PHYSICOCHEMICAL AND NMR RELAXOMETRIC CHARACTERIZATION OF FREEZE AND SPRAY DRIED GLYCATED SOY PROTEINS

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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

PHYSICOCHEMICAL AND NMR RELAXOMETRIC CHARACTERIZATION OF FREEZE AND SPRAY DRIED GLYCATED SOY PROTEINS

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Rare sugar is a type of a sugar group consisting of monosaccharides that is not widely found in nature as its name implies. D-psicose that is C-3 epimer of fructose is one of the rare sugars, and its sweetness is equivalent to 70% of the sweetness of sucrose. Researches showed that gylcation with sugars improves their physical and chemical properties. In this study, two different glycation techniques were conducted (freeze drying and spray drying) for soy proteins. In addition to D-Psicose, glycation was performed using glucose and fructose controls. Effect of two different pHs (7,10) and 5 different soy protein : sugar ratios (1:1, 2:1, 3:1, 5:1 and 10:1) were tested. For the glycated samples, emulsification activity, hydration behavior by NMR Relaxometery, free amino groups by OPA, protein solubility by Lowry Method, glycation degree, FTIR analysis, reducing sugar and antioxidant activity by DPPH experiments were conducted. According to the results obtained, the best combination to have the highest glycation degree was found to be the samples with pH 7 buffer, 1:1 soy protein to sugar ratio and freeze drying (FD) as the glycation type. This study showed that application of glycation on soy protein improved its functional properties. Indeed, developed properties of D-Psicose helped to improve the functional properties of the

soy protein. Also, it was seen that NMR Relaxometry could give insight on the degree of glycation by considering the hydration behavior.

Keywords: Soy Protein, D-Psicose, NMR Relaxometry, Freeze Drying, Spray Drying

ÖZ

DONDURULARAK VE PÜSKÜRTÜLEREK KURUTULMUŞ GLİKE SOY PROTEİNLERİNİN FİZİKOKİMYASAL VE NMR RELAKSOMETRİK KARAKTERİZASYONU

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Nadir şekerler, isminden de anlaşıldığı üzere, monosakkaritlerden oluşan bir şeker grubudur. Fruktozun C-3 epimer olan D-Psikoz, nadir şekerlerden biridir ve tatlılığı, sakarozun tatlılığının 70%'ine eşittir. Araştırmalara göre şekerler ile glikasyona uğradığında proteinlerin fiziksel ve kimyasal özelliklerinin geliştiği gözlemlenmiştir. Bu çalışmada, soya proteinleri için iki farklı glikasyon tekniği uygulanmıştır (dondurarak kurutma ve püskürterek kurutma). D-Psikoz'a ek olarak, glikoz ve fruktoz indirgeyici şekerler kullanılarak glikasyon yapılmıştır. İki farklı pH (7,10) ve 5 farklı soya proteini: şeker oranının (1:1, 2:1, 3:1, 5:1 ve 10:1) etkisi test edilmiştir. Glike olmuş örnekler için, emülsifikasyon aktivitesi, NMR Relaksometre ile hidrasyon davranışı, OPA ile serbest amino grupları, Lowry Yöntemi ile protein çözünürlüğü, glikasyon derecesi, FTIR analizi, indirgen şeker tayini ve antioksidan aktivite deneylerinin DPPH deneyleri ile belirlenmesi testleri uygulanmıştır. Deneylerden elde edilen sonuçlara göre, numunelerin en yüksek glikasyonu için en iyi kombinasyonun, pH 7 tamponu, 1: 1 soya proteini şeker oranı ve glikasyon tipi olarak dondurularak kurutma (FD) olduğuna karar verilmiştir. Bu çalışma, soya proteini üzerine glikasyon uygulamasının işlevsel özelliklerini geliştirdiğini göstermiştir. Gerçekten de, D-Psicose'un gelişmiş özellikleri, soya proteininin işlevsel özelliklerini geliştirmeye

yardımcı olduğu saptanmıştır. Ayrıca, NMR Relaksometre'nin hidrasyon davranışını dikkate alarak glikasyon derecesini belirlemek için kullanılabileceği görülmüştür.

Anahtar Kelimeler: Soya Proteini, D-Psikoz, NMR Relaksometre, Dondurarak Kurutma, Püskürterek Kurutma

To the ones that deserve best, especially my beloved family.

