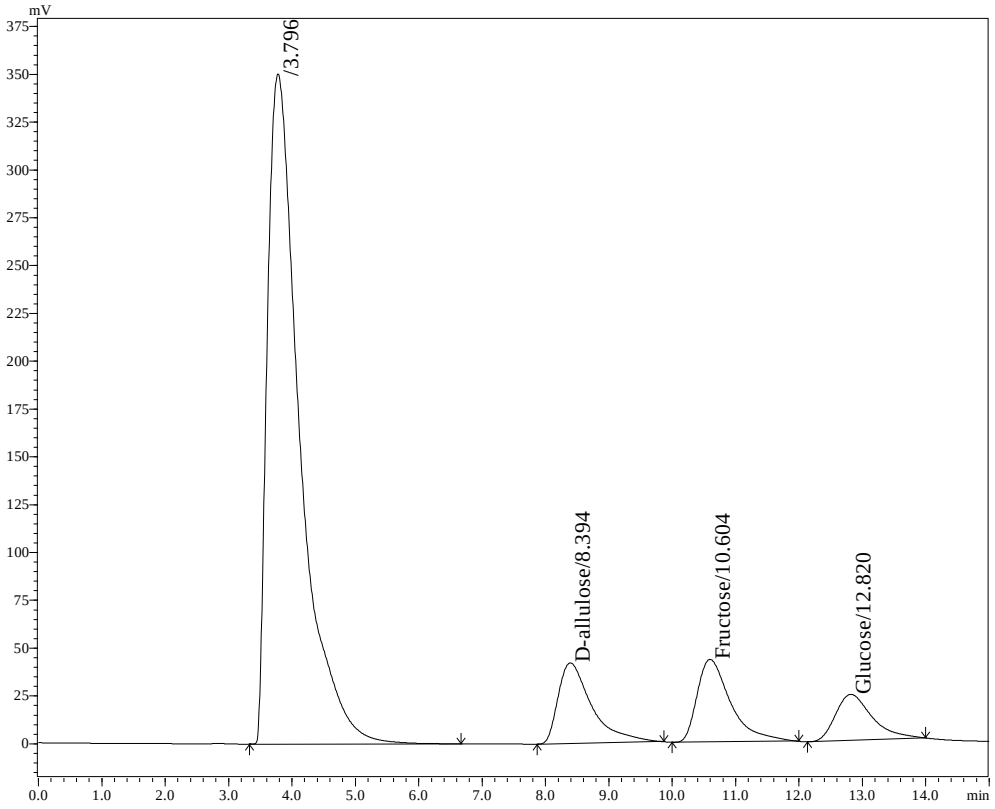

Figure 2.1. *Chromatogram of sugars for the concentration of 10 g/L*

Table 2.1. *Calibration curves and retention times of sugars*

Sugar Type	Retention Time (min)	Calibration Curve*	R^2
D-allulose	8.4	y = 0.000006x ± 0.0755	0.992
Fructose	10.6	y = 0.000006x ± 0.0633	1.000
Glucose	12.8	y = 0.000009x ± 0.6708	0.996

*y represents the concentration as g/L and x represents the area under peak.

2.2.6. Solubility of Glycated Proteins

Sample preparation for determination of soluble proteins included dissolving of 100 mg glycated samples in 20 mL of corresponding buffer solutions (pH 7 or pH 10). Solutions were stirred in an orbital shaker (Daihan Scientific Co., Ltd., Korea) for overnight to obtain complete hydration. Mixed solutions were then centrifuged (MF-80, Hanil Science Industrial Co. Ltd., South Korea) at 4,000 rpm for 5 minutes. Finally, ½ dilution was applied to the supernatant for further spectrophotometric analysis. Control samples consisted of native soy protein isolate and the same procedures were applied.

For the quantification of soluble proteins, Lowry method was used (Lowry et al., 1951). The reagents used to prepare Lowry solution are given in Table 2.2. Lowry solution was prepared by mixing reagents A:B:C at a volume ratio of 100:1:1, respectively. 0.5 mL sample was mixed with 2.5 mL Lowry reagent and incubated 10 min at room temperature. Then, 0.25 mL Folin-Ciocalteu's phenol reagent (diluted with a ratio of 1:1 from 2N stock solution) added to the tubes, mixed well, and incubated at dark for 30 minutes. Finally, the absorbance values were recorded at 680 nm by a UV/VIS Spectrophotometer Optizen Pop Nano Bio (Mecasys Co. LTD, Korea). The calibration curve was obtained by BSA for the concentrations of 0.03125, 0.0625, 0.125, 0.25, 0.5 g/L. Calibration curve was obtained by absorbance vs g/L BSA concentration ($y=1.9754x + 0.0598$, $R^2=0.99$) (Appendix B, Figure B.5.). All the measurements were performed as triplicates.

Table 2.2. *The reagents used to prepare Lowry solution*

Reagent A	2% (w/v) Na_2CO_3 dissolved in 0.1 N NaOH
Reagent B	2% (w/v) $CuSO_4 \cdot 5H_2O$
Reagent C	2% (w/v) $C_4H_4KNaO_6 \cdot 4H_2O$

2.2.7. Browning Intensity

Melanoidins are formed at the final stage of the Maillard reaction and cause brownish components. Measurement of browning intensity was performed by spectrophotometric analysis.

Sample preparation was the same with OPA method. The supernatant was also used for determination of browning intensity with UV/VIS Spectrophotometer Optizen Pop Nano Bio (Mecasys Co. LTD, Korea) at 420 nm. Control samples consisted of native soy protein isolate and the same procedures were applied. The measurements were performed as triplicates.

2.2.8. Fourier Transform Infrared Spectroscopy (FT-IR) Experiments

Changes in the protein structure was measured with IR Affinity-1 Spectrometer with Attenuated Total Reflectance (ATR) (Shimadzu Corporation, Kyoto, Japan). Freeze-dried samples were analyzed in the spectra of 4000-600 cm^{-1} with 32 scans, at a resolution of 4 cm^{-1}. Native soy protein isolate, fructose, glucose, and D-allulose were also measured as control samples. All measurements were done as triplicates.

2.2.9. Time Domain Nuclear Magnetic Resonance (TD-NMR) Relaxometry Analyses

Time Domain Nuclear Magnetic Resonance Relaxometry experiments were performed with a 0.5 Tesla (20.34 MHz) NMR system (Spin Track GmbH, Kircheim/Teck, Germany) equipped with a 10mm RF probe. Spin-spin (T_2) relaxation time measurements were done using CPMG (Carr-Purcell-Meiboom-Gill) sequence. Number of echoes was set to 1,200 with an echo time of 500 μs. Relaxation period was set to 1,100 ms. T_2 relaxation times of samples were measured before and after glycation. Experiments were done as duplicates.

2.2.10. Statistical Analysis

Statistical analysis was done for all of the experimental data to check the significant difference of factors used. All measurements were performed as duplicates or triplicates. Analysis of variance (ANOVA) was performed using the general linear model by using Minitab V17 (Minitab Inc., Coventry, UK) at 5% significance level. For the comparisons, Tukey's comparison test was performed with 95% confidence interval. All of the assumptions of ANOVA were checked prior to analysis and irrelevant data were removed if necessary. The letters indicate significant difference among samples ($p<0.05$).

2.3. Experimental Design

A summary of the factors, levels and the responses are given in Table 2.3.

Table 2.3. *Experimental design with factors, levels, and responses*

Factors	**Levels**	**Responses**
Glycation Method	Microwave, Water Bath*	1. Quantification of Free Amino Groups 2. Quantification of Reducing Sugar Content by HPLC 3. Solubility of Glycated Proteins 4. Browning Intensity 5. FT-IR Experiments 6. NMR Relaxometry Analyses
Glycation pH	7, 10	
Sugar Type	Fructose, Glucose, D-allulose	
Protein-Sugar Ratio (w/w)	1.6, 7.2	

*****Water bath glycation was only applied for fructose sugar at pH 10

CHAPTER 3

RESULTS AND DISCUSSION

3.1. Measurement of Free Amino Group Concentration

Glycation occurs between the reducing end of the sugar and free amino group of the protein to form Schiff base and causes permanent depletion of available amino groups of proteins. Therefore, when protein is glycated, the concentration of the free amino group of the protein is expected to decrease. In this study, as stated, the free amino group concentration of glycated protein was measured by the OPA method. The reaction of OPA with free amino groups of protein produces 1-alkylthio-2-alkylisoindole when β-mercaptoethanol and SDS involve the reaction (Tsubokura et al., 2009). The formed component has the maximum absorbance at 340 nm. Results were compared with the native soy protein isolate since no modified amino group exist at the beginning.

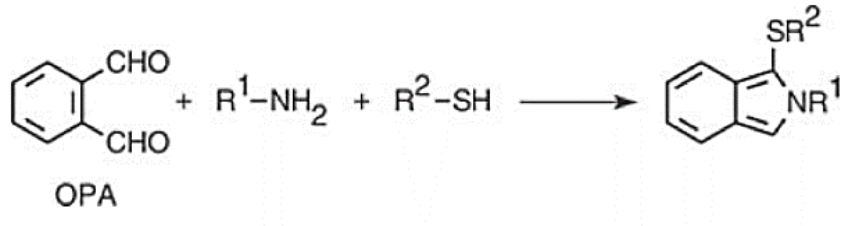

Figure 3.1. *OPA reaction with primary amino groups and β-mercaptoethanol. Adapted from Oliveira et al. (2016)*

ANOVA analysis of microwave glycation were conducted with the factors of pH (7 and 10), sugar type (fructose, glucose, D-allulose), and the weight ratios of soy protein-sugar (1.6 and 7.2). According to the results, effects of pH, sugar type, and protein-sugar ratio and their interactions were all found to be significant ($p<0.05$).

Experimental results (Table A.1.) and statistical analysis results (Table C.1.) are given in Appendix section.

Figure 3.2. represents mg free amino group/g free amino group in native soy protein isolate at the corresponding pH (mg FAG/g FAG in native SPI). According to the results, glycation pH had a significant effect on the concentration of the free amino groups ($p<0.05$). Increasing the pH value caused a decrease in free amino group concentration as expected (Table A.1.). Maillard reaction rate is known to occur faster at alkaline conditions since the reactivity of carbonyl and amine groups is increased. Especially, at the pH above the isoelectric point, conjugation between the free amino group of soy protein and the reducing end of sugar is expected to be accelerated (Abd El-Salam & El-Shibiny, 2018; Perez-Locas & Yaylayan, 2010). The past studies also observed that higher pH values caused faster binding of sugar to protein at the initial stages of the Maillard reaction (Ajandouz, Tchiakpe, Dalle Ore, Benajiba, & Puigserver, 2001).

On the other hand, neutral pHs caused an interesting frame in terms of free amino concentration (Figure 3.2.). At pH 7, free amino group concentration increased or remained the same for all types of sugars. This profile could be explained by two reasons. First of all, in contrary to studies in the literature, in this study, glycation proceeded at very higher temperatures (Qi, Liao, Yin, Zhu, & Yang, 2010; Zhu, Damodaran, & Lucey, 2010; Zhu, Damodaran, & Lucey, 2008). In the presented study, heating was performed at 90 ºC which was higher than the denaturation temperature of soy β-conglycinin (78º C) and close to that of soy glycinin (94 ºC) (Wang, Liu, Ma, & Zhao, 2019). Also, the wet glycation method has a disadvantage that higher temperatures at aqueous solutions enhance protein denaturation and polymerization (de Oliveira, Coimbra, de Oliveira, Zuñiga, & Rojas, 2016). Besides, it is known that the denaturation of globular proteins reveals amino groups from the inner structure (de Oliveira et al., 2016b; Nasrollahzadeh et al., 2017). Also, globular proteins create gels at lower protein concentrations in the presence of reducing sugars (Oliver et al., 2006a). Moreover, since denaturation of protein could lead to gel

formation at neutral pH, it may decelerate the condensation reaction between the free amino group and carbonyl group after 80 ºC (Boostani et al., 2017; Tarone, Fasolin, Perrechil, Hubinger, & Cunha, 2013). The high temperature of 90 ºC was chosen to decrease the reaction time. Over and above, protein denaturation did not hinder the glycation reaction, since reducing sugar concentration decreased at that pH value. The sharpest increase in free amino group concentration was observed for D-allulose glycated (pH 7) samples (Figure 3.2.). Besides, a study about egg white protein showed a higher aggregation of proteins when heated with D-allulose (Sun, Hayakawa, & Izumori, 2004). Therefore, more aggregation might cause more denaturation, thus increased free amino concentration. The other reason for the increased free amino group concentration might be the partial hydrolysis of protein caused by microwave heating. Microwave heating is known as a useful and rapid method for protein hydrolysis into peptides or amino acids at acidic medium within shorter times (<1 min) (Chen, Wang, & Li, 2014; Zhong, Marcus, & Li, 2005).

One of the major factors in glycation reactions is the type of the reducing sugar. ANOVA analysis showed that sugar type had a significant effect on the free amino group concentration of glycated soy protein isolate ($p<0.05$). At the alkaline conditions, free amino group concentrations were found same for fructose and glucose glycated proteins ($p>0.05$). However, egg white protein glycation with glucose, fructose, and D-allulose demonstrated different results indicating that glucose was more reactive than others (Sun, Hayakawa, & Izumori, 2004). On the other hand, β-lactoglobulin glycation with fructose and D-allulose showed no significant difference in the concentration of free amino groups (Zeng et al., 2013). The reason for different results could be explained by the protein and sugar interaction and/or reaction conditions (Benjakul, Lertittikul, & Bauer, 2005).

Different protein-sugar weight ratio had a significant effect on the free amino concentration of glycated soy protein isolate ($p<0.05$). Similar results were found for different protein-sugar weight ratios at pH 10 (except for D-allulose, protein-sugar weight ratio of 7.2). When 50-70% of the lysine is attached to the sugar, a no loss

period of lysine occurs because of the decrease in pH value or restriction of reactants in the solution (Ajandouz et al., 2001). Moreover, increasing protein concentration might have caused an increase in the viscosity of the solution and thus decreased the mobility and the reaction rate (Zhu et al., 2008).

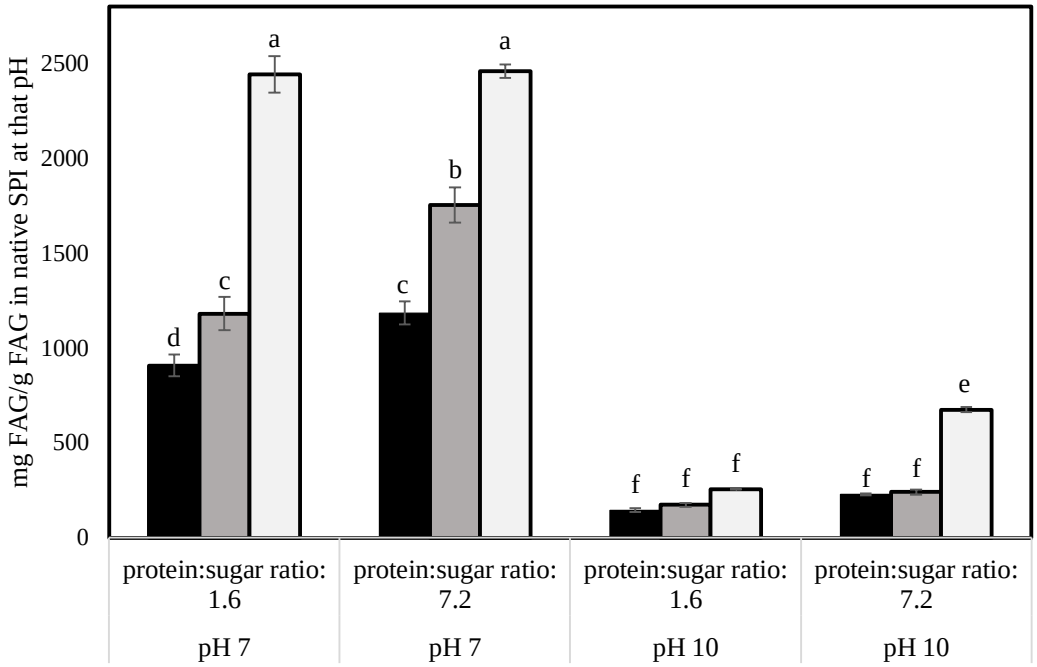

Figure 3.2. *mg FAG/g FAG in native SPI at that pH after microwave glycation with fructose* (■), *glucose* (▒), *and D-allulose* (□)

The effects of microwave and water bath heating on glycation reaction were also investigated. For the comparison, pH 10 was selected for glycation reaction and fructose was used as the reducing sugar. Experimental results (Table A.2.) and statistical analysis results (Table C.2.) are given in Appendix section.

Figure 3.3. represents mg FAG/g FAG in native SPI after microwave and water bath glycation of soy protein isolate with fructose at pH 10. ANOVA results showed a significant effect of glycation method on free amino group concentration ($p<0.05$). Free amino group concentration was much lower at microwave glycated samples for

the same treatment time. The result was parallel to studies in the literature that microwave glycation of ovalbumin and bovine serum albumin had higher glycation degree than conventional glycated ones (Nasrollahzadeh et al., 2017; Tsubokura et al., 2009). It was assumed that different results obtained by microwave and conventional glycation processes were resulted by changes in the thermodynamic parameters of the systems since microwave heating causes the rotation of dipole molecules and provides efficient contact between the molecules and atoms and thus decreases the activation energy (Nasrollahzadeh et al., 2017). Moreover, different mechanisms caused by microwave heating and conventional heating were explained by the effect of microwaves on protein folding and unfolding kinetics resulting in altered reactivity of the proteins (Bohr & Bohr, 2000). Since conventional heating caused a higher degree of secondary agglomeration than microwave heating, less free amino groups were reacted with carbonyl groups (Guan et al., 2011). Besides, microwave heating induces rapid Maillard reaction rates since ions and electrolytes in food systems are affected by microwave irradiation (Nasrollahzadeh et al., 2017; Yeo & Shibamoto, 1991).

The weight ratio of protein-sugar also had a significant effect on free amino group concentration ($p<0.05$). While free amino group concentrations were the same for microwave heating, much higher glycation reaction occurred in protein-sugar ratio of 1.6 at water bath heated samples. Nevertheless, increase on the free amino concentration was observed at water bath heating. The reason might be the denaturation of soy protein in water bath heating due to the increased protein content causing an increase in protein-protein interaction such as formation of disulfide linkages (Guan et al., 2011).

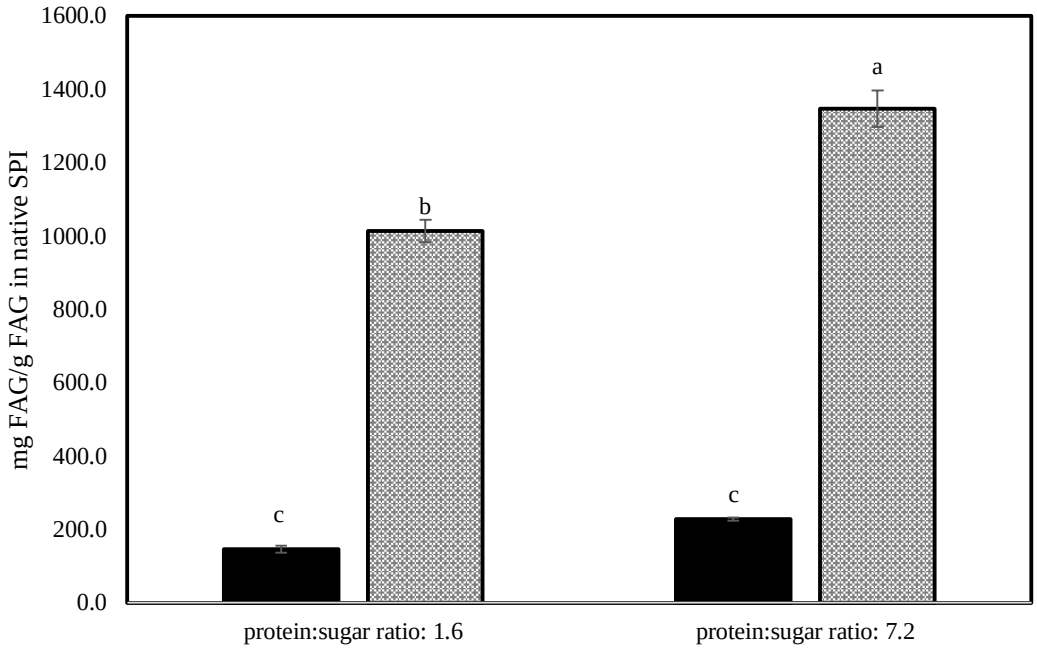

Figure 3.3. *mg FAG/g FAG in native SPI after microwave* (■) *and water bath glycation* (▨) *with fructose at pH 10*

3.2. Analysis of Reducing Sugar Concentration

Glycation is known as the initial condensation reaction between the carbonyl group of the reducing sugar and the free amino group of the protein. Therefore, the decrease in reducing sugar concentration is considered as an indicator of glycation reaction. HPLC was used to measure reducing sugar concentrations and the concentrations were determined by calibration curves prepared with standard sugar solutions and types of sugars were identified according to their retention times in the column. Native soy protein isolate was also analyzed by HPLC and there were no sugars found. Interestingly, sugar analysis by HPLC revealed the isomerization of monosaccharides when heated at certain conditions. Isomerization reaction converts aldose to another aldose or ketose. The reaction could be proceeded by itself when sugar is heated at alkaline conditions (Lobry de Bruyn-Alberda van Ekenstein) (Angyal, 2001). Also, it was found that microwave heating increased the rate of isomerization of sugar

reactions (Nooshkam & Madadlou, 2016). Since the reactivity of sugars differs in the glycation process, isomerization could affect Maillard reaction rates. In the present study, as expected, there was no isomerization reaction at pH 7 samples (Table 3.1.). On the other hand, the isomerization of fructose to glucose and D-allulose, D-allulose to fructose, and glucose to fructose were observed at alkaline conditions. Sugar isomerization in the presence of proteins was also mentioned in the literature. In a study it was found that, heating in the presence of casein protein caused the isomerization of fructose to glucose and vice versa (van Boekel & Brands, 1998). Moreover, it was pointed out that higher sugar concentration caused a higher isomerization degree of fructose to D-allulose in heated food products (Oshima et al., 2014). Corresponding results were also found in the presented study that isomerization was higher at a protein-sugar weight ratio of 1.6. On the other hand, there was no isomerization of fructose when it was heated in a water bath at pH 10. The reason might be the rapid heating effect of microwave heating and its ability to ease the chemical reactions.

Table 3.1. *Percentage mean values of remained reducing sugars after microwave heating and water bath heating*

Method	pH	Sugar Type	Protein-sugar Ratio	Fructose (%)	Glucose (%)	D-Allulose (%)
MW	10	Fructose	1.6	70.9 ± 2.0	21.2 ± 1.8	8.2 ± 0.7
	10	Fructose	7.2	100.0 ± 0.0	nd	nd
	10	D-allulose	1.6	19.9 ± 1.5	nd	80.1 ± 1.5
	10	D-allulose	7.2	2.1 ± 0.1	nd	97.9 ± 0.1
	10	Glucose	1.6	26.2 ± 1.0	73.8 ± 1.0	nd
	10	Glucose	7.2	15.1 ± 0.2	84.9 ± 0.2	nd
	7	Fructose	1.6	100.0 ± 0.0	nd	nd
	7	Fructose	7.2	100.0 ± 0.0	nd	nd
	7	D-allulose	1.6	nd	nd	100.0 ± 0.0
	7	D-allulose	7.2	nd	nd	100.0 ± 0.0
	7	Glucose	1.6	nd	100.0 ± 0.0	nd
	7	Glucose	7.2	nd	100.0 ± 0.0	nd
WB	10	Fructose	1.6	100.0 ± 0.0	nd	nd
	10	Fructose	7.2	100.0 ± 0.0	nd	nd

MW: Microwave heating, WB: Water bath heating, nd: not detected. The results were expressed as the mean of three replicates ± standard error

Since some isomerization of sugars was encountered in the analysis, remained reducing sugar concentration was obtained by adding all sugar isomer concentrations present in the solution. Therefore, it was called total reducing sugar remained. ANOVA analysis of reducing sugar concentrations were conducted with the factors of pH (7 and 10), sugar type (fructose, glucose, D-allulose), and the weight ratio of soy protein-sugar (1.6 and 7.2). Experimental results (Table A.3.) and statistical analysis results (Table C.3.) are given in Appendix. Percentage of remained reducing sugar was given by the formula:

$$Total\ reducing\ sugar\ remained\ (\%) = \frac{Total\ sugar\ concentration\ after\ glycation\ (g/L)}{Initial\ sugar\ concentration\ (g/L)} \times 100$$

Glycation pH had a significant effect on the total reducing sugar concentration remained ($p<0.05$). It was observed that at the same conditions, alkaline pH caused more depletion in reducing sugar concentration (Figure 3.4.). Since higher pH increased the reactivity of carbonyl and amine groups, more glycation reaction occurred at that pH. Studies established that loss of fructose at pH 10 was more than at pH 6, and even higher loss occurred at pH 12 (Ajandouz et al., 2001).

Sugar type also had a significant effect on reducing sugar content of glycated samples ($p<0.05$). It is known that different sugars form different types and amounts of Maillard products as well as exhibiting different reaction rates. According to the percentage of remained sugars, the reactivity of sugars could be ordered as D-allulose > fructose > glucose at pH 10 (Figure 3.4.). Similar results about sugar reactivity were found at dry glycation of egg white protein with D-allulose, fructose, and glucose (Sun et al., 2004).

Protein-sugar weight ratio had no significant effect on remained sugar concentration ($p>0.0.5$). The percentage of remained sugar was not changed for the same sugar types of different protein sugar ratios at the same pH values (except fructose at pH 7). This might indicate that the maximum sugar concentration was used at the ratio of 1.6, and

increasing protein concentration did not affect glycation extent in terms of covalent bonding of sugar to protein.

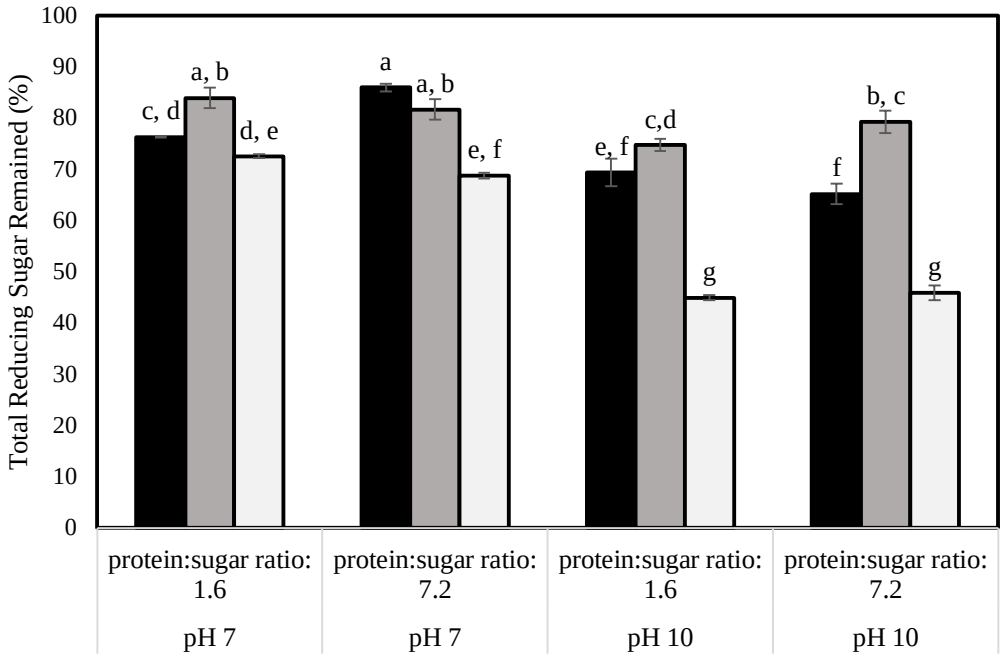

Figure 3.4. *Remained reducing sugar after microwave glycation with fructose* (■), *glucose* (▣), *and D-allulose* (□)

The effects of microwave and water bath heating on reducing sugar content were also investigated (Figure 3.5.). ANOVA results indicated a significant difference in the methods used ($p<0.05$). However, there was no significant effect of the protein-sugar weight ratio on remained reducing sugar concentration ($p>0.05$). Experimental results (Table A.4.) and statistical analysis results (Table C.4.) are given in Appendix section.

Remained sugar concentration results were in accordance with free amino group concentrations. Water bath heating induced insufficient rate of glycation reaction when compared to microwave heating. It was expected since microwave glycation was found to decrease the activation energy of Maillard reaction compared to water

bath heating by inducing effective touch between molecules and atoms, and by changing the free energy of the system through altering the entropy (Guan et al., 2011; Nasrollahzadeh et al., 2017; Tu et al., 2015). Besides, Guan et al. conducted microwave glycation of soy protein isolate with lactose, maltose, dextran, and soluble starch and concluded that microwave heating accelerated the glycation reactions compared to water bath heating (2006).

The weight ratio of protein-sugar had no significant effect on remained sugar concentration ($p>0.05$). For water bath heating, it was expected since there was no/or little amount of glycation occurred. On the other hand, microwave glycation probably caused the maximum amount of sugar attached to protein and an increase in protein concentration did not affect remained sugar concentration.

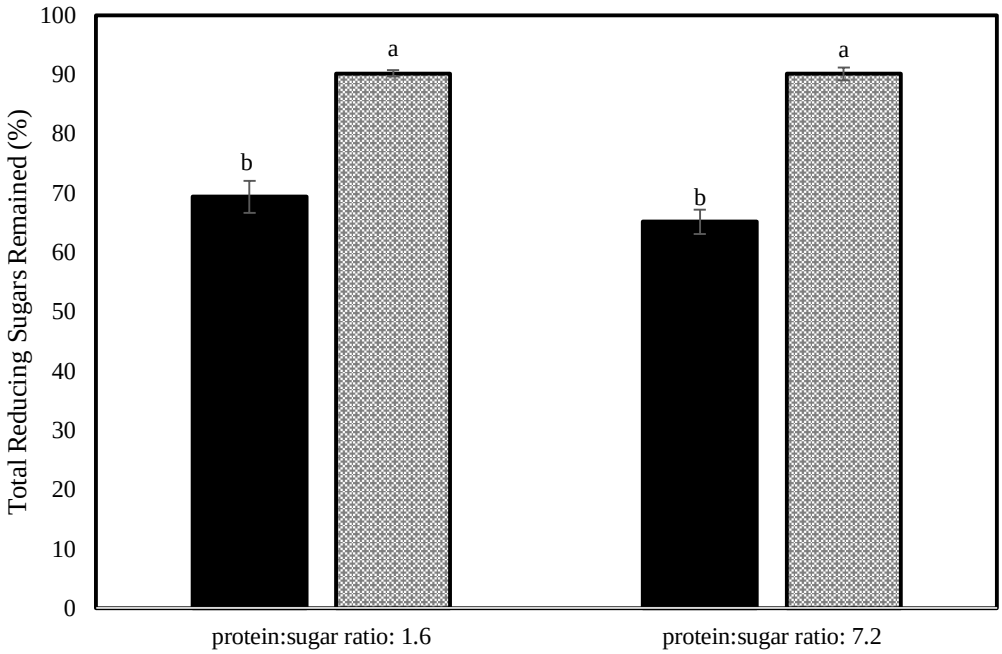

Figure 3.5. *Remained total reducing sugar after microwave* (■) *and water bath glycation* (▨) *with fructose at pH 10*

3.3. Analysis of Browning

Glycation is known as the initial steps of the Maillard reaction. In glycation studies, although it was desired to stop the reaction at the initial stages, some of the intermediate and final products of the Maillard reactions might be produced (Nasrollahzadeh et al., 2017; Li et al., 2014; Sun et al., 2004). The condensation reaction between sugars and proteins produces Amadori rearrangement products and develops Schiff base components. Maillard reaction products formed at the advanced stage of the Maillard do not only possess antioxidant, antimicrobial, and antigenicity properties but also they are related to mutagenic, carcinogenic and cytotoxic compounds (de Oliveira et al., 2016a). Besides, color and flavor formation are observed at the final stages of the reaction and they are undesirable at proteins designed for food production. Therefore, glycation reactions should be performed under controlled conditions to avoid advanced Maillard products (de Oliveira et al., 2016).

Melanoidins are formed at the final stage of the Maillard reaction, cause brownish components, and quantified by measurement of absorbance at 420 nm. Microwave glycation analyses were performed with the factors of pH, sugar type, and the weight ratio of soy protein-sugar. ANOVA analysis was conducted and experimental results (Table A.5.) and statistical results (Table C.5.) are given in Appendix. It was determined that pH, sugar type, protein-sugar ratio, and their interactions had significant effects on browning intensity ($p<0.05$).

The most increase in browning intensity was found at glucose glycated soy protein isolates at pH 7 (Figure 3.6.). However, while free amino concentration was increasing, a slight decrease in sugar concentration was observed for glucose at that pH. Caramelization is another non-enzymatic browning reaction and it may take place at the same time with Maillard reaction at high temperatures (Ajandouz et al., 2001). Although sugar-related reactions occur at very high temperatures when they are present alone in the solution, the presence of protein lowers the reaction temperature

since sugars react with amino acids (Mauron, 1981). Therefore, Maillard and caramelization reactions are both responsible for compounds that promote browning intensity at heated food systems (Guan et al., 2011). Since browning intensity could also increase as a result of caramelization of the sugar, that circumstance at pH 7 for glucose can be explained by caramelization instead of Maillard reaction. Moreover, sugar concentration was higher at the protein-sugar ratio of 1.6 and that could be the reason for more browning intensity at that pH (Benjakul et al., 2005).

D-allulose generally induced higher browning intensity than fructose at certain conditions. The result was consistent with past studies. β-lactoglobulin glycation with D-allulose showed a higher browning degree than fructose although they had similar reaction rates at the initial stages (Zeng et al., 2013). Similarly, Cheetangdee & Fukada (2014) also studied the glycation of bovine β-lactoglobulin proteins with hexoses. While the initial stage of the Maillard reaction was quicker in glucose than fructose and D-allulose, D-allulose caused more brown color development than the others. They claimed that aldohexoses were more reactive than ketohexoses at the initial stage of the Maillard since carbonyl carbon of the aldehyde group is more electrophilic than ketose group. Moreover, Sun et al. (2004) found out that D-allulose exhibited intense cross-linking chemistry including covalent bonding of carbonyl-amino groups and intermolecular SS bonds caused by sulfhydryl groups. They claimed that intense crosslinking caused some changes in the partial conformation of the proteins. Therefore, it could be concluded as D-allulose was more reactive at the final stages of the Maillard reaction.

Although it was reported as the browning intensity was altered by sugar type in the decreasing order of aldopentoses > aldohexoses > ketohexoses > disaccharides, different results were obtained in microwave glycation of soy protein isolate (Benjakul et al., 2005). However, it was reported that browning intensity of sugar-protein solutions could be found divergent depending on heating conditions such as temperature, time, and pH of the solutions (Ajandouz et al., 2001).

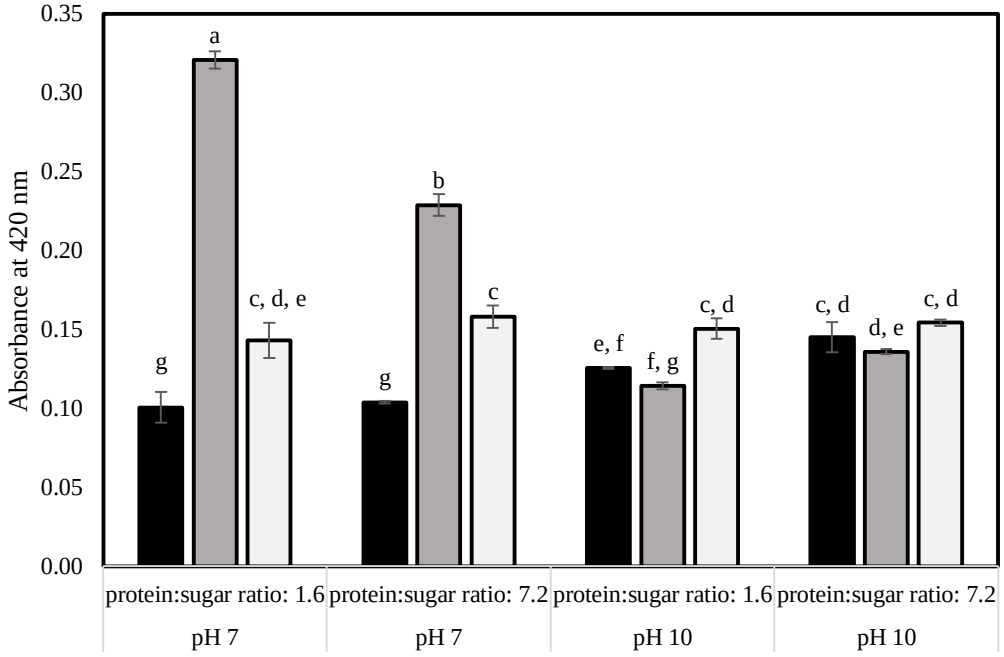

Figure 3.6. *Absorbance of glycated soy proteins at 420 nm after microwave glycation with fructose* (■) *glucose* (▩), *and D-allulose* (□)

The effects of microwave and water bath heating on browning intensity were also investigated. ANOVA results indicated a significant difference in the methods used and protein-sugar ratio ($p<0.05$). Experimental results (Table A.6.) and statistical analysis results (Table C.6.) are given in Appendix section.

According to the results, water bath heating caused more browning intensity than microwave heating (Figure 3.7.). However, free amino group and reducing sugar concentrations were higher in water bath glycated samples. The results indicated that browning in water bath heating occurred as a result of the caramelization reaction. Besides, it was found that microwave heating repressed the caramelization reaction to some extent (Guan et al., 2011). In the literature, it was found that microwave glycation of ovalbumin protein caused a lower degree of browning than conventional heating (Wang et al., 2013). Moreover, in another study, the absorbance of microwave glycated ovalbumin at 420 nm was not changed significantly for the first 5 minutes

(Tu et al., 2015). Therefore, it was concluded that the formation of brown color mostly depended on glycation type (wet and dry glycation), protein type, sugar type, pH and reaction time. Besides, an increase in reaction time might have caused an increase in the browning intensity of microwave glycated protein samples.