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INTRODUCTION

1.1. Soy Protein

1.1.1. General View

In today's world, the idea that protein consumption is a healthier way rather than consumption of fat and sugar has attracted the consumer's attention significantly. To meet this demand, addition of protein to the food formulations has been growing at unexpected rates in recent years. Soy protein due to its health effects on human body has gained popularity among food products. A recent study that gathered the opinion of the people from all over the world found that people who do not prefer to eat meat or animal based food products (vegetarians or vegans) are more likely to consume soy based food products and the number of people who choose this lifestyle increases day by day (Mojica, Dia, & De Mejía, 2014).

Soy protein is isolated from soybean and the history of planting soybean started in 1911 by Francis J.-G. Beltzer, who did his research in Vietnam to obtain vegetable casein from soybean (Shurtleff & Aoyagi, 2016). Since then, there is an enormous amount of food on the shelf in which soy protein is included.

Soy proteins are classified according to their protein content on a dry basis. The protein content of soy flour is around 40-50%, protein content of concentrate is around 70% and the highest protein content belongs to the soy protein isolate with a protein content of 90%. Having the highest protein content, soy protein isolate is the one that

is the most widely used as soy protein source (Lucia, Jin, Hubbe, Pawlak, & Rojas, 2012).

Soy protein isolate (SPI) has been used in the food industry since 1959 due to its functional properties. SPI is obtained through refining or purifying (Sipos, 1988). SPI is usually combined with other food ingredients. It is mainly used to improve the texture of food products, to increase protein content, to enhance the retention of moisture, and also is included in the formulations as an emulsifier (Singh, Kumar, Sabapathy, & Bawa, 2008).

According to the late surveys conducted on soy-based products, it was pointed out that there are 15,000 soy products on the market, and every other day many more coming out. The bottom line of the survey is that the soy protein market currently tops $5.1 billion a year and is planned to keep on growing as the need for the world's expanding population (Fraser, 2017).

1.1.2. Soy Protein & Health

Soybeans are different from the other vegetable foods with the fact that soybeans contain all eight essential amino acids that are required for human body. Soybeans are also found to have great number of vitamins and minerals. Soybeans are high in fiber, iron, calcium, zinc, and B vitamins (Sacks et al., 2006a).

One of the crucial feature of the soy protein is that including soy protein in the diet decreases Low Density Lipoprotein (LDL) whereas increases High Density Lipoprotein (HDL) levels (Blesso & Fernandez, 2018). Moreover, as reported by the

American Cancer Research Institute, soy protein decreases osteoporosis, breast, colon and prostate cancer risk in human body (Anderson, Johnstone, & Cook-Newell, 2002).

In another research which was conducted on rats, it was shown that including SPI on the diet reduced the concentrations of circulating estrogens in the female rats. This result of the research came to the conclusion that the risk of endocrine cancers in women and adverse effects of estrogen deficiency such as heart disease and osteoporosis can be prevented by the addition of soy protein to the diet (Badger, Ronis, & Hakkak, 1997).

When soy protein is included in the diet, not only consumption of low saturated fats and cholesterol decreases but also getting high polyunsaturated fats that are considered more healthier leads to the prevention from obesity and cardiovascular diseases (Sacks et al., 2006b). Moreover, it was found that, isoflavones in the soy proteins helped to lowered the amount of lipids on the blood (Anderson et al., 2002).

People who do sports regularly in their life or athletes use proteins as supplements in their diets. Soy protein is a good alternative as a supplement especially for the vegan persons. Therefore, the demand for soy protein intake as a supplement is also growing up very fast. In addition to its high protein content, soy protein contains naturally occurring compounds such as isoflavones and saponins which act as antioxidant, immune-regulatory, against cancer, and cardiovascular diseases. Moreover, soy protein that includes compounds like isoflavones helps to prevent free radical damage to the muscle while doing exercises. According to a research, for people who have been given soy protein isolate for four weeks as supplement; decrease in muscle damage and enhanced muscle recovery was observed compared the ones who did not use soy protein as supplement (Shenoy, Dhawan, & Sandhu, 2016).

A reasonable daily intake is calculated as 15 g soy protein and around 50 mg isoflavones to act as protector (Messina, 2016). Also, researchers suggest that higher amounts may be needed ; 25 g/day soy protein is thought to be used for cholesterol reduction (States & Chinese, 1999).