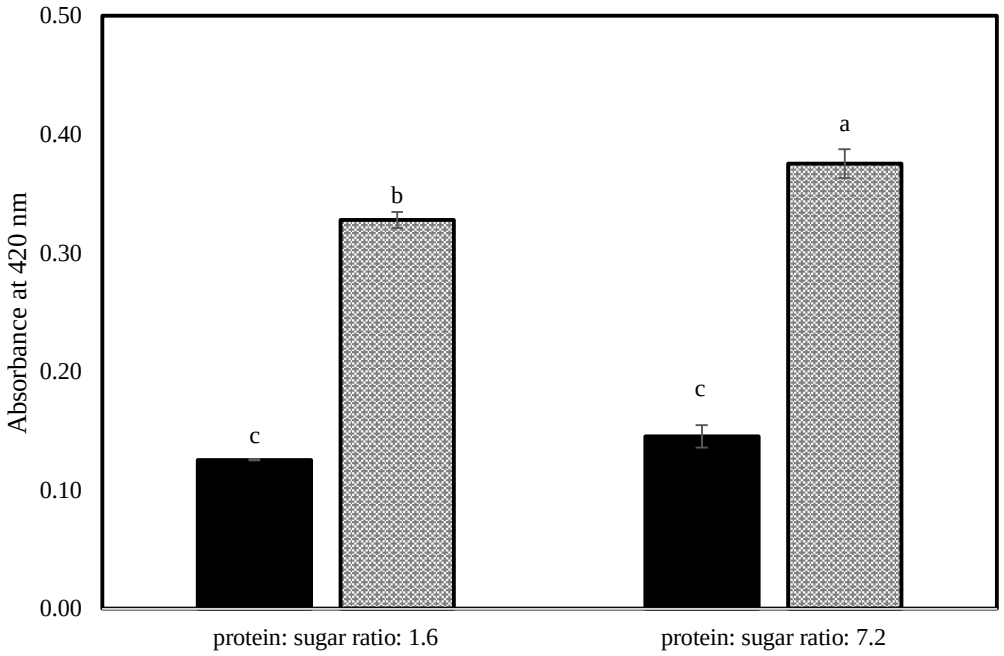

Figure 3.7. *Absorbance at 420 nm after microwave* (■)*and water bath glycation* (▨) *with fructose at pH 10*

3.4. Evaluation of Soy Protein Isolate Glycation by Means of Sugar Types

Microwave glycation of soy protein isolate with sugars followed different paths when examined in terms of individual sugars (Figure 3.8., 3.9., and 3.10.). Fructose was found more reactive at pH 10. Total sugar concentration and free amino concentration of glycated soy protein with fructose decreased while browning intensity was increasing. At pH 10, there was no significant difference in terms of remained sugar and free amino group concentration for different protein-sugar weight ratios ($p>0.05$).

However, the increase in browning intensity was observed at the protein-sugar ratio of 7.2, at pH 10. The reason for the browning could be the caramelization reaction at that condition. Therefore, it could be concluded that the glycation reaction was successful at the alkaline condition for fructose than neutral pH value. Also, it was observed that free amino group concentration at pH 7 was significantly higher than at pH 10 ($p<0.05$). Besides, it was expected since fructose related studies showed higher fructose loss at higher pH values (Ajandouz et al., 2001).

At pH 7, free amino group concentration of protein glycated with glucose increased while reducing sugar concentration decreased. On the other hand, browning intensity was the highest at that pH. Therefore, browning degree at that pH could be associated with caramelization rather than Maillard reaction. However, less browning was observed at alkaline conditions where glycation was dominated. It could be explained as glucose was more reactive at initial stage than the final stage, so it produced brown pigments in a lesser extent as a result of the Maillard reaction.

At the initial stages of the Maillard reaction at pH 10 samples, glucose and D-allulose glycated proteins showed a higher degree of glycation as it was interpreted from the decrease of free amino group and reducing sugar concentration. However, an increase in browning intensity was not seen in those conditions. The reaction rate between the free amino groups of proteins and carbonyl groups of sugars was not directly related to the development of polymeric substances produced at the later stages of the Maillard reaction (Sun et al., 2004). Therefore, browning intensity may not be directly related to the Maillard reaction rate. Moreover, repressing the melanoidins formation while the carbonyl group attaches to free amino group could be a better way of enhancing functional properties of proteins (Zhong et al., 2019).

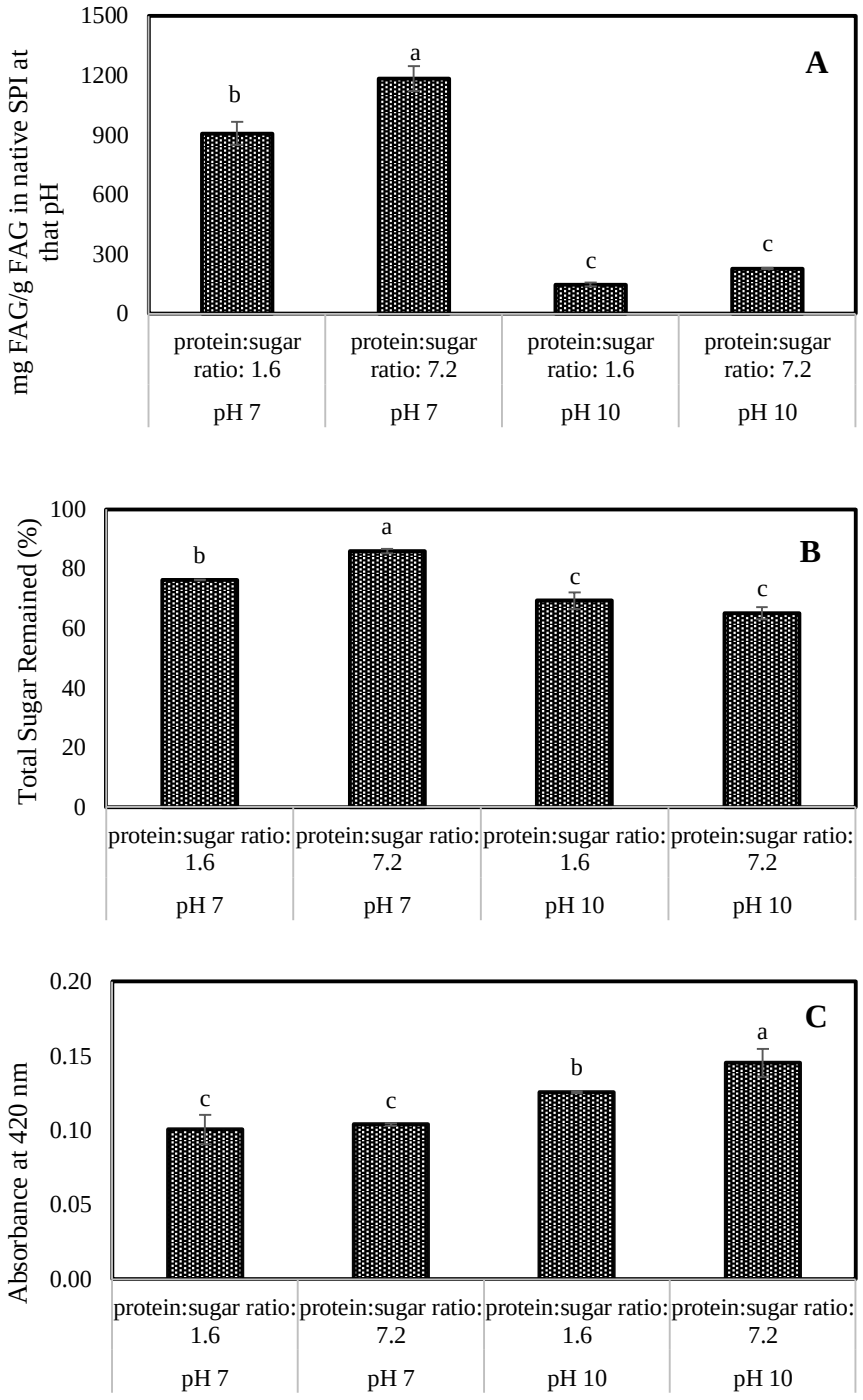

Figure 3.8. *mg FAG/g FAG in native SPI at that pH (A), percentage of total reducing sugar (B), and absorbance value at 420 nm (C) for microwave glycated soy protein isolate with* ***fructose***

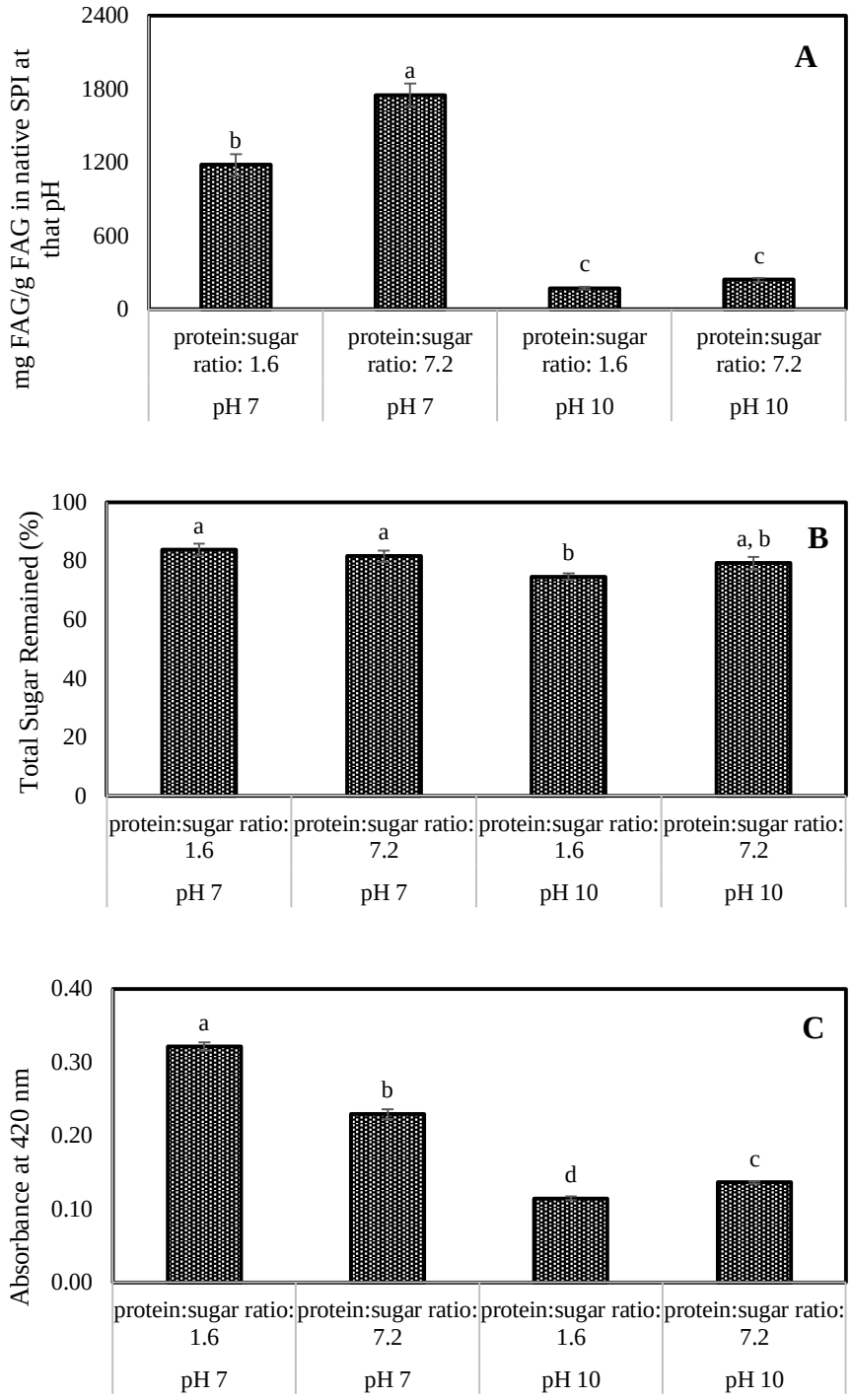

Figure 3.9. *mg FAG/g FAG in native SPI at that pH (A), percentage of total reducing sugar (B), and absorbance value at 420 nm (C) for microwave glycated soy protein isolate with* ***glucose***

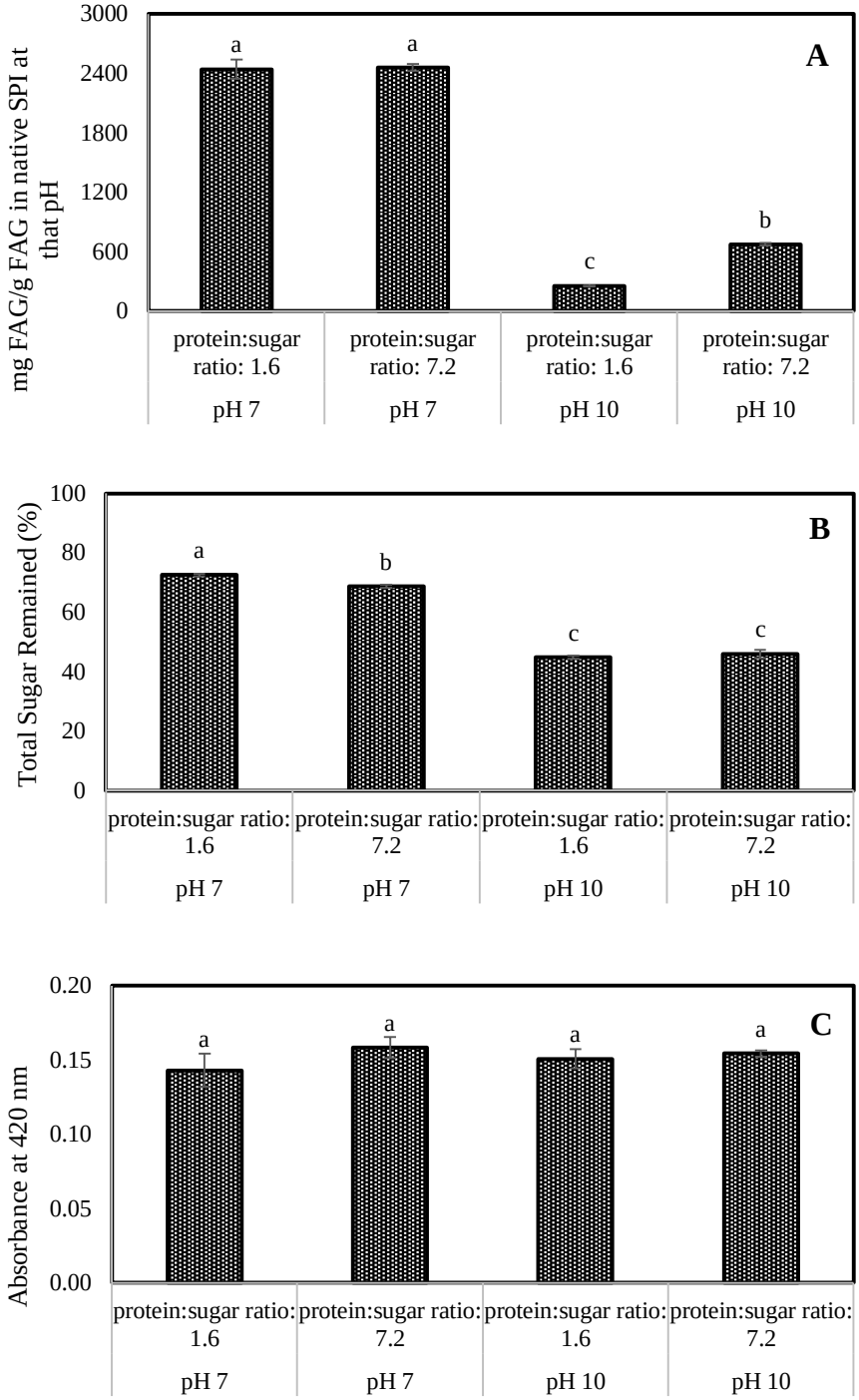

Figure 3.10. *mg FAG/g FAG in native SPI at that pH (A), percentage of total reducing sugar (B), and absorbance value at 420 nm (C) for microwave glycated soy protein isolate with* ***D-allulose***

3.5. Solubility of Proteins

Solubility is an important parameter for proteins used in food processes. It affects not only the composition of auxiliary ingredients but also the processing conditions of the products. It is known from the literature that controlled Maillard reactions may alter some of the functional properties of proteins including solubility (de Oliveira et al., 2016a). Moreover, solubility has a direct effect on other functional properties such as emulsifying activity, foaming capacity, and gelling property (de Oliveira et al., 2016a). Protein solubility was measured by the Lowry method according to the nitrogen content in the solution.

$$Protein\ solubility\ (\%) = \frac{Nitrogen\ content\ in\ the\ glycated\ protein\ solution\ (g/l)}{Nitrogen\ content\ in\ the\ control\ protein\ solution\ (g/l)} \times 100$$

ANOVA analysis was conducted and experimental results (Table A.7.) and statistical results (Table C.10 and C.11) are given in Appendix. The solubility of control protein solutions was measured as the solubility of native soy protein isolates at that pH value. Therefore, solubility graphs were reported for different pH values for clarity. It was determined that sugar type, protein-sugar ratio, and their interactions had significant effects on the solubility of proteins ($p<0.05$).

At both pH values, both sugar type and protein-sugar weight ratio were found significant in terms of solubility ($p<0.05$). An increase in solubility was observed at pH 7 (Figure 3.11.). However, from the free amino group and sugar concentration, it was concluded that there was a limited degree of glycation occurred at pH 7. Yet, it is known that the protein solubility decreases near the isoelectric point due to the electrostatic interactions causing aggregation of proteins (Mu, Zhao, Zhao, Cui, & Liu, 2011). The initial condensation reaction between protein and sugar likely to hinder protein-protein interactions and increases the interaction between protein and water molecules (Boostani et al., 2017). Moreover, it was suggested that the limited degree of glycation causes more soluble proteins because of the limited attack of sugars to water molecules decreases the self-association of proteins (de Oliveira et al., 2016).

Therefore, in the presented study, since glycation at pH 7 was near the isoelectric point of soy protein isolate (pI of 4.5), glycation could increase the solubility of protein at that pH (Tian et al., 2011).

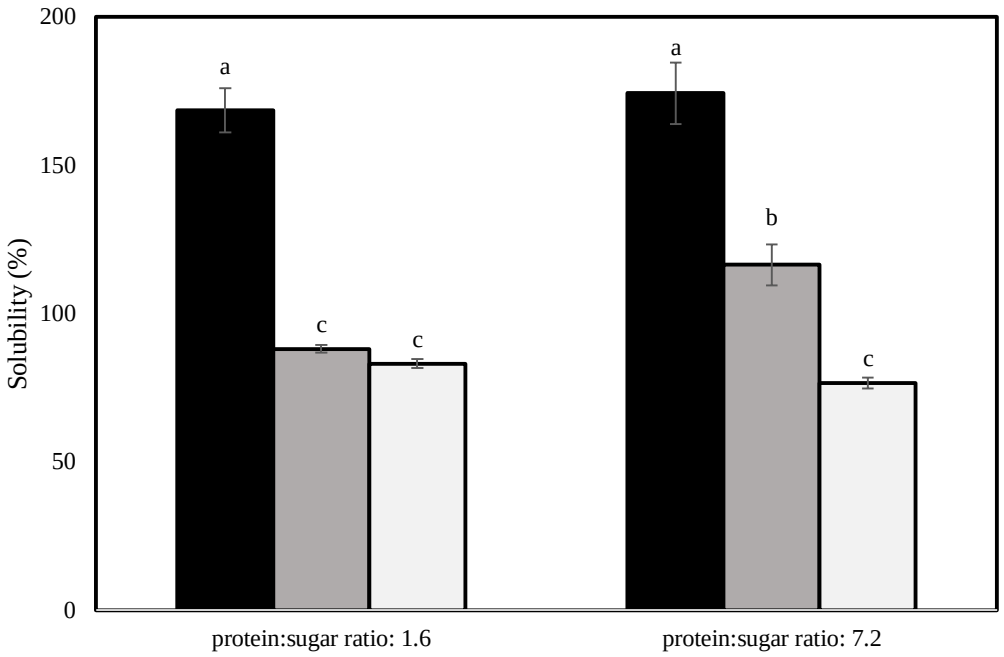

Figure 3.11. *Solubility of glycated soy protein isolate at pH 7, after microwave glycation with fructose* (■) *glucose* (▣), *and D-allulose* (□)

Although studies showed increased solubility of ovalbumin and bovine serum albumin after microwave glycation, a reverse trend was seen in the presented study at pH 10 as can be seen in figure 3.12. (Nasrollahzadeh et al., 2017; Tsubokura et al., 2009). The first reason for conflicting results might be the biochemical differences in proteins and sugars (Oliver et al., 2006). Yet, egg white protein conjugated with pectin also showed decreased solubility after glycation (Al-Hakkak & Al-Hakkak, 2010). The other reason might be the occurrence of a higher degree of glycation and it was reported that only a specific degree of glycation enhances the solubility of proteins since some of the advanced Maillard products cause insoluble components and decrease solubility (Álvarez, García, Rendueles, & Díaz, 2012).

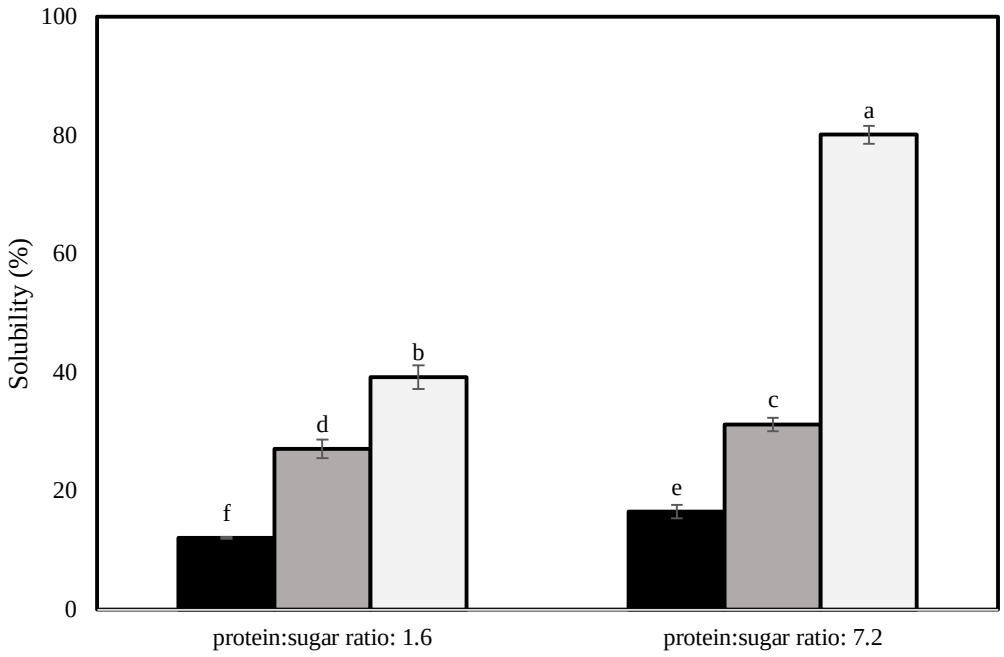

Figure 3.12. *Solubility of glycated soy protein isolate at pH 10, after microwave glycation with fructose* (■) *glucose* (▣), *and D-allulose* (□)

The effects of microwave and water bath heating on protein solubility were also investigated. There was a significant effect of methods used ($p<0.05$), however, the weight ratio of protein-sugar showed no significant effects on protein solubility ($p>0.05$). Experimental results (Table A.8.) and statistical analysis results (Table C.12.) are given in Appendix section.

Microwave heating was found to decrease soy protein isolate solubility as compared to water bath heating (Figure 3.13.). However, as discussed before, water bath heating caused a very limited glycation extent in the presented study. Therefore, a higher degree of glycation at microwave heating might have caused a decrease in solubility. Besides, it is known that solubility of soy proteins decreased with microwave heating (Hafez, Mohamed, Hewedy, & Singh, 1985). Moreover, denaturation in water bath

heating might have caused soluble aggregation of β-conglycinin upon heating and higher solubility was observed in that case (Wang et al., 2019).

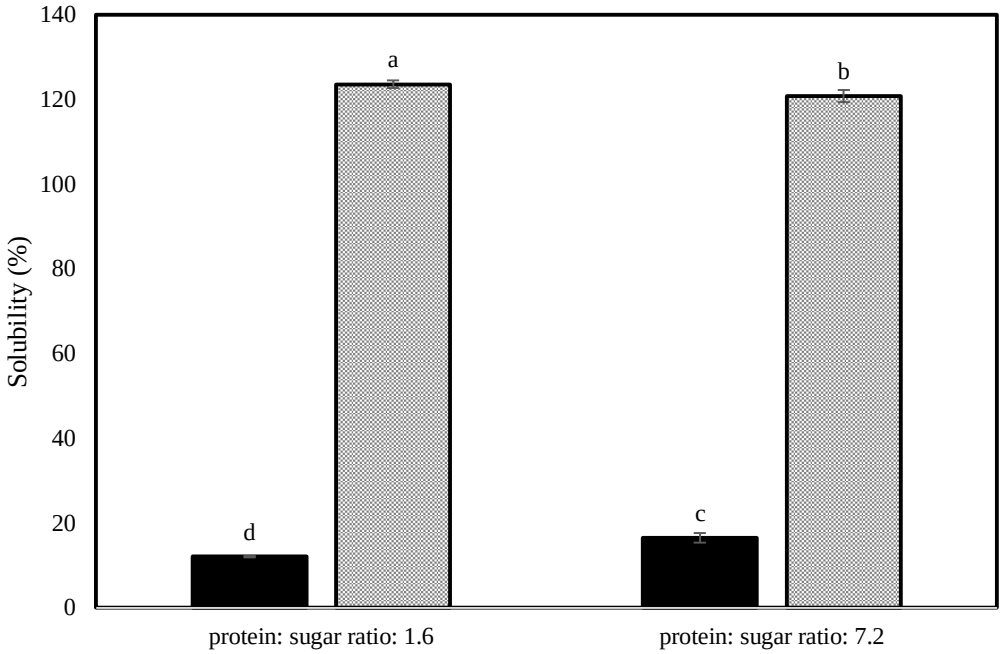

Figure 3.13. *Solubility of soy protein isolate after microwave* (■) *and water bath glycation* (▨) *with fructose at pH 10*

3.6. Fourier Transform Infrared Spectroscopy (FT-IR) Analysis

Changes that occurred by Maillard reactions in chemical properties and functional groups of the proteins could be detected by mid-infrared spectra. Fourier transform infrared (FT-IR) is a useful technique to get valuable information about changes in protein structure due to glycation (Oliver, Kher, McNaughton, & Augustin, 2009). The changes in protein structure are observed by the formation of new peaks or changing of the intensity of the peaks.

Graphs are given separately for different pH values and weight ratios of protein-sugar to distinguish the differences. Native soy protein absorbance demonstrated the normal features of the protein spectrum zones (Figure 3.14.). Amide I bands of native soy

protein isolate formed between 1700-1600 (1638), cm^{-1}, amide II bands formed between 1550-1450 (1527) cm^{-1} and amide III bands formed between 1450-1200 (1238) cm^{-1} as mentioned in the literature (Boostani et al., 2017). Amide I bands correspond to C=O stretching, amide II bands indicate N-H bending while amide III bands represent C-N stretching and N-H bending (Boostani et al., 2017). Moreover, the peaks between 850-740 cm^{-1} were assumed as characteristic bands of sugar types (Figure 3.14.).

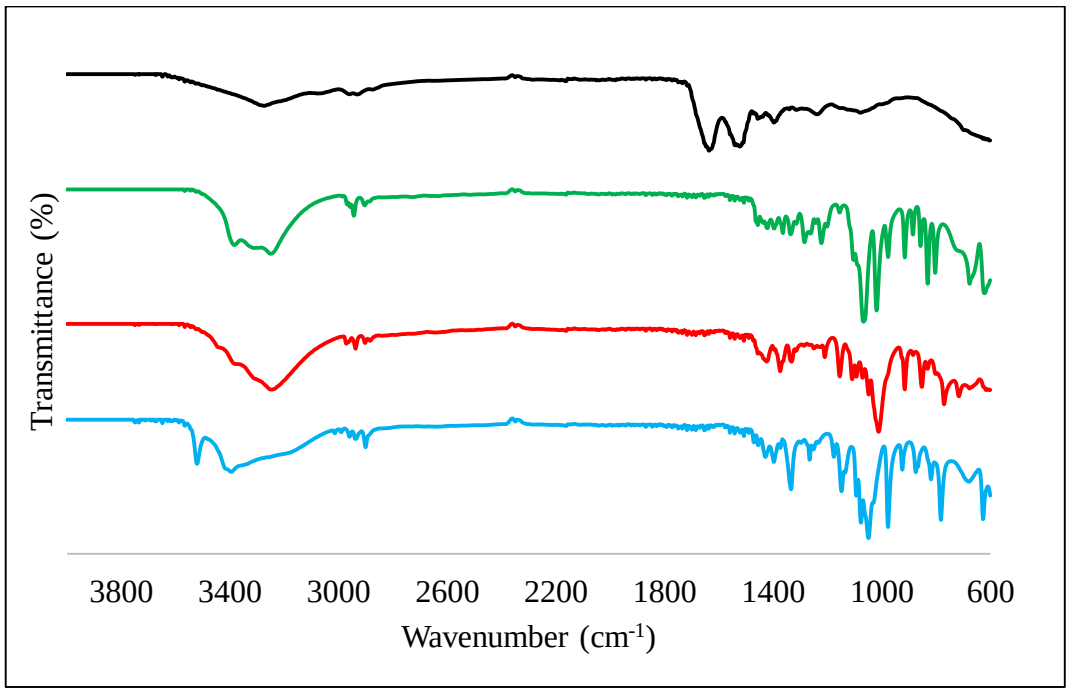

Figure 3.14. *The Fourier transform infrared spectroscopy of native soy protein (black), D-allulose (green), glucose (red), and fructose (blue)*

Since Maillard reaction occurs between the free amino group of the protein and the carbonyl group of sugar, the bands of NH_2 groups decreases. On the other hand, the formation of Amadori products and Schiff base products cause formation the of bands regarding C=O and C=N stretching (Boostani et al., 2017). Therefore, increased absorbance at 1631 cm^{-1} may indicate the formation of Schiff Base products. Besides,

new absorption bands in the amide I region were considered as bands caused by Amadori products. Moreover, the peaks between 3300-3200 cm^{-1} were related to O-H stretching vibrations (Boostani et al., 2017). That large bands indicated free and bound –OH and N-H groups which were ready to make conjugation with the proteins (Xu et al., 2019). The bands between 900-862 cm^{-1} were caused by C-H bending of soy protein isolate side chains (Zhong et al., 2019b). Glycation also caused an increase in –C-O stretching absorbance and –OH deformation vibrations at 1100-1050 cm^{-1}, and –OH stretching vibration at 3300-3200 cm^{-1}.

According to the results, glycation of soy protein isolate with fructose, glucose, and D-allulose could be seen from FT-IR spectra (Figure 3.15., 3.16., 3.17., and 3.18.). However, more information about glycation degree could be obtained by further investigations of FT-IR data such as secondary structure distributions of native soy protein isolate and glycated samples to have information about α-helix and β-sheet structure of the samples.

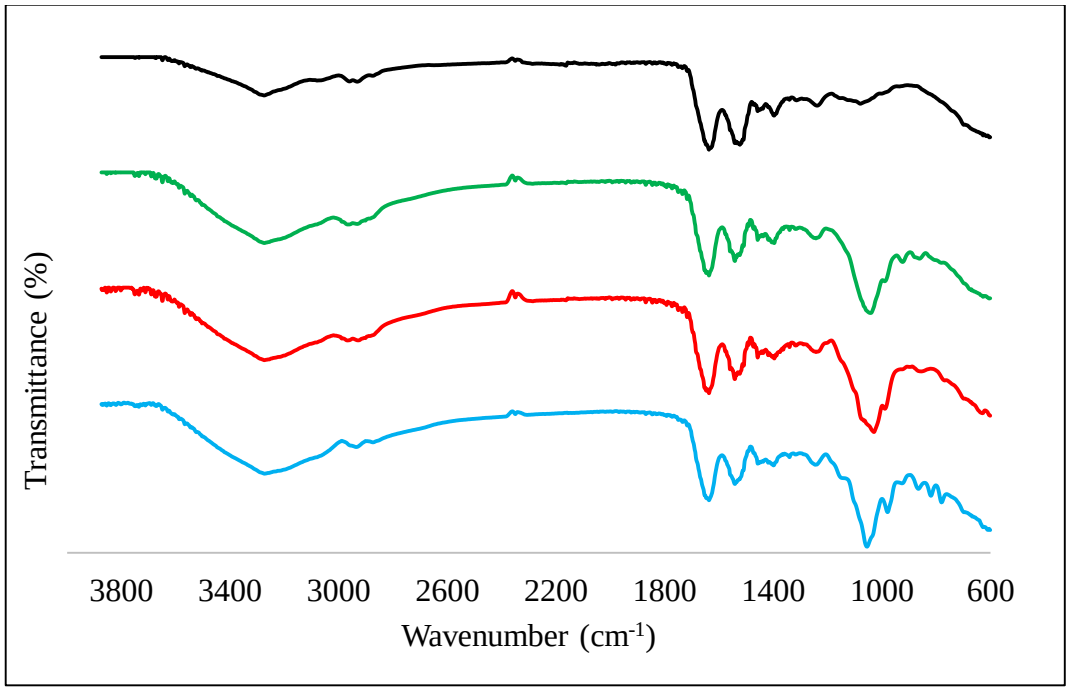

Figure 3.15. *The Fourier transform infrared spectroscopy of native soy protein (black), and glycated samples at pH 7, protein-sugar weight ratio of 1.6 of D-allulose (green), glucose (red), and fructose (blue)*

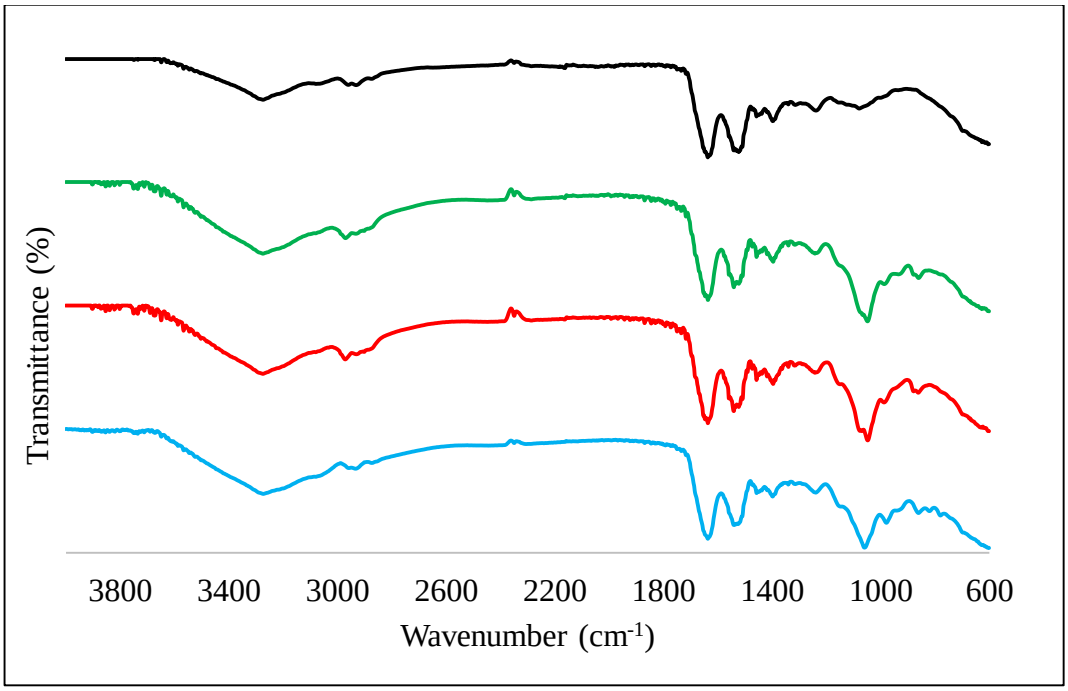

Figure 3.16. *The Fourier transform infrared spectroscopy of native soy protein (black), and glycated samples at pH 7, protein-sugar weight ratio of 7.2 of D-allulose (green), glucose (red), and fructose (blue)*

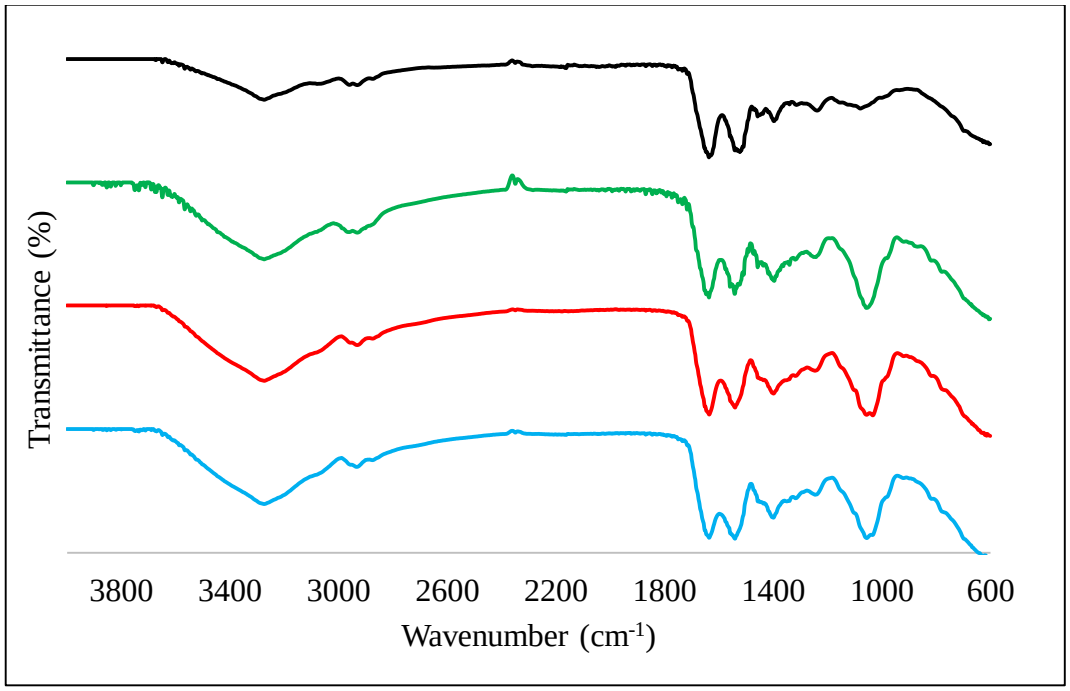

Figure 3.17. *The Fourier transform infrared spectroscopy of native soy protein (black), and glycated samples at pH 10, protein-sugar weight ratio of 1.6 of D-allulose (green), glucose (red), and fructose (blue)*

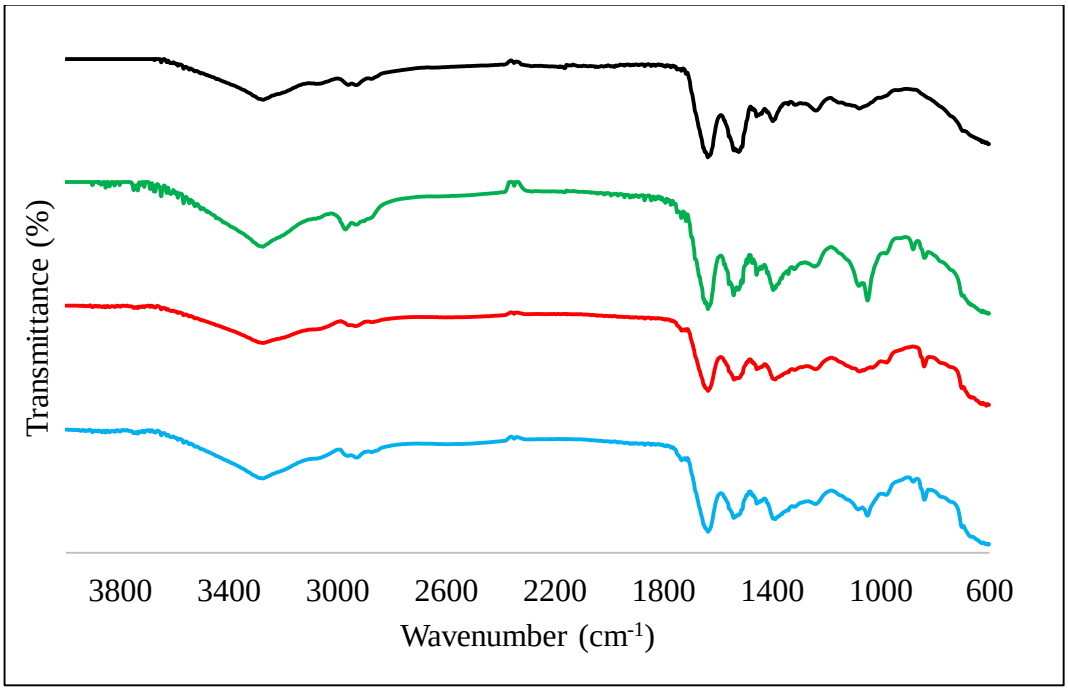

Figure 3.18. *The Fourier transform infrared spectroscopy of native soy protein (black), and glycated samples at pH 10, protein-sugar weight ratio of 7.2 of D-allulose (green), glucose (red), and fructose (blue)*

Comparison of FT-IR spectra of microwave and water bath glycation of soy protein isolate with fructose was also investigated at pH 10 value (Figure 3.19.). Since glycation is mainly related to –C-O stretching and –OH deformation at 1100-1050 cm^{-1}, it was clearly seen that conjugation in microwave glycated samples was much higher than that of a water bath glycated ones. Also new absorption bands at amide I regions (1700-1600 cm^{-1}) were observed in microwave glycated samples.