Among these all good benefits of soybean and soy proteins, there are some drawbacks of soy-based foods as well. Some people are allergic to soy-based foods. Although soy protein allergy is seen mostly in infancy, some of them carry the allergy into adulthood. According to historical evidences, the first allergic reactions of soy protein in humans were described in 1934. In the study, anti-soy IgE antibodies were identified but allergen specificity patterns were considered as more complex to be identified (Symptoms, 2011). People who suffer from soy protein allergy showed symptoms such as tingling in the mouth, swelling of the lips, face, tongue and throat. Apart from those, abdominal pain, diarrhea, nausea or vomiting were also the outcomes. However, very small number of fatal allergic reactions of soy protein have been reported to this date. In addition, in all these cases patients also had severe peanut allergies and asthma (Various, 2004).

On the other hand, although soy protein's allergen is not vital for human beings, researchers try to develop to prevent this problem by doing modifications on the proteins. Thanks to modification strategies on the proteins, the allergen characteristic of the protein is eliminated while its beneficial features are still remaining. One of the modification techniques is glycation of the protein with sugars. Glycated soy proteins were found to show a decrease in the allergenicity of soy proteins (van de Lagemaat, Manuel Silván, Javier Moreno, Olano, & Dolores del Castillo, 2007a). Glycation will be discussed in detail in the upcoming sections.

Soy foods are nutrient rich and considered as alternative sources to animal-based foods to fulfill the demand of growing population. Moreover, soy-based foods have potential to solve issues such as starvation and malnutrition all over the world. Nevertheless, the effects of soy-based foods to the human health is still a huge topic that needs comprehensive investigation.

1.1.3. Functional Properties of Soy Protein

Soy proteins in their different forms such as concentrated and isolated have functional properties which make them appropriate for food applications and consumer acceptance. Those properties are the intrinsic physicochemical characteristics that have an impact on the behavior of protein in food systems during processing, preparation, manufacturing and storage. These properties are needed to be investigated crucially since they are easily affected by the composition and conformational changes of the proteins and also their interactions with other food components. In addition to these intrinsic factors, proteins' functional properties are easily influenced by processing and the environmental changes occurred during treatment (Wolf, 1970).

1.1.3.1. Emulsifying Activity and Stability

One of the most important functional properties of soy protein is their emulsifying activity. Soy protein has an emulsion activity due to the fact that it contains both lipophilic and hydrophilic groups in the polymer chains. If a compound contains both these groups, the compound is named as amphiphilic. Thanks to this feature, soy protein can act as an effective emulsifier to form and even stabilize oil-in-water emulsions in a great extent (Tang, 2017).

Emulsifying properties of soy protein is related with the high conformational flexibility at the quaternary structural level. Indeed, the conformational flexibility

plays crucial role in the emulsifying properties of soy proteins. The emulsifying character of soy protein is actually coming from the two major protein fractions that are named as 11S and 7S globulins, or meaningly glycinin and β-conglycinin. These proteins place 87% of the total protein amount in soy bean with more glycinin compared to β-conglycinin (Adachi et al., 2003). The ratio of 11S:7S strongly effects the emulsifying ability of soy proteins. Furthermore, 11S:7S ratio could be important for the stability of emulsion (Mujoo, Trinh, & Ng, 2003).

Another important criterion for emulsions is the stability. Emulsion stability is defined as the ability of an emulsion to resist changes upon storage conditions. Stabilization of proteins in emulsions is actually related to high electrical charge state and more hydrophilic-lipophilic groups within the protein (Roesch & Corredig, 2003). Moreover, when protein concentration is increased adsorption behavior of protein is also increased. Hence, the emulsion stability could be increased. In that regard, soy protein facilitates the emulsion stability because of its high protein content in varied forms (Zayas & Cliffs, 1991).

1.1.3.2. Solubility

Determination of solubility is an important criterion for potential applications in food industry to observe how other physicochemical and functional properties are affected. Solubility of soy protein is highly affected by the applications such as heating, drying, and other processing treatments during manufacture and storage conditions. Furthermore, the environmental conditions including pH, ionic strength, and temperature strongly impact the soy protein solubility (Lee, Ryu, & Rhee, 2003a). Therefore, for all different conditions, solubility profile of the soy protein should be evaluated individually.

In a research where solubility behavior of soy protein in different conditions was examined, it was found out that soy protein solubility was enhanced with the increase of pH from 6 to 8. On the other hand, solubility was the lowest around pH 4-5 due to the fact that this pH range was actually close to the isoelectric point of soy protein (pI) (Hefnawy & Ramadan, 2011). When the pH of the protein solution is close to its pI value, the net electric charge of that protein is nearly equal to zero which causes decrease in the electric attractive force between polypeptide chains. Therefore, around pI range of soy protein, the solubility would be lowest (Wang & Zayas, 1991). In order to get better solubility of soy protein, pH should be increased to the values above 7. Jung and Murphy (2005) also pointed out in their study that increasing pH to the alkali conditions generally increased protein solubility in soy protein concentrate.