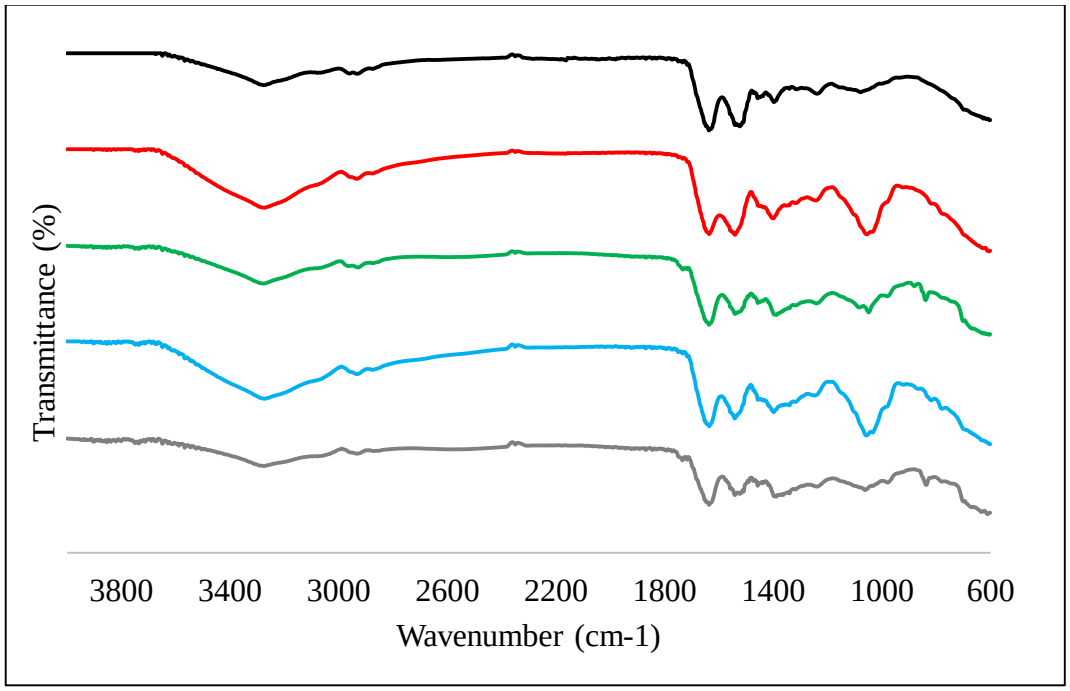

Figure 3.19. *The Fourier transform infrared spectroscopy of native soy protein (black), glycated soy protein isolates with fructose at pH 10, microwave glycation; protein-sugar ratio of 1.6 (red) and 7.2 (green), water bath glycation; protein-sugar ratio of 1.6 (blue) and 7.2 (gray)*

3.7. Time Domain Nuclear Magnetic Resonance Analysis

Time-domain nuclear magnetic resonance (TD-NMR) provides basic information about chemical, structural and molecular states of food samples. It has been a popular method in food science due to its non-destructive and non-invasive nature, and not requiring any treatment before analysis, and giving qualitative and quantitative information about food systems. Main outcomes from the TD-NMR are spin-lattice relaxation time (T_1) and spin-spin relaxation time (T_2). Spin-spin relaxation time, also known as transverse relaxation time, is obtained by the time required for transverse magnetization to decay zero (Kirtil & Oztop, 2016). Since T_2 relaxation time of liquid water is relatively higher than that of macromolecules, it dominates the signal comes from NMR (Gidley, 2014). In other words, T_2 relaxation times could be used to quantify water mobility in the system (Kirtil et al., 2017). From the T_2 relaxation

times, changes in the hydration behavior of a component could be interpreted. Therefore, T_2 relaxation time provides information about the water content of food samples and interactions of water molecules with adjacent macromolecules (Kirtil et al., 2014) and it could be used to understand protein-sugar interactions in glycation studies.

T_2 relaxation times of the mixtures of soy protein isolate and sugars before glycation and after microwave glycation are given at different pH values (Table 3.2 and 3.3.). Moreover, a comparative table for different pH values after microwave glycation are also given for making results easily understandable (Table 3.4.). ANOVA analysis were conducted separately for different pH values. The weight ratio of protein-sugar and type of sugar were the factors. Statistical analysis results are given in Appendix (Table C. 13.-C. 33.)

Before glycation, effects of both protein-sugar weight ratio and sugar type were found significant ($p<0.05$). Glycation of soy protein isolate caused a significant increase of T_2 relaxation times at pH 7 ($p<0.05$) (Table 3.2.). Glycation is the initial step of the Maillard reaction, occurs between the free amino group of the protein and carbonyl group of the sugar, and reveals water molecules as a result of the condensation reaction. Moreover, from the results of the free amino group and sugar concentration, the denaturation of soy protein was expected at that pH. As a globular protein, the denaturation of soy protein reveals hydrophobic parts from the inside of the protein structure. Therefore, an increase in T_2 relaxation rate could be related to the chemical interchange of the water molecule with hydroxyl and amino groups and the release of water molecules to the system (Oztop, Rosenberg, Rosenberg, McCarthy, & McCarthy, 2010). Moreover, the weight ratio of protein-sugar had a significant effect before and after glycation at both pH values ($p<0.05$). Generally, as protein concentration increased, T_2 values decreased. The decrease was expected since soy protein is a macromolecule and water forms hydrogen bonds with soy protein resulting in the creation of immobilized water in the system (Oztop et al., 2010). Also, results showed that glycation at pH 7 decreased the hydration ability of the soy protein isolate.

There was a decrease in T_2 values of the samples after glycation at pH 10 (except fructose 7.2 and glucose 1.6) (Table 3.3.). Although glycation occurred at a greater extent at that pH, lower T_2 values were observed. Glycation in aqueous solution also causes gelation at higher temperatures (de Oliveira et al., 2016). Moreover, Amadori rearrangement adds water molecules to the structure to produce Schiff base compounds. Therefore, it was expected that at the later stages of the Maillard reaction, it resulted in restriction of water mobility, thus decreased T_2 relaxation times. Moreover, the results could be interpreted as glycation at pH 10 increased the hydration ability of the soy protein isolate.

A comparison of T_2 relaxation times of water bath and microwave glycation of soy protein isolate with fructose at pH 10 was also investigated (Table 3.5). ANOVA results indicated that there was no significant effect of the glycation method on T_2 values ($p>0.05$). However, increased protein concentration at water bath heating also had a decreasing effect on T_2 values, as expected ($p<0.05$). Statistical analysis results are given in Appendix section (Table C. 34.).

Table 3.2. *T_2 relaxation times of soy protein isolate-sugar mixtures before and after microwave glycation at pH 7*

Sugar Type	Protein-Sugar Ratio	Before Glycation T_2 (ms)	After Glycation T_2 (ms)
Fructose	1.6	$98.44 \pm 0.10^{b,A}$	$192.55 \pm 12.65^{b,B}$
Fructose	7.2	$66.85 \pm 0.05^{f,A}$	$152.32 \pm 4.45^{c,B}$
Glucose	1.6	$95.72 \pm 0.08^{c,A}$	$161.71 \pm 3.56^{c,B}$
Glucose	7.2	$70.59 \pm 0.03^{d,A}$	$126.58 \pm 3.91^{d,B}$
D-Allulose	1.6	$99.30 \pm 0.07^{a,A}$	$231.39 \pm 13.24^{a,B}$
D-Allulose	7.2	$68.52 \pm 0.12^{e,A}$	$138.29 \pm 7.45^{cd,B}$

Small letters indicate significant difference in each column (a-f) while capital letters indicate significant difference in a row (A-B)

Table 3.3. *T_2 relaxation times of soy protein isolate-sugar mixtures before and after microwave glycation at pH 10*

Sugar Type	Protein-Sugar Ratio	Before Glycation T_2 (ms)	After Glycation T_2 (ms)
Fructose	1.6	$98.44 \pm 0.10^{b,A}$	$88.38 \pm 2.87^{b,B}$
Fructose	7.2	$66.85 \pm 0.05^{f,A}$	$67.13 \pm 3.26^{c,A}$
Glucose	1.6	$95.72 \pm 0.08^{c,A}$	$97.40 \pm 4.52^{a,A}$
Glucose	7.2	$70.59 \pm 0.03^{d,A}$	$53.69 \pm 0.79^{d,B}$
D-Allulose	1.6	$99.30 \pm 0.07^{a,A}$	$93.79 \pm 3.37^{ab,B}$
D-Allulose	7.2	$68.52 \pm 0.12^{e,A}$	$47.59 \pm 1.21^{d,B}$

Small letters indicate significant difference in each column (a-f) while capital letters indicate significant difference in a row (A-B)

Table 3.4. *T_2 relaxation times of microwave glycated soy protein isolate at different pH values*

Sugar Type	Protein-Sugar Ratio	pH 7 T_2 (ms)	pH 10 T_2 (ms)
Fructose	1.6	$192.55 \pm 12.65^{b,A}$	$88.38 \pm 2.87^{b,B}$
Fructose	7.2	$152.32 \pm 4.45^{c,A}$	$67.13 \pm 3.26^{c,B}$
Glucose	1.6	$161.71 \pm 3.56^{c,A}$	$97.40 \pm 4.52^{a,B}$
Glucose	7.2	$126.58 \pm 3.91^{d,A}$	$53.69 \pm 0.79^{d,B}$
D-Allulose	1.6	$231.39 \pm 13.24^{a,A}$	$93.79 \pm 3.37^{ab,B}$
D-Allulose	7.2	$138.29 \pm 7.45^{cd,A}$	$47.59 \pm 1.21^{d,B}$

Small letters indicate significant difference in each column (a-f) while capital letters indicate significant difference in a row (A-B)

Table 3.5. *T_2 relaxation times of microwave and water bath glycated soy protein isolate with fructose at pH 10*

Protein-Sugar Ratio	Microwave Heating T_2 (ms)	Water Bath Heating T_2 (ms)
1.6	88.38 ± 2.87^{a}	86.84 ± 3.41^{a}
7.2	67.13 ± 3.26^{b}	70.24 ± 4.13^{b}

CHAPTER 4

CONCLUSION AND RECOMMENDATION

In the presented study, microwave glycation of soy protein isolate with fructose, glucose, and D-allulose at different pH values (pH 7 and pH 10), and the different weight ratio of protein: sugar (1.6 and 7.2) were investigated. Microwave and water bath glycation of soy protein isolate with fructose at pH 10 was also studied with different protein-sugar weight ratio (1.6 and 7.2) for comparison.

Free amino group concentration implied higher glycation degree at alkaline conditions than neutral pH value. On the other hand, it was concluded that there might be denaturation or partial hydrolysis of protein occurred at neutral pH.

HPLC results revealed more sugar reduction at pH 10 than pH 7. Reactivity of sugars for glycation reaction was ordered as D-allulose > fructose > glucose. Also, it was observed that microwave glycation at alkaline pH caused the isomerization of sugars. Moreover, microwave glycation significantly decreased sugar concentration compared to water bath glycation ($p<0.05$).

Browning intensity was found higher at microwave glycated soy protein isolate with glucose at pH 7, and the reason might be the caramelization reaction. Since glycation temperature is high, side reactions might take place like caramelization and contributed to browning intensity. D-allulose induced higher browning intensity at certain conditions and the result was parallel with the literature. Moreover, higher browning intensity obtained by water bath glycation was also associated with caramelization.

The solubility results showed that microwave glycation of soy protein isolate at pH 10 decreased solubility of the protein. It was argued that the higher extent of glycation might decrease the solubility of the protein due to the formation of insoluble

compounds. Also, microwave glycation was found to decrease the solubility of soy protein isolate when compared to water bath glycation.

FT-IR results demonstrated the glycation reaction of sugars with soy protein isolate. Increased absorbance at 1631 cm^{-1} was associated with the formation of Schiff base products. Moreover, new absorption bands at the amide I region implied the production of Amadori compounds. Besides, microwave glycation was observed at a higher extent than water bath glycation.

Nuclear Magnetic Resonance experiments showed that microwave glycation increased T_2 values at pH 7 and decreased T_2 values at pH 10. It was concluded that glycation at pH 10 increased the hydration ability of soy protein isolate. Moreover, the glycation method (water bath and microwave) had no significant effect on T_2 relaxation times ($p>0.05$).

To conclude, microwave glycation of soy protein isolate was found more efficient at pH 10 value. The free amino group and reducing sugar concentration decreased more efficiently at that pH value. However, browning intensity was not higher, since the reaction was not able to proceed at the final stage of the Maillard. The reactivity of the sugars was ordered as D-allulose > fructose > glucose. Moreover, the isomerization of sugars at microwave heating was observed at alkaline conditions. Microwave glycation caused a decrease in solubility of protein at pH 10 while an increase was seen at pH 7. Moreover, FT-IR results demonstrated the conjugation reaction between sugars and soy protein isolate. NMR results provided valuable information about the structural modification of glycated soy protein isolate and water mobility in the system.

More detailed results might be obtained by amino acid analysis by reverse phase high performance liquid chromatography. Sodium-dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis and MALDI-TOF-MS experiments might supply information about changes in the molecular weight of the protein. Moreover,

Circular dichroism (CD) test might provide additional information about the structural changes.

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APPENDICES

A. EXPERIMENTAL RESULTS

Table A.1. *Experimental results of free amino group concentration after microwave glycation*

pH	Sugar Type	Protein-Sugar Ratio (w/w)	Concentration (g/L)
7	Control	1.6	0.0285 ± 0.0041
7	Control	7.2	0.0271 ± 0.0017
7	Fructose	1.6	0.0258 ± 0.0016
7	Fructose	7.2	0.0337 ± 0.0018
7	Glucose	1.6	0.0321 ± 0.0024
7	Glucose	7.2	0.0476 ± 0.0025
7	D-allulose	1.6	0.0695 ± 0.0027
7	D-allulose	7.2	0.0653 ± 0.0053
10	Control	1.6	0.0667 ± 0.0006
10	Control	7.2	0.0726 ± 0.0029
10	Fructose	1.6	0.0098 ± 0.0006
10	Fructose	7.2	0.0152 ± 0.0003
10	Glucose	1.6	0.0116 ± 0.0008
10	Glucose	7.2	0.0161 ± 0.0009
10	D-allulose	1.6	0.0171 ± 0.0002
10	D-allulose	7.2	0.0451 ± 0.0009

Table A.2. *Comparison of free amino group concentration of microwave and water bath glycated soy protein isolate with fructose at pH 10*

Method	Protein-Sugar Ratio (w/w)	Concentration (g/L)
Microwave	1.6	0.0098 ± 0.0006
Microwave	7.2	0.0152 ± 0.0003
Water Bath	1.6	0.0677 ± 0.0021
Water Bath	7.2	0.0900 ± 0.0034

Table A.3. *Experimental results of reducing sugar concentration after microwave glycation*

pH	Sugar Type	Protein-Sugar Ratio (w/w)	Concentration (g/L)
7	Control	1.6	19.23 ± 0.00
7	Control	7.2	6.10 ± 0.00
7	Fructose	1.6	14.95 ± 0.48
7	Fructose	7.2	5.24 ± 0.05
7	Glucose	1.6	16.53 ± 0.75
7	Glucose	7.2	4.98 ± 0.12
7	D-allulose	1.6	13.94 ± 0.07
7	D-allulose	7.2	4.19 ± 0.03
10	Control	1.6	19.23 ± 0.00
10	Control	7.2	6.10 ± 0.00
10	Fructose	1.6	13.34 ± 0.53
10	Fructose	7.2	4.18 ± 0.37
10	Glucose	1.6	14.37 ± 0.22
10	Glucose	7.2	4.83 ± 0.13
10	D-allulose	1.6	8.63 ± 0.09
10	D-allulose	7.2	2.80 ± 0.09

Table A.4. *Comparison of reducing sugar concentration of microwave and water bath glycated soy protein isolate with fructose at pH 10*

Method	Protein-Sugar Ratio (w/w)	Concentration (g/L)
Microwave	1.6	13.34 ± 0.53
Microwave	7.2	4.18 ± 0.37
Water Bath	1.6	17.34 ± 0.11
Water Bath	7.2	5.50 ± 0.07

Table A.5. *Browning intensity at 420 nm after microwave glycation*

pH	Sugar Type	Protein-Sugar Ratio (w/w)	Absorbance at 420 nm
7	Fructose	1.6	0.101 ± 0.010
7	Fructose	7.2	0.104 ± 0.001
7	Glucose	1.6	0.321 ± 0.006
7	Glucose	7.2	0.229 ± 0.007
7	D-allulose	1.6	0.143 ± 0.011
7	D-allulose	7.2	0.158 ± 0.007
10	Fructose	1.6	0.126 ± 0.001
10	Fructose	7.2	0.145 ± 0.010
10	Glucose	1.6	0.114 ± 0.002
10	Glucose	7.2	0.136 ± 0.002
10	D-allulose	1.6	0.151 ± 0.006
10	D-allulose	7.2	0.154 ± 0.002

Table A.6. *Comparison of browning intensity at 420 nm of microwave and water bath glycated soy protein isolate with fructose at pH 10*

Method	Protein-Sugar Ratio (w/w)	Absorbance at 420 nm
Microwave	1.6	0.126 ± 0.001
Microwave	7.2	0.145 ± 0.010
Water Bath	1.6	0.328 ± 0.007
Water Bath	7.2	0.375 ± 0.012

Table A.7. *Experimental results of solubility after microwave glycation*

pH	Sugar Type	Protein-Sugar Ratio (w/w)	Concentration (g/L)
7	Control	1.6	0.198 ± 0.025
7	Control	7.2	0.188 ± 0.003
7	Fructose	1.6	0.317 ± 0.023
7	Fructose	7.2	0.346 ± 0.021
7	Glucose	1.6	0.166 ± 0.002
7	Glucose	7.2	0.231 ± 0.014
7	D-allulose	1.6	0.156 ± 0.003
7	D-allulose	7.2	0.152 ± 0.004
10	Control	1.6	0.555 ± 0.003
10	Control	7.2	0.624 ± 0.018
10	Fructose	1.6	0.067 ± 0.001
10	Fructose	7.2	0.103 ± 0.007
10	Glucose	1.6	0.150 ± 0.009
10	Glucose	7.2	0.195 ± 0.007
10	D-allulose	1.6	0.218 ± 0.011
10	D-allulose	7.2	0.500 ± 0.010

Table A.8. *Comparison of solubility of microwave and water bath glycated soy protein isolate with fructose at pH 10*

Method	Protein-Sugar Ratio (w/w)	Concentration (g/L)
Microwave	1.6	0.067 ± 0.001
Microwave	7.2	0.103 ± 0.007
Water Bath	1.6	0.686 ± 0.005
Water Bath	7.2	0.754 ± 0.009

B. CALIBRATION CURVES

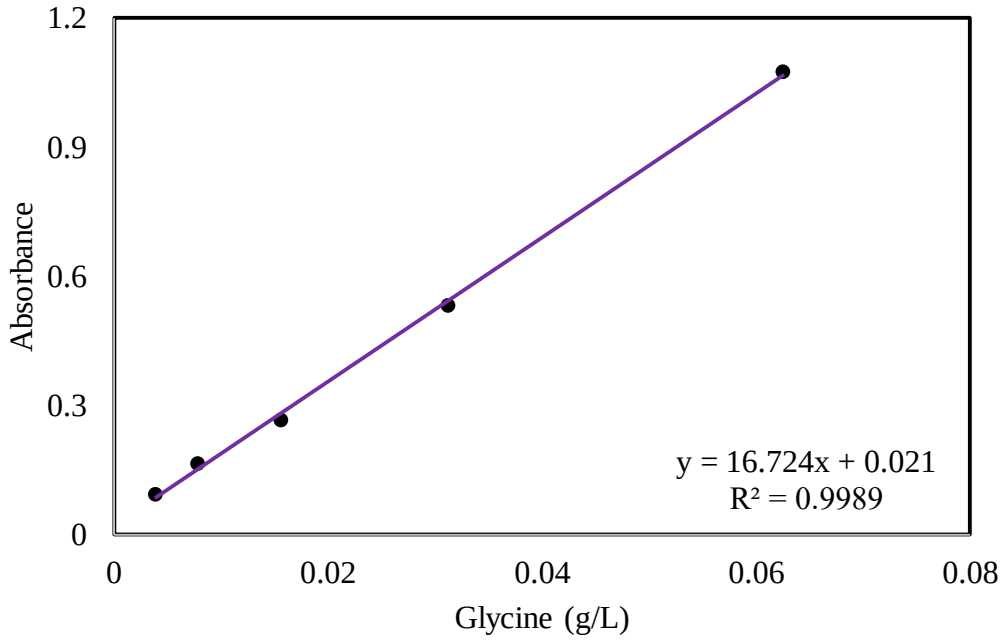

Figure B.1. *Example of a calibration curve of OPA method*

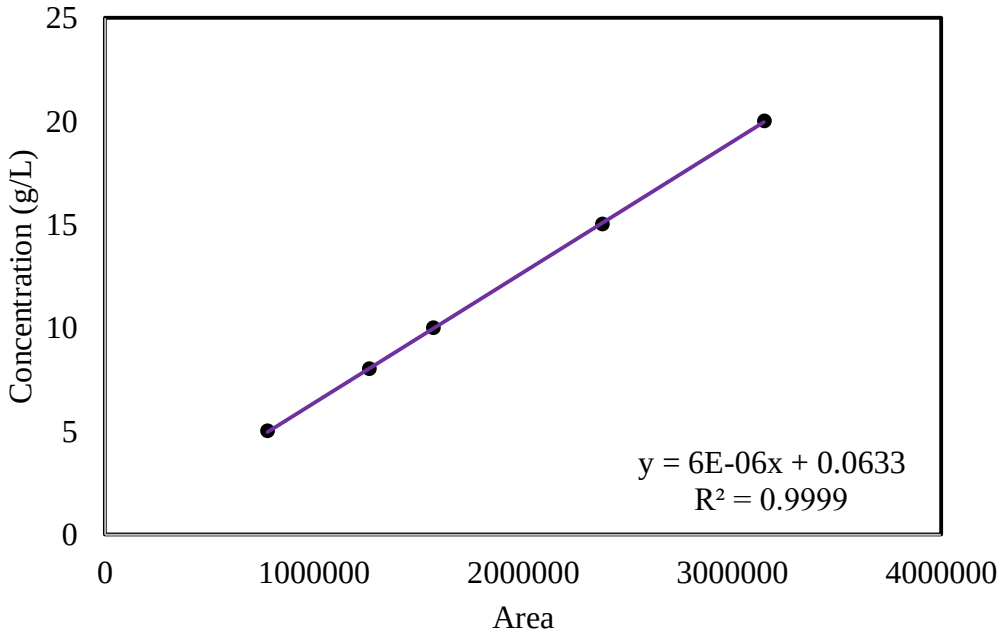

Figure B.2. *Calibration curve of fructose*

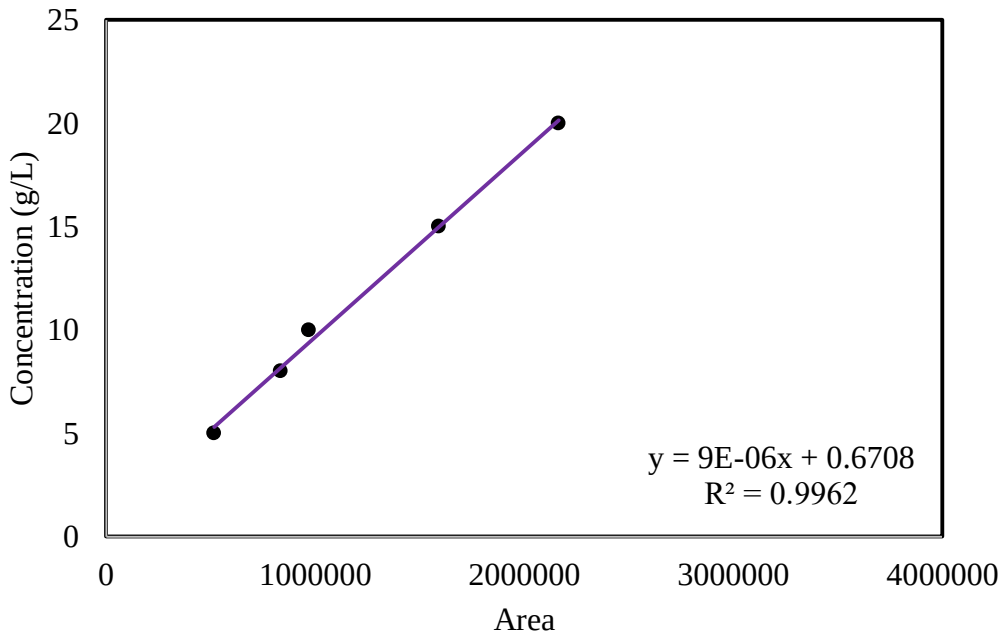

Figure B.3. *Calibration curve of glucose*

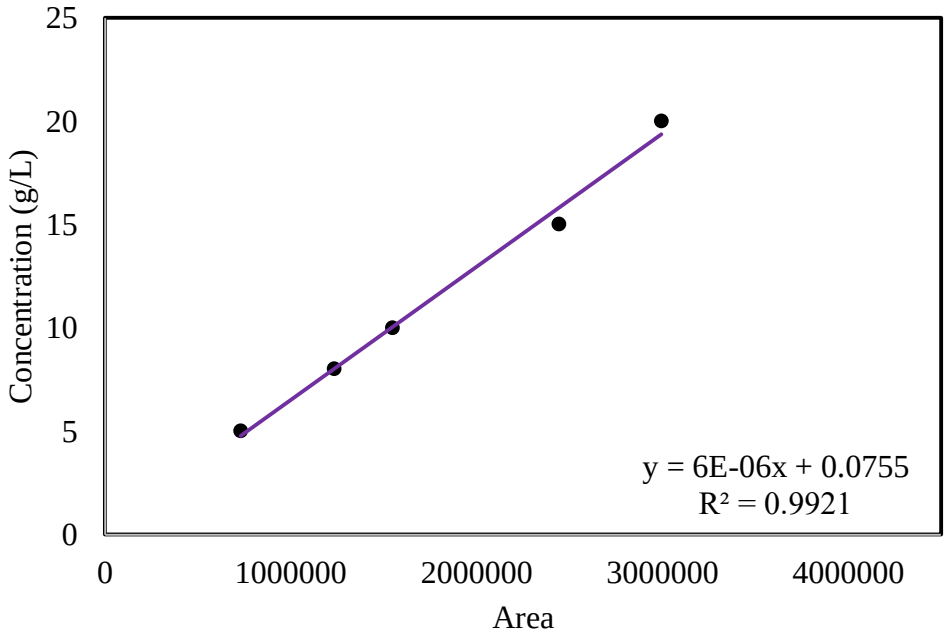

Figure B.4. *Calibration curve of D-allulose*

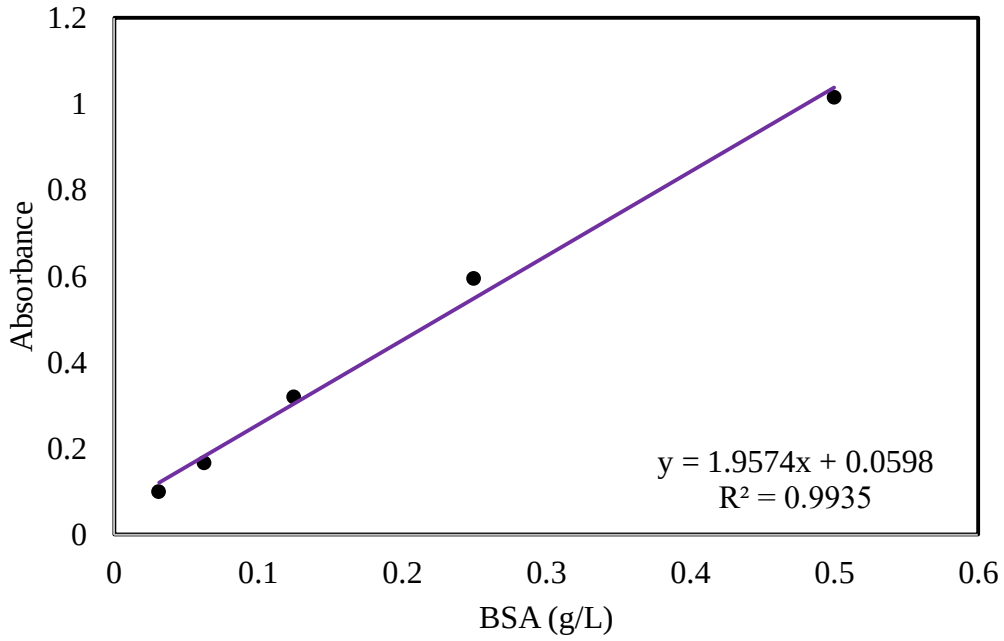

Figure B.5. *Calibration curve of Lowry method*

C. ANOVA TABLES

Table C.1. *Analysis of variance for mg FAG/g FAG in native SPI at that pH of microwave glycated soy protein isolate*

```
Factor               Type   Levels  Values
pH                   fixed       2  7, 10
Sugar Type           fixed       3  D-allulose, Fructose, Glucose
Protein:Sugar Ratio  fixed       2  1.6, 7.2


Analysis of Variance for mg FAG/g native FAG, using Adjusted SS for Tests

Source                                DF    Seq SS    Adj SS    Adj MS        F
pH                                     1  15243377  16161873  16161873  5421.14
Sugar Type                             2   3991562   4234086   2117043   710.11
Protein:Sugar Ratio                    1    446707    493769    493769   165.62
pH*Sugar Type                          2   1922350   1820364    910182   305.30
pH*Protein:Sugar Ratio                 1     30314     21038     21038     7.06
Sugar Type*Protein:Sugar Ratio         2     29900     31769     15885     5.33
pH*Sugar Type*Protein:Sugar Ratio      2    297172    297172    148586    49.84
Error                                 23     68569     68569      2981
Total                                 34  22029949

Source                                    P
pH                                    0.000
Sugar Type                            0.000
Protein:Sugar Ratio                   0.000
pH*Sugar Type                         0.000
pH*Protein:Sugar Ratio                0.014
Sugar Type*Protein:Sugar Ratio        0.013
pH*Sugar Type*Protein:Sugar Ratio     0.000
Error
Total


S = 54.6010   R-Sq = 99.69%   R-Sq(adj) = 99.54%


Grouping Information Using Tukey Method and 95.0% Confidence

pH   N    Mean  Grouping
 7  17  1654.7  A
10  18   287.0    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

Sugar Type   N    Mean  Grouping
D-allulose  11  1457.9  A
Glucose     12   837.7    B
Fructose    12   617.0      C

Means that do not share a letter are significantly different.
```

Grouping Information Using Tukey Method and 95.0% Confidence

Protein:Sugar Ratio	N	Mean	Grouping
7.2	17	1090.4	A
1.6	18	851.3	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

pH	Sugar Type	N	Mean	Grouping
7	D-allulose	5	2450.1	A
7	Glucose	6	1467.5	B
7	Fructose	6	1046.5	C
10	D-allulose	6	465.8	D
10	Glucose	6	207.8	E
10	Fructose	6	187.5	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

pH	Protein:Sugar Ratio	N	Mean	Grouping
7	7.2	8	1798.9	A
7	1.6	9	1510.5	B
10	7.2	9	381.9	C
10	1.6	9	192.2	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Sugar Type	Protein:Sugar Ratio	N	Mean	Grouping
D-allulose	7.2	5	1566.8	A
D-allulose	1.6	6	1349.1	B
Glucose	7.2	6	997.8	C
Fructose	7.2	6	706.6	D
Glucose	1.6	6	677.5	D
Fructose	1.6	6	527.4	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

pH	Sugar Type	Protein:Sugar Ratio	N	Mean	Grouping
7	D-allulose	7.2	2	2458.3	A
7	D-allulose	1.6	3	2442.0	A
7	Glucose	7.2	3	1753.7	B
7	Fructose	7.2	3	1184.8	C
7	Glucose	1.6	3	1181.3	C
7	Fructose	1.6	3	908.3	D
10	D-allulose	7.2	3	675.4	E

```
10  D-allulose  1.6           3   256.2           F
10  Glucose     7.2           3   241.9           F
10  Fructose    7.2           3   228.4           F
10  Glucose     1.6           3   173.8           F
10  Fructose    1.6           3   146.6           F

Means that do not share a letter are significantly different.
```

Table C.2. *Analysis of variance for mg FAG/g FAG in native SPI of microwave and water bath glycated soy protein isolate with fructose at pH 10*

```
Factor               Type   Levels  Values
Method               fixed       2  MW, WB
Protein:Sugar Ratio  fixed       2  1.6, 7.2


Analysis of Variance for mg FAG/g native FAG, using Adjusted SS for Tests

Source                       DF   Seq SS   Adj SS   Adj MS        F      P
Method                        1  2959571  2959571  2959571  3280.29  0.000
Protein:Sugar Ratio           1   129616   129616   129616   143.66  0.000
Method*Protein:Sugar Ratio    1    47688    47688    47688    52.86  0.000
Error                         8     7218     7218      902
Total                        11  3144093


S = 30.0371   R-Sq = 99.77%   R-Sq(adj) = 99.68%


Grouping Information Using Tukey Method and 95.0% Confidence

Method  N    Mean  Grouping
WB      6  1180.7  A
MW      6   187.5    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

Protein:Sugar
Ratio          N   Mean  Grouping
7.2            6  788.0  A
1.6            6  580.2    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

        Protein:Sugar
Method  Ratio          N    Mean  Grouping
WB      7.2            3  1347.7  A
WB      1.6            3  1013.7    B
MW      7.2            3   228.4      C
MW      1.6            3   146.6        D
Means that do not share a letter are significantly different.
```

Table C.3. *Analysis of variance for remained reducing sugar (%) of microwave glycated soy protein isolate*

```
Factor               Type   Levels  Values
pH                   fixed       2  7; 10
Sugar Type           fixed       3  D-allulose; Fructose; Glucose
Protein:Sugar Ratio  fixed       2  1,6; 7,2


Analysis of Variance for Remained Reducing Sugar (%), using Adjusted SS for
     Tests

Source                                DF   Seq SS   Adj SS   Adj MS       F
P
pH                                     1  1812,29  1789,08  1789,08  751,79
0,000
Sugar Type                             2  3097,51  2933,01  1466,50  616,24
0,000
Protein:Sugar Ratio                    1     1,37     5,56     5,56    2,33
0,141
pH*Sugar Type                          2   559,34   541,20   270,60  113,71
0,000
pH*Protein:Sugar Ratio                 1     0,01     1,19     1,19    0,50
0,487
Sugar Type*Protein:Sugar Ratio         2    24,16    22,93    11,47    4,82
0,019
pH*Sugar Type*Protein:Sugar Ratio      2   162,80   162,80    81,40   34,21
0,000
Error                                 21    49,97    49,97     2,38
Total                                 32  5707,46


S = 1,54264   R-Sq = 99,12%   R-Sq(adj) = 98,67%


Grouping Information Using Tukey Method and 95,0% Confidence

pH   N  Mean  Grouping
 7  16  78,2  A
10  17  63,2    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Sugar Type   N  Mean  Grouping
Glucose     11  79,9  A
Fructose    10  74,2    B
D-allulose  12  58,0      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence
```

Protein:Sugar Ratio	N	Mean	Grouping
7,2	17	71,1	A
1,6	16	70,3	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

pH	Sugar Type	N	Mean	Grouping
7	Glucose	5	82,8	A
7	Fructose	5	81,1	A
10	Glucose	6	77,0	B
7	D-allulose	6	70,6	C
10	Fructose	5	67,3	D
10	D-allulose	6	45,4	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

pH	Protein:Sugar Ratio	N	Mean	Grouping
7	7,2	9	78,8	A
7	1,6	7	77,6	A
10	7,2	8	63,4	B
10	1,6	9	63,0	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Sugar Type	Protein:Sugar Ratio	N	Mean	Grouping
Glucose	7,2	6	80,5	A
Glucose	1,6	5	79,3	A
Fructose	7,2	5	75,5	B
Fructose	1,6	5	72,8	B
D-allulose	1,6	6	58,7	C
D-allulose	7,2	6	57,3	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

pH	Sugar Type	Protein:Sugar Ratio	N	Mean	Grouping
7	Fructose	7,2	3	85,9	A
7	Glucose	1,6	2	83,9	A B
7	Glucose	7,2	3	81,7	A B
10	Glucose	7,2	3	79,2	B C
7	Fructose	1,6	2	76,3	C D
10	Glucose	1,6	3	74,7	C D
7	D-allulose	1,6	3	72,5	D E
10	Fructose	1,6	3	69,4	E F
7	D-allulose	7,2	3	68,8	E F

```
10  Fructose    7,2            2  65,2            F
10  D-allulose  7,2            3  45,9              G
10  D-allulose  1,6            3  44,9              G

Means that do not share a letter are significantly different.
```

Table C.4. *Analysis of variance for remained reducing sugar (%) of microwave and water bath glycated soy protein isolate with fructose at pH 10*

```
Factor               Type   Levels  Values
Method               fixed       2  MW; WB
Protein:Sugar Ratio  fixed       2  1,6; 7,2


Analysis of Variance for Remained Reducing Sugar (%), using Adjusted SS for
     Tests

Source                       DF   Seq SS   Adj SS   Adj MS       F      P
Method                        1  1376,05  1396,46  1396,46  441,36  0,000
Protein:Sugar Ratio           1     9,94    12,31    12,31    3,89  0,089
Method*Protein:Sugar Ratio    1    11,41    11,41    11,41    3,60  0,099
Error                         7    22,15    22,15     3,16
Total                        10  1419,54


S = 1,77876   R-Sq = 98,44%   R-Sq(adj) = 97,77%


Grouping Information Using Tukey Method and 95,0% Confidence

Method  N  Mean  Grouping
WB      6  90,1  A
MW      5  67,3    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Protein:Sugar
Ratio          N  Mean  Grouping
1,6            6  79,8  A
7,2            5  77,6  A

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

        Protein:Sugar
Method  Ratio          N  Mean  Grouping
WB      1,6            3  90,2  A
WB      7,2            3  90,1  A
MW      1,6            3  69,4    B
```

```
MW      7,2            2  65,2    B

Means that do not share a letter are significantly different.
```