There are many researches conducted to evaluate the effect of temperature on the soy protein solubility. Soy protein is known to be more heat stable compared to animal-based protein sources. Therefore, the solubility could be improved by high temperature like 70–80°C. In one study, when temperature was increased from 25°C to 50°C, protein solubility increased by more than 20%. This was explained by unfolding behavior of the protein with temperature. By doing so, the structure was changed to a straight chain form that resulted in more protein-water interactions (Lee, Ryu, & Rhee, 2003b).

The contribution of ionic strength on soy protein solubility depends on the concentration of the buffer solution and the pH of the media together. In one study, when the ionic strength was increased from 0 to 0.5 (0.2M with $CaCl_2$ buffer), the water holding capacity (WHC) of the gels prepared by soy protein decreased by almost 30%. Moreover, in order to understand the effect of pH and ionic strength on the solubility, pH was selected in one case as acidic and in the other as alkali while keeping all the other factors constant. The results of this examination found that WHC

of the gels decreased in alkali medium. As WHC decreases, the protein-water exposure increases. Thus, solubility of the soy protein increases as exposure occurs (Puppo & Añón, 1998). As a conclusion, if both pH of the medium and ionic strength of the buffer increase simultaneously, the solubility of the soy protein can be enhanced.

1.1.3.3. Gelation

Formation of a gel is a complex process in which several changes like denaturation, dissociation or association and aggregation occurs respectively. Soy protein's major two proteins that are glycinin and β-conglycinin have the ability to form gels in ordered structures. Gel formation is very useful in food industry since the ability of a protein to form a gel contributes to water holding, encapsulation of flavors or other compounds (Hermansson, 1985).

Gel strength of the soy protein is related with the protein concentration. Soy isolates that had the highest protein concentration among soy protein variations form firm, tough and hard gels. On the other hand, soy protein forms that have less than 70% protein tend to form soft fragile gels (Govindaraju, 2003).

According to a research, the gelation of the soy protein starts after heating to 60 °C. However, it should be pointed out that, in order to obtain a gel from the soy proteins at least 8% protein concentration is needed. When the protein concentration increased for example from 8 to 20% temperature required for maximum gel formation also increased from 70 °C to 100 °C (Nishinari, Fang, Guo, & Phillips, 2014). In addition, the firmness of the gels increased with increasing protein concentration (Inaba, Hoshizawa, Adachi, Matsumura, & Mori, 1994).

When the protein fraction's gelation abilities are considered it is seen that, gelation of glycinin are influenced by electrostatic interactions and disulfide bonds in the protein matrix. Furthermore, in the case of β-conglycinin mostly hydrogen bonding and hydrophobic interactions affect the gel formation and its strength. Also, when the hardness value of the gels was compared, gels formed with β-conglycinin were found to be harder than the gels formed by glycinin (Utsumi & Kinsella, 1985).

It is known that soy protein is affected by pH and ionic strength of the medium relatively. The strength of the gel is also affected by the pH and the ions. There are some studies that investigated the effect of salt (ionic strength) and pH on gelation properties of soy protein. According to the findings, gelation behavior of soy proteins was influenced by salt and pH substantially. Also, the strong gels were formed at salt concentration of 0.4–0.5 M NaCl and at a pH range of 3–5 (Shan et al., 2015).

1.1.3.4. Water absorption and binding

Soy protein includes some polar components in it such as hydroxyl, amino, carboxyl, and ionic components. These polar components of soy protein interact with water. It is a fact that soy protein is one of the hydrophilic polymers with high absorbing when soaked in water. Moreover, to determine water binding properties of soy protein in an accurate way, water activity should be determined. The water absorption by soy proteins was explored in many studies and in one study, water absorption pattern was obtained at a water activity range of 0.3-0.9 (Roesch & Corredig, 2003).

Water binding of soy protein is important in many food product formulations. Experimental results of one research found that enzyme treatment with transglutaminase resulted in a positive impact on water binding of the protein. Indeed, soybean 7S and 11S globulin proteins showed the ability to swell and bind water

(Zhang & Zhao, 2013). The effect of temperature on water absorption behavior of soy protein was also examined in one study. The study was based on heating of the soy protein isolate to 80 °C and 100 °C. The results showed that temperature had a little effect on moisture sorption pattern (Inaba et al., 1994).