Table C.5. *Analysis of variance for browning intensity of microwave glycated soy protein isolate at 420 nm*

```
Factor           Type   Levels  Values
ph               fixed       2  7; 10
Sugar type       fixed       3  D-allulose; fructose; glucose
pro:sugar ratio  fixed       2  1,6; 7,2


Analysis of Variance for absorbance, using Adjusted SS for Tests

Source                          DF    Seq SS    Adj SS    Adj MS       F      P
ph                               1  0,013187  0,013187  0,013187  318,82  0,000
Sugar type                       2  0,040030  0,040030  0,020015  483,90  0,000
pro:sugar ratio                  1  0,000201  0,000201  0,000201    4,85  0,037
ph*Sugar type                    2  0,057474  0,057474  0,028737  694,78  0,000
ph*pro:sugar ratio               1  0,003501  0,003501  0,003501   84,64  0,000
Sugar type*pro:sugar ratio       2  0,004177  0,004177  0,002088   50,49  0,000
ph*Sugar type*pro:sugar ratio    2  0,006492  0,006492  0,003246   78,47  0,000
Error                           24  0,000993  0,000993  0,000041
Total                           35  0,126052


S = 0,00643126   R-Sq = 99,21%   R-Sq(adj) = 98,85%


Grouping Information Using Tukey Method and 95,0% Confidence

ph   N  Mean  Grouping
 7  18   0,2  A
10  18   0,1    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Sugar type   N  Mean  Grouping
glucose     12   0,2  A
D-allulose  12   0,2    B
fructose    12   0,1      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

pro:sugar
ratio       N  Mean  Grouping
1,6        18   0,2  A
7,2        18   0,2    B
```

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

ph	Sugar type	N	Mean	Grouping
7	glucose	6	0,3	A
10	D-allulose	6	0,2	B
7	D-allulose	6	0,2	B
10	fructose	6	0,1	C
10	glucose	6	0,1	C
7	fructose	6	0,1	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

ph	pro:sugar ratio	N	Mean	Grouping
7	1,6	9	0,2	A
7	7,2	9	0,2	B
10	7,2	9	0,1	C
10	1,6	9	0,1	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Sugar type	pro:sugar ratio	N	Mean	Grouping
glucose	1,6	6	0,2	A
glucose	7,2	6	0,2	B
D-allulose	7,2	6	0,2	C
D-allulose	1,6	6	0,1	C
fructose	7,2	6	0,1	D
fructose	1,6	6	0,1	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

ph	Sugar type	pro:sugar ratio	N	Mean	Grouping
7	glucose	1,6	3	0,3	A
7	glucose	7,2	3	0,2	B
7	D-allulose	7,2	3	0,2	C
10	D-allulose	7,2	3	0,2	C D
10	D-allulose	1,6	3	0,2	C D
10	fructose	7,2	3	0,1	C D
7	D-allulose	1,6	3	0,1	C D E
10	glucose	7,2	3	0,1	D E
10	fructose	1,6	3	0,1	E F
10	glucose	1,6	3	0,1	F G
7	fructose	7,2	3	0,1	G
7	fructose	1,6	3	0,1	G

Means that do not share a letter are significantly different.

Table C.6. *Analysis of variance for browning intensity of microwave and water bath glycated soy protein isolate with fructose at pH 10*

```
Factor               Type   Levels  Values
Method               fixed       2  MW; WB
Protein:Sugar Ratio  fixed       2  1,6; 7,2


Analysis of Variance for Absrbance, using Adjusted SS for Tests

Source                       DF    Seq SS    Adj SS    Adj MS        F      P
Method                        1  0,140184  0,140184  0,140184  2009,81  0,000
Protein:Sugar Ratio           1  0,003367  0,003367  0,003367    48,27  0,000
Method*Protein:Sugar Ratio    1  0,000574  0,000574  0,000574     8,23  0,021
Error                         8  0,000558  0,000558  0,000070
Total                        11  0,144683


S = 0,00835165   R-Sq = 99,61%   R-Sq(adj) = 99,47%


Grouping Information Using Tukey Method and 95,0% Confidence

Method  N  Mean  Grouping
WB      6   0,4  A
MW      6   0,1    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Protein:Sugar
Ratio          N  Mean  Grouping
7,2            6   0,3  A
1,6            6   0,2    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

        Protein:Sugar
Method  Ratio          N  Mean  Grouping
WB      7,2            3   0,4  A
WB      1,6            3   0,3    B
MW      7,2            3   0,1      C
MW      1,6            3   0,1      C

Means that do not share a letter are significantly different.
```

Table C.7. *Analysis of variance for microwave glycated soy protein isolate with fructose (mg FAG/g FAG in native SPI, remained sugar concentration (%), and browning intensity at 420 nm, respectively)*

```
Factor               Type   Levels  Values
pH                   fixed       2  7; 10
Protein:Sugar Ratio  fixed       2  1.6; 7.2


Analysis of Variance for mg FAG/g FAG in native SPI, using Adjusted SS for
     Tests

Source                   DF   Seq SS   Adj SS   Adj MS        F      P
pH                        1  2213938  2213938  2213938  1218.52  0.000
Protein:Sugar Ratio       1    96276    96276    96276    52.99  0.000
pH*Protein:Sugar Ratio    1    28439    28439    28439    15.65  0.004
Error                     8    14535    14535     1817
Total                    11  2353188


S = 42.6252   R-Sq = 99.38%   R-Sq(adj) = 99.15%


Grouping Information Using Tukey Method and 95.0% Confidence

pH  N    Mean  Grouping
 7  6  1046.5  A
10  6   187.5    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

Protein:Sugar
Ratio          N   Mean  Grouping
7.2            6  706.6  A
1.6            6  527.4    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

    Protein:Sugar
pH  Ratio          N    Mean  Grouping
 7  7.2            3  1184.8  A
 7  1.6            3   908.3    B
10  7.2            3   228.4      C
10  1.6            3   146.6      C

Means that do not share a letter are significantly different.
```

Factor	Type	Levels	Values
pH	fixed	2	7; 10
Protein:Sugar Ratio	fixed	2	1.6; 7.2

Analysis of Variance for % remained sugar, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
pH	1	516.88	459.49	459.49	136.37	0.000
Protein:Sugar Ratio	1	17.63	17.63	17.63	5.23	0.062
pH*Protein:Sugar Ratio	1	115.17	115.17	115.17	34.18	0.001
Error	6	20.22	20.22	3.37		
Total	9	669.90				

S = 1.83559 R-Sq = 96.98% R-Sq(adj) = 95.47%

Grouping Information Using Tukey Method and 95.0% Confidence

pH	N	Mean	Grouping
7	5	81.1	A
10	5	67.3	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Protein:Sugar Ratio	N	Mean	Grouping
7.2	5	75.5	A
1.6	5	72.8	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

pH	Protein:Sugar Ratio	N	Mean	Grouping
7	7.2	3	85.9	A
7	1.6	2	76.3	B
10	1.6	3	69.4	C
10	7.2	2	65.2	C

Means that do not share a letter are significantly different.

Factor	Type	Levels	Values
pH	fixed	2	7; 10
Protein:Sugar Ratio	fixed	2	1.6; 7.2

Analysis of Variance for Browning, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
pH	1	0.0033001	0.0033001	0.0033001	70.21	0.000
Protein:Sugar Ratio	1	0.0003967	0.0003967	0.0003967	8.44	0.020
pH*Protein:Sugar Ratio	1	0.0002001	0.0002001	0.0002001	4.26	0.073

```
Error                    8  0.0003760  0.0003760  0.0000470
Total                   11  0.0042729


S = 0.00685565   R-Sq = 91.20%   R-Sq(adj) = 87.90%


Grouping Information Using Tukey Method and 95.0% Confidence

pH  N  Mean  Grouping
10  6   0.1  A
 7  6   0.1    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

Protein:Sugar
Ratio          N  Mean  Grouping
7.2            6   0.1  A
1.6            6   0.1    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

    Protein:Sugar
pH  Ratio          N  Mean  Grouping
10  7.2            3   0.1  A
10  1.6            3   0.1    B
 7  7.2            3   0.1      C
 7  1.6            3   0.1      C

Means that do not share a letter are significantly different.
```

Table C.8 *Analysis of variance for microwave glycated soy protein isolate with glucose (mg FAG/g FAG in native SPI, remained sugar concentration (%), and browning intensity at 420 nm, respectively)*

```
Factor               Type   Levels  Values
pH                   fixed       2  7; 10
Protein:Sugar Ratio  fixed       2  1.6; 7.2


Analysis of Variance for mg FAG/g FAG in native SPI, using Adjusted SS for
     Tests

Source                   DF   Seq SS   Adj SS   Adj MS        F      P
pH                        1  4760223  4760223  4760223  1124.80  0.000
Protein:Sugar Ratio       1   307751   307751   307751    72.72  0.000
pH*Protein:Sugar Ratio    1   190684   190684   190684    45.06  0.000
Error                     8    33857    33857     4232
Total                    11  5292515
```

S = 65.0543   R-Sq = 99.36%   R-Sq(adj) = 99.12%

Grouping Information Using Tukey Method and 95.0% Confidence

pH	N	Mean	Grouping
7	6	1467.5	A
10	6	207.8	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Protein:Sugar Ratio	N	Mean	Grouping
7.2	6	997.8	A
1.6	6	677.5	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

pH	Protein:Sugar Ratio	N	Mean	Grouping
7	7.2	3	1753.7	A
7	1.6	3	1181.3	B
10	7.2	3	241.9	C
10	1.6	3	173.8	C

Means that do not share a letter are significantly different.

Factor	Type	Levels	Values
pH	fixed	2	7; 10
Protein:Sugar Ratio	fixed	2	1.6; 7.2

Analysis of Variance for % remained sugar, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
pH	1	84.806	89.735	89.735	25.95	0.001
Protein:Sugar Ratio	1	6.272	3.509	3.509	1.01	0.347
pH*Protein:Sugar Ratio	1	30.695	30.695	30.695	8.88	0.021
Error	7	24.205	24.205	3.458		
Total	10	145.978				

S = 1.85953   R-Sq = 83.42%   R-Sq(adj) = 76.31%

Grouping Information Using Tukey Method and 95.0% Confidence

pH	N	Mean	Grouping
7	5	82.8	A
10	6	77.0	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Protein:Sugar
Ratio N Mean Grouping
7.2 6 80.5 A
1.6 5 79.3 A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Protein:Sugar
pH Ratio N Mean Grouping
7 1.6 2 83.9 A
7 7.2 3 81.7 A
10 7.2 3 79.2 A B
10 1.6 3 74.7 B

Means that do not share a letter are significantly different.

Factor Type Levels Values
pH fixed 2 7; 10
Protein:Sugar Ratio fixed 2 1.6; 7.2

Analysis of Variance for Browning, using Adjusted SS for Tests

Source DF Seq SS Adj SS Adj MS F P
pH 1 0.067350 0.067350 0.067350 3049.82 0.000
Protein:Sugar Ratio 1 0.003710 0.003710 0.003710 168.00 0.000
pH*Protein:Sugar Ratio 1 0.009690 0.009690 0.009690 438.80 0.000
Error 8 0.000177 0.000177 0.000022
Total 11 0.080927

S = 0.00469929 R-Sq = 99.78% R-Sq(adj) = 99.70%

Grouping Information Using Tukey Method and 95.0% Confidence

pH N Mean Grouping
7 6 0.3 A
10 6 0.1 B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Protein:Sugar
Ratio N Mean Grouping
1.6 6 0.2 A
7.2 6 0.2 B

Means that do not share a letter are significantly different.

```
Grouping Information Using Tukey Method and 95.0% Confidence

    Protein:Sugar
pH  Ratio          N  Mean  Grouping
 7  1.6            3   0.3  A
 7  7.2            3   0.2    B
10  7.2            3   0.1      C
10  1.6            3   0.1        D

Means that do not share a letter are significantly different.
```

Table C.9. *Analysis of variance for microwave glycated soy protein isolate with D-allulose (mg FAG/g FAG in native SPI, remained sugar concentration (%), and browning intensity at 420 nm, respectively)*

```
Factor               Type   Levels  Values

pH                   fixed       2  7; 10
Protein:Sugar Ratio  fixed       2  1,6; 7,2


Analysis of Variance for % remained free amino, using Adjusted SS for Tests

Source                  DF  Seq SS  Adj SS  Adj MS        F      P
pH                       1  112739  111043  111043  3665,22  0,000
Protein:Sugar Ratio      1    1501    1286    1286    42,46  0,000
pH*Protein:Sugar Ratio   1     562     562     562    18,56  0,004
Error                    7     212     212      30
Total                   10  115014


S = 5,50421   R-Sq = 99,82%   R-Sq(adj) = 99,74%


Grouping Information Using Tukey Method and 95,0% Confidence

pH  N   Mean  Grouping
 7  5  247,9  A
10  6   43,9    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Protein:Sugar
Ratio          N   Mean  Grouping
7,2            5  156,9  A
1,6            6  134,9    B

Means that do not share a letter are significantly different.
```

Grouping Information Using Tukey Method and 95,0% Confidence

pH	Protein:Sugar Ratio	N	Mean	Grouping
7	7,2	2	251,6	A
7	1,6	3	244,2	A
10	7,2	3	62,1	B
10	1,6	3	25,6	C

Means that do not share a letter are significantly different.

Factor	Type	Levels	Values
pH	fixed	2	7; 10
Protein:Sugar Ratio	fixed	2	1,6; 7,2

Analysis of Variance for % remained sugar, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
pH	1	1908,99	1908,99	1908,99	2749,96	0,000
Protein:Sugar Ratio	1	5,53	5,53	5,53	7,96	0,022
pH*Protein:Sugar Ratio	1	16,96	16,96	16,96	24,43	0,001
Error	8	5,55	5,55	0,69		
Total	11	1937,03				

S = 0,833180   R-Sq = 99,71%   R-Sq(adj) = 99,61%

Grouping Information Using Tukey Method and 95,0% Confidence

pH	N	Mean	Grouping
7	6	70,6	A
10	6	45,4	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Protein:Sugar Ratio	N	Mean	Grouping
1,6	6	58,7	A
7,2	6	57,3	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

pH	Protein:Sugar Ratio	N	Mean	Grouping
7	1,6	3	72,5	A
7	7,2	3	68,8	B
10	7,2	3	45,9	C
10	1,6	3	44,9	C

Means that do not share a letter are significantly different.

Factor	Type	Levels	Values
pH	fixed	2	7; 10
Protein:Sugar Ratio	fixed	2	1,6; 7,2

Analysis of Variance for Browning, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
pH	1	0,0000101	0,0000101	0,0000101	0,18	0,680
Protein:Sugar Ratio	1	0,0002708	0,0002708	0,0002708	4,92	0,057
pH*Protein:Sugar Ratio	1	0,0001021	0,0001021	0,0001021	1,86	0,210
Error	8	0,0004400	0,0004400	0,0000550		
Total	11	0,0008229				

S = 0,00741620 R-Sq = 46,53% R-Sq(adj) = 26,48%

Grouping Information Using Tukey Method and 95,0% Confidence

pH	N	Mean	Grouping
10	6	0,2	A
7	6	0,2	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Protein:Sugar Ratio	N	Mean	Grouping
7,2	6	0,2	A
1,6	6	0,1	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

pH	Protein:Sugar Ratio	N	Mean	Grouping
7	7,2	3	0,2	A
10	7,2	3	0,2	A
10	1,6	3	0,2	A
7	1,6	3	0,1	A

Means that do not share a letter are significantly different.

Table C.10. *Analysis of variance for solubility (%) of microwave glycated soy protein isolate at pH 7*

```
actor                Type   Levels  Values
Sugar Type           fixed       3  D-allulose; Fructose; Glucose
Protein:Sugar Ratio  fixed       2  1,6; 7,2


Analysis of Variance for % Solubility, using Adjusted SS for Tests

Source                          DF   Seq SS   Adj SS   Adj MS       F      P
Sugar Type                       2  26099,4  25430,5  12715,3  367,21  0,000
Protein:Sugar Ratio              1    243,5    207,5    207,5    5,99  0,032
Sugar Type*Protein:Sugar Ratio   2   1025,7   1025,7    512,8   14,81  0,001
Error                           11    380,9    380,9     34,6
Total                           16  27749,6


S = 5,88447   R-Sq = 98,63%   R-Sq(adj) = 98,00%


Grouping Information Using Tukey Method and 95,0% Confidence

Sugar Type  N   Mean  Grouping
Fructose    5  174,6  A
Glucose     6  102,3     B
D-allulose  6   79,8       C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Protein:Sugar
Ratio          N   Mean  Grouping
7,2            9  122,4  A
1,6            8  115,4     B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

            Protein:Sugar
Sugar Type  Ratio          N   Mean  Grouping
Fructose    1,6            2  174,9  A
Fructose    7,2            3  174,3  A
Glucose     7,2            3  116,5     B
Glucose     1,6            3   88,1       C
D-allulose  1,6            3   83,1       C
D-allulose  7,2            3   76,6       C

Means that do not share a letter are significantly different.
```

Table C.11. *Analysis of variance for solubility (%) of microwave glycated soy protein isolate at pH 10*

```
Factor               Type   Levels  Values
Sugar Type           fixed       3  D-allulose; Fructose; Glucose
Protein:Sugar Ratio  fixed       2  1,6; 7,2


Analysis of Variance for % Solubility, using Adjusted SS for Tests

Source                           DF  Seq SS  Adj SS  Adj MS        F      P
Sugar Type                        2  6406,7  6406,7  3203,3  1662,43  0,000
Protein:Sugar Ratio               1  1225,7  1225,7  1225,7   636,11  0,000
Sugar Type*Protein:Sugar Ratio    2  1341,7  1341,7   670,9   348,15  0,000
Error                            12    23,1    23,1     1,9
Total                            17  8997,2


S = 1,38813   R-Sq = 99,74%   R-Sq(adj) = 99,64%


Grouping Information Using Tukey Method and 95,0% Confidence

Sugar Type  N  Mean  Grouping
D-allulose  6  59,6  A
Glucose     6  29,2    B
Fructose    6  14,3      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Protein:Sugar
Ratio          N  Mean  Grouping
7,2            9  42,6  A
1,6            9  26,1    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

            Protein:Sugar
Sugar Type  Ratio          N  Mean  Grouping
D-allulose  7,2            3  80,1  A
D-allulose  1,6            3  39,2    B
Glucose     7,2            3  31,3      C
Glucose     1,6            3  27,1        D
Fructose    7,2            3  16,5          E
Fructose    1,6            3  12,1            F

Means that do not share a letter are significantly different.
```

Table C.12. *Analysis of variance for solubility (%) of microwave and water bath glycated soy protein isolate with fructose at pH 10*

```
Factor               Type   Levels  Values
Method               fixed       2  MW; WB
Protein:Sugar Ratio  fixed       2  1,6; 7,2


Analysis of Variance for % Solubility, using Adjusted SS for Tests

Source                       DF  Seq SS  Adj SS  Adj MS         F      P
Method                        1   34919   34919   34919  32899,80  0,000
Protein:Sugar Ratio           1       2       2       2      1,86  0,210
Method*Protein:Sugar Ratio    1      40      40      40     37,37  0,000
Error                         8       8       8       1
Total                        11   34969


S = 1,03023   R-Sq = 99,98%   R-Sq(adj) = 99,97%


Grouping Information Using Tukey Method and 95,0% Confidence

Method  N   Mean  Grouping
WB      6  122,2  A
MW      6   14,3    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Protein:Sugar
Ratio          N  Mean  Grouping
7,2            6  68,7  A
1,6            6  67,9  A

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

        Protein:Sugar
Method  Ratio          N   Mean  Grouping
WB      1,6            3  123,6  A
WB      7,2            3  120,8    B
MW      7,2            3   16,5      C
MW      1,6            3   12,1        D

Means that do not share a letter are significantly different.
```

Table C.13. *Analysis of variance for T_2 relaxation times before glycation*

```
Factor                Type   Levels  Values
protein: sugar ratio  fixed       2  1,6; 7,2
Sugar Type            fixed       3  D-Allulose; Fructose; Glucose


Analysis of Variance for T2, using Adjusted SS for Tests

Source                            DF   Seq SS   Adj SS   Adj MS          F
protein: sugar ratio               1  3827,98  3827,98  3827,98  586714,33
Sugar Type                         2     4,90     4,90     2,45     375,70
protein: sugar ratio*Sugar Type    2    37,13    37,13    18,57    2845,52
Error                             12     0,08     0,08     0,01
Total                             17  3870,09

Source                               P
protein: sugar ratio             0,000
Sugar Type                       0,000
protein: sugar ratio*Sugar Type  0,000
Error
Total


S = 0,0807739   R-Sq = 100,00%   R-Sq(adj) = 100,00%


Grouping Information Using Tukey Method and 95,0% Confidence

protein:
sugar
ratio     N  Mean  Grouping
1,6       9  97,8  A
7,2       9  68,7    B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Sugar Type  N  Mean  Grouping
D-Allulose  6  83,9  A
Glucose     6  83,2    B
Fructose    6  82,6      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

protein:
sugar
ratio     Sugar Type  N  Mean  Grouping
1,6       D-Allulose  3  99,3  A
1,6       Fructose    3  98,4    B
1,6       Glucose     3  95,7      C
7,2       Glucose     3  70,6        D
7,2       D-Allulose  3  68,5          E
7,2       Fructose    3  66,8            F
Means that do not share a letter are significantly different.
```

Table C.14. *Analysis of variance for T_2 relaxation times after microwave glycation at pH 7*

```
Factor                Type   Levels  Values
protein: sugar ratio  fixed       2  1,6; 7,2
Sugar Type            fixed       3  D-Allulose; Fructose; Glucose


Analysis of Variance for T2, using Adjusted SS for Tests

Source                           DF   Seq SS   Adj SS   Adj MS       F      P
protein: sugar ratio              1  14187,7  14187,7  14187,7  193,99  0,000
Sugar Type                        2   5221,0   5221,0   2610,5   35,69  0,000
protein: sugar ratio*Sugar Type   2   3090,2   3090,2   1545,1   21,13  0,000
Error                            12    877,6    877,6     73,1
Total                            17  23376,5


S = 8,55203   R-Sq = 96,25%   R-Sq(adj) = 94,68%



Grouping Information Using Tukey Method and 95,0% Confidence

protein:
sugar
ratio     N   Mean  Grouping
1,6       9  195,2  A
7,2       9  139,1    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Sugar Type  N   Mean  Grouping
D-Allulose  6  184,8  A
Fructose    6  172,4  A
Glucose     6  144,1    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

protein:
sugar
ratio     Sugar Type  N   Mean  Grouping
1,6       D-Allulose  3  231,4  A
1,6       Fructose    3  192,5    B
1,6       Glucose     3  161,7      C
7,2       Fructose    3  152,3      C
7,2       D-Allulose  3  138,3      C D
7,2       Glucose     3  126,6        D
Means that do not share a letter are significantly different.
```

Table C.15. *Analysis of variance for T_2 relaxation times of microwave glycated soy protein isolate with fructose at pH 7, protein-sugar weight ratio of 1.6*

```
Factor  Type   Levels  Values
Status  fixed       2  After Glycation; Before Glycation


Analysis of Variance for T2, using Adjusted SS for Tests

Source  DF  Seq SS  Adj SS  Adj MS       F      P
Status   1   13286   13286   13286  166,15  0,000
Error    4     320     320      80
Total    5   13606


S = 8,94222   R-Sq = 97,65%   R-Sq(adj) = 97,06%


Grouping Information Using Tukey Method and 95,0% Confidence

Status            N   Mean  Grouping
After Glycation   3  192,5  A
Before Glycation  3   98,4    B

Means that do not share a letter are significantly different.
```

Table C.16. *Analysis of variance for T_2 relaxation times of microwave glycated soy protein isolate with fructose at pH 7, protein-sugar weight ratio of 7.2*

```
Factor  Type   Levels  Values
Status  fixed       2  After Glycation; Before Glycation


Analysis of Variance for T2, using Adjusted SS for Tests

Source  DF  Seq SS  Adj SS  Adj MS        F      P
Status   1   10960   10960   10960  1108,03  0,000
Error    4      40      40      10
Total    5   11000


S = 3,14509   R-Sq = 99,64%   R-Sq(adj) = 99,55%


Grouping Information Using Tukey Method and 95,0% Confidence

Status            N   Mean  Grouping
After Glycation   3  152,3  A
Before Glycation  3   66,8    B

Means that do not share a letter are significantly different.
```

Table C.17. *Analysis of variance for T_2 relaxation times of microwave glycated soy protein isolate with glucose at pH 7, protein-sugar weight ratio of 1.6*

```
Factor  Type   Levels  Values
Status  fixed       2  After Glycation; Before Glycation


Analysis of Variance for T2, using Adjusted SS for Tests

Source  DF  Seq SS  Adj SS  Adj MS        F      P
Status   1  6532,2  6532,2  6532,2  1032,65  0,000
Error    4    25,3    25,3     6,3
Total    5  6557,5


S = 2,51509   R-Sq = 99,61%   R-Sq(adj) = 99,52%


Grouping Information Using Tukey Method and 95,0% Confidence

Status            N   Mean  Grouping
After Glycation   3  161,7  A
Before Glycation  3   95,7    B

Means that do not share a letter are significantly different.
```

Table C.18. *Analysis of variance for T_2 relaxation times of microwave glycated soy protein isolate with glucose at pH 7, protein-sugar weight ratio of 7.2*

```
Factor  Type   Levels  Values
Status  fixed       2  After Glycation; Before Glycation


Analysis of Variance for T2, using Adjusted SS for Tests

Source  DF  Seq SS  Adj SS  Adj MS       F      P
Status   1  4702,3  4702,3  4702,3  615,56  0,000
Error    4    30,6    30,6     7,6
Total    5  4732,8


S = 2,76388   R-Sq = 99,35%   R-Sq(adj) = 99,19%


Grouping Information Using Tukey Method and 95,0% Confidence

Status            N   Mean  Grouping
After Glycation   3  126,6  A
Before Glycation  3   70,6    B

Means that do not share a letter are significantly different.
```

Table C.19. *Analysis of variance for T_2 relaxation times of microwave glycated soy protein isolate with D-allulose at pH 7, protein-sugar weight ratio of 1.6*

```
Factor  Type   Levels  Values
Status  fixed       2  After Glycation; Before Glycation


Analysis of Variance for T2, using Adjusted SS for Tests

Source  DF  Seq SS  Adj SS  Adj MS       F      P
Status   1   26171   26171   26171  298,61  0,000
Error    4     351     351      88
Total    5   26521


S = 9,36172   R-Sq = 98,68%   R-Sq(adj) = 98,35%


Grouping Information Using Tukey Method and 95,0% Confidence

Status            N   Mean  Grouping
After Glycation   3  231,4  A
Before Glycation  3   99,3    B

Means that do not share a letter are significantly different.
```

Table C.20. *Analysis of variance for T_2 relaxation times of microwave glycated soy protein isolate with glucose at pH 7, protein-sugar weight ratio of 7.2*

```
Factor  Type   Levels  Values
Status  fixed       2  After Glycation; Before Glycation


Analysis of Variance for T2, using Adjusted SS for Tests

Source  DF  Seq SS  Adj SS  Adj MS       F      P
Status   1  7302,1  7302,1  7302,1  261,07  0,000
Error    4   111,9   111,9    28,0
Total    5  7414,0


S = 5,28867   R-Sq = 98,49%   R-Sq(adj) = 98,11%


Grouping Information Using Tukey Method and 95,0% Confidence

Status            N   Mean  Grouping
After Glycation   3  138,3  A
Before Glycation  3   68,5    B

Means that do not share a letter are significantly different.
```

Table C.21. *Analysis of variance for T_2 relaxation times after microwave glycation at pH 10*

```
Factor                Type   Levels  Values
protein: sugar ratio  fixed       2  1,6; 7,2
Sugar Type            fixed       3  D-Allulose; Fructose; Glucose


Analysis of Variance for T2, using Adjusted SS for Tests

Source                           DF  Seq SS  Adj SS  Adj MS       F      P
protein: sugar ratio              1  6176,9  6176,9  6176,9  703,18  0,000
Sugar Type                        2   156,8   156,8    78,4    8,92  0,004
protein: sugar ratio*Sugar Type   2   566,5   566,5   283,3   32,25  0,000
Error                            12   105,4   105,4     8,8
Total                            17  7005,6


S = 2,96383   R-Sq = 98,50%   R-Sq(adj) = 97,87%


Grouping Information Using Tukey Method and 95,0% Confidence

protein:
sugar
ratio     N  Mean  Grouping
1,6       9  93,2  A
7,2       9  56,1    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Sugar Type  N  Mean  Grouping
Fructose    6  77,8  A
Glucose     6  75,5  A
D-Allulose  6  70,7    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

protein:
sugar
ratio     Sugar Type  N  Mean  Grouping
1,6       Glucose     3  97,4  A
1,6       D-Allulose  3  93,8  A B
1,6       Fructose    3  88,4    B
7,2       Fructose    3  67,1      C
7,2       Glucose     3  53,7        D
7,2       D-Allulose  3  47,6        D

Means that do not share a letter are significantly different.
```

Table C.22. *Analysis of variance for T_2 relaxation times of microwave glycated soy protein isolate with fructose at pH 10, protein-sugar weight ratio of 1.6*

```
Factor  Type   Levels  Values
Status  fixed       2  After Glycation; Before Glycation


Analysis of Variance for T2, using Adjusted SS for Tests

Source  DF  Seq SS  Adj SS  Adj MS      F      P
Status   1  151,66  151,66  151,66  36,81  0,004
Error    4   16,48   16,48    4,12
Total    5  168,14


S = 2,02972   R-Sq = 90,20%   R-Sq(adj) = 87,75%


Grouping Information Using Tukey Method and 95,0% Confidence

Status            N  Mean  Grouping
Before Glycation  3  98,4  A
After Glycation   3  88,4    B

Means that do not share a letter are significantly different.
```

Table C.23. *Analysis of variance for T_2 relaxation times of microwave glycated soy protein isolate with fructose at pH 10, protein-sugar weight ratio of 7.2*

```
Factor  Type   Levels  Values
Status  fixed       2  After Glycation; Before Glycation


Analysis of Variance for T2, using Adjusted SS for Tests

Source  DF  Seq SS  Adj SS  Adj MS     F      P
Status   1   0,124   0,124   0,124  0,02  0,886
Error    4  21,196  21,196   5,299
Total    5  21,320


S = 2,30196   R-Sq = 0,58%   R-Sq(adj) = 0,00%


Grouping Information Using Tukey Method and 95,0% Confidence

Status            N  Mean  Grouping
After Glycation   3  67,1  A
Before Glycation  3  66,8  A

Means that do not share a letter are significantly different.
```

Table C.24. *Analysis of variance for T_2 relaxation times of microwave glycated soy protein isolate with glucose at pH 10, protein-sugar weight ratio of 1.6*

```
Factor  Type   Levels  Values
Status  fixed       2  After Glycation; Before Glycation


Analysis of Variance for T2, using Adjusted SS for Tests

Source  DF  Seq SS  Adj SS  Adj MS     F      P
Status   1    4,21    4,21    4,21  0,41  0,556
Error    4   40,84   40,84   10,21
Total    5   45,05


S = 3,19515   R-Sq = 9,35%   R-Sq(adj) = 0,00%


Grouping Information Using Tukey Method and 95,0% Confidence

Status            N  Mean  Grouping
After Glycation   3  97,4  A
Before Glycation  3  95,7  A

Means that do not share a letter are significantly different.
```

Table C.25. *Analysis of variance for T_2 relaxation times of microwave glycated soy protein isolate with glucose at pH 10, protein-sugar weight ratio of 7.2*

```
Factor  Type   Levels  Values
Status  fixed       2  After Glycation; Before Glycation


Analysis of Variance for T2, using Adjusted SS for Tests

Source  DF  Seq SS  Adj SS  Adj MS        F      P
Status   1  428,31  428,31  428,31  1356,09  0,000
Error    4    1,26    1,26    0,32
Total    5  429,57


S = 0,561997   R-Sq = 99,71%   R-Sq(adj) = 99,63%


Grouping Information Using Tukey Method and 95,0% Confidence

Status            N  Mean  Grouping
Before Glycation  3  70,6  A
After Glycation   3  53,7    B

Means that do not share a letter are significantly different
```

Table C.26. *Analysis of variance for T_2 relaxation times of microwave glycated soy protein isolate with D-allulose at pH 10, protein-sugar weight ratio of 1.6*

```
Factor  Type   Levels  Values
Status  fixed       2  After Glycation; Before Glycation


Analysis of Variance for T2, using Adjusted SS for Tests

Source  DF  Seq SS  Adj SS  Adj MS     F      P
Status   1  45,558  45,558  45,558  8,00  0,047
Error    4  22,774  22,774   5,693
Total    5  68,332


S = 2,38610   R-Sq = 66,67%   R-Sq(adj) = 58,34%


Grouping Information Using Tukey Method and 95,0% Confidence

Status            N  Mean  Grouping
Before Glycation  3  99,3  A
After Glycation   3  93,8    B

Means that do not share a letter are significantly different.
```

Table C.27. *Analysis of variance for T_2 relaxation times of microwave glycated soy protein isolate with D-allulose at pH 10, protein-sugar weight ratio of 7.2*

```
Factor  Type   Levels  Values
Status  fixed       2  After Glycation; Before Glycation


Analysis of Variance for T2, using Adjusted SS for Tests

Source  DF  Seq SS  Adj SS  Adj MS       F      P
Status   1  657,10  657,10  657,10  893,44  0,000
Error    4    2,94    2,94    0,74
Total    5  660,04


S = 0,857597   R-Sq = 99,55%   R-Sq(adj) = 99,44%


Grouping Information Using Tukey Method and 95,0% Confidence

Status            N  Mean  Grouping
Before Glycation  3  68,5  A
After Glycation   3  47,6    B

Means that do not share a letter are significantly different.
```

Table C.28. *Analysis of variance for the effects of pH on T_2 relaxation times of microwave glycated soy protein isolate with fructose, protein-sugar weight ratio of 1.6*

```
Factor  Type   Levels  Values
pH      fixed       2  7; 10


Analysis of Variance for T2, using Adjusted SS for Tests

Source  DF  Seq SS  Adj SS  Adj MS       F      P
pH       1   16277   16277   16277  193,61  0,000
Error    4     336     336      84
Total    5   16613


S = 9,16908   R-Sq = 97,98%   R-Sq(adj) = 97,47%


Grouping Information Using Tukey Method and 95,0% Confidence

pH  N   Mean  Grouping
 7  3  192,5  A
10  3   88,4    B

Means that do not share a letter are significantly different.
```

Table C.29. *Analysis of variance for the effects of pH on T_2 relaxation times of microwave glycated soy protein isolate with fructose, protein-sugar weight ratio of 7.2*

```
Factor  Type   Levels  Values
pH      fixed       2  7; 10


Analysis of Variance for T2, using Adjusted SS for Tests

Source  DF  Seq SS  Adj SS  Adj MS       F      P
pH       1   10886   10886   10886  716,76  0,000
Error    4      61      61      15
Total    5   10947


S = 3,89725   R-Sq = 99,45%   R-Sq(adj) = 99,31%

Grouping Information Using Tukey Method and 95,0% Confidence

pH  N   Mean  Grouping
 7  3  152,3  A
10  3   67,1    B

Means that do not share a letter are significantly different.
```

Table C.30. *Analysis of variance for the effects of pH on T_2 relaxation times of microwave glycated soy protein isolate with glucose, protein-sugar weight ratio of 1.6*

```
Factor  Type   Levels  Values
pH      fixed       2  7; 10


Analysis of Variance for T2, using Adjusted SS for Tests

Source  DF  Seq SS  Adj SS  Adj MS       F      P
pH       1  6204,6  6204,6  6204,6  375,40  0,000
Error    4    66,1    66,1    16,5
Total    5  6270,7


S = 4,06544   R-Sq = 98,95%   R-Sq(adj) = 98,68%


Grouping Information Using Tukey Method and 95,0% Confidence

pH  N   Mean  Grouping
 7  3  161,7  A
10  3   97,4    B

Means that do not share a letter are significantly different.
```

Table C.31. *Analysis of variance for the effects of pH on T_2 relaxation times of microwave glycated soy protein isolate with glucose, protein-sugar weight ratio of 7.2*

```
Factor  Type   Levels  Values
pH      fixed       2  7; 10


Analysis of Variance for T2, using Adjusted SS for Tests

Source  DF  Seq SS  Adj SS  Adj MS        F      P
pH       1  7968,9  7968,9  7968,9  1001,87  0,000
Error    4    31,8    31,8     8,0
Total    5  8000,7


S = 2,82029   R-Sq = 99,60%   R-Sq(adj) = 99,50%


Grouping Information Using Tukey Method and 95,0% Confidence

pH  N   Mean  Grouping
 7  3  126,6  A
10  3   53,7    B

Means that do not share a letter are significantly different.
```

Table C.32. *Analysis of variance for the effects of pH on T_2 relaxation times of microwave glycated soy protein isolate with D-allulose, protein-sugar weight ratio of 1.6*

```
Factor  Type   Levels  Values
pH      fixed       2  7; 10


Analysis of Variance for T2, using Adjusted SS for Tests

Source  DF  Seq SS  Adj SS  Adj MS       F      P
pH       1   28400   28400   28400  304,30  0,000
Error    4     373     373      93
Total    5   28773


S = 9,66074   R-Sq = 98,70%   R-Sq(adj) = 98,38%


Grouping Information Using Tukey Method and 95,0% Confidence

pH  N   Mean  Grouping
 7  3  231,4  A
10  3   93,8    B

Means that do not share a letter are significantly different.
```

Table C.33. *Analysis of variance for the effects of pH on T_2 relaxation times of microwave glycated soy protein isolate with D-allulose, protein-sugar weight ratio of 7.2*

```
Factor  Type   Levels  Values
pH      fixed       2  7; 10


Analysis of Variance for T2, using Adjusted SS for Tests

Source  DF  Seq SS  Adj SS  Adj MS       F      P
pH       1   12340   12340   12340  430,09  0,000
Error    4     115     115      29
Total    5   12455


S = 5,35652   R-Sq = 99,08%   R-Sq(adj) = 98,85%

Grouping Information Using Tukey Method and 95,0% Confidence

pH  N   Mean  Grouping
 7  3  138,3  A
10  3   47,6    B

Means that do not share a letter are significantly different.
```

Table C.34. *Analysis of variance for T_2 relaxation times of microwave and water bath glycated soy protein isolate with fructose at pH 10*

Factor	Type	Levels	Values
Method	fixed	2	MW; WB
Protein: sugar ratio	fixed	2	1,6; 7,2

Analysis of Variance for T2, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Method	1	1,84	1,84	1,84	0,16	0,704
Protein: sugar ratio	1	1074,36	1074,36	1074,36	90,46	0,000
Method*Protein: sugar ratio	1	16,19	16,19	16,19	1,36	0,277
Error	8	95,01	95,01	11,88		
Total	11	1187,40				

S = 3,44623 R-Sq = 92,00% R-Sq(adj) = 89,00%

Grouping Information Using Tukey Method and 95,0% Confidence

Method	N	Mean	Grouping
WB	6	78,5	A
MW	6	77,8	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Protein: sugar ratio	N	Mean	Grouping
1,6	6	87,6	A
7,2	6	68,7	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Method	Protein: sugar ratio	N	Mean	Grouping
MW	1,6	3	88,4	A
WB	1,6	3	86,8	A
WB	7,2	3	70,2	B
MW	7,2	3	67,1	B

Means that do not share a letter are significantly different.