1.1.3.5. Antioxidant Activity

In the last decade, the attention for natural compounds that have antioxidant activity increased. The most important concern of this situation is due to the usage of synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tert-butyl hydroquinone (TBHQ) having potential side effect of toxicity and carcinogenicity in human body. According to the findings of the researches, natural antioxidants have beneficial health effects on coronary heart diseases and cancer (Loganayaki, Siddhuraju, & Manian, 2013).

Antioxidants are defined as the substances that inhibit oxidation. Oxidation is one of the chemical reaction that could produce free radicals. Those free radicals may lead to chain reactions that cause damage the cells of organisms (Ahn, Min, & Ahn, 2005). Oxidation is also one of the serious problems faced by the food industry because with oxidation not only off-flavors are produced in the food but also the nutritional quality and shelf-life of the foods are badly affected. To eliminate those risks in the food systems, antioxidants are added to the foods (Jin et al., 2015).

Soy protein is classified as one of the natural antioxidants among the plant-based protein sources. Soy protein contains components such as phenolics, flavonoids, tannins and isoflavones that are actually responsible for powerful antioxidant activity. For instance, flavonoids have double bonds that include C-ring that supplies higher antioxidant activity (Loganayaki et al., 2013).

Researches about antioxidant activity of the soy proteins is still a major topic since there are still some compounds that are also responsible of the antioxidant activity. For instance, according to Pratt (1972) in addition to isoflavones, aromatic amines, sulfhydryl compounds are also counted to act as antioxidants in plant tissues and these substances are in synergistic effect with other.

1.1.4. Soy Protein Modification Techniques

The usage of soy-based foods and soy proteins in the food industry is on the increase these days. However, there are some drawbacks of soy protein in terms of both functional properties and health concerns. For instance, the solubility of soy protein is limited. Moreover, there are some people that are allergenic to soy protein. To eliminate those problems and to keep the benefits coming from soy protein, some modifications are needed to be applied on the proteins. By the help of those modifications, not only the drawbacks of the protein would be eliminated but also its functional properties may be improved. The nutritive, physicochemical and functional properties of soy proteins' can be modified by physical, chemical and biochemical treatments (Schmohl & Schwarzer, 2014).

1.1.4.1. Physical Modification

Thermal modification is one of the oldest and most frequently used physical modification technique for soy protein. There are various ways of applying thermal treatment to soy protein. By heat treatment protease inhibitor activity and lipoxygenase activity could be reduced (Csapó & Albert, 2019). In addition, by the help of heat some undesirable volatile compounds can be eliminated. The effects of heating also increase the digestibility of the soy proteins (Kumar, Choudhary, Mishra, Varma, & Mattiason, 2002). Vnučec et al. (2015) stated that heating the solution prepared with soy protein at around 80°C increased the adhesive bonding strength.

The investigations on soy protein demonstrated that the soy protein functionality depends on the degree of dissociation, denaturation and aggregation of 7S and 11S globulins. When soy protein is exposed to heat, those globulins are denatured and some functional properties were improved such as solubility, water absorption binding, gelation, emulsification activity. Wang & Johnson (2001) showed that the direct steam–infusion treatment as an alternative way to improve solubility, foaming and emulsifying properties of the soy protein. In their experiment, they suggested that after 30 seconds of steam infusion, the solubility of the protein increased to 60% while emulsifying properties increased about four times.

1.1.4.2. Chemical Modification

It is known that the effect of high or low pH values on soy protein is crucial. It was pointed out that around the isoelectric point of the soy protein the solubility is lowest. Therefore, acidification of the soy protein especially to 4.0-5.0 causes insolubilization of the protein nearly about 80%. On the other hand, solubility of soy proteins is substantially increased around pH of 11-12. However, toxic substances like lysinoalanine could be formed at this high pH values. In order to eliminate these toxic components, mild alkaline modification (pH 8.0) at slightly increased temperatures (50-60°C) might be preferred to have better solubility of the soy proteins.

Acetylation and succinylation of proteins with acid anhydrides are the mostly used chemical modification techniques for soy proteins (Lundblad, 2010). Acetylation with acetic anhydride actually is the covalent attachment of neutral acetyl functions to the protein amino group. This reaction results in a partial unfolding of the protein. Acetylation reaction of the protein contributes to a slight increase in solubility, reduced isoelectric point, and decreased tendency to gel upon heating (Kester & Richardson, 2010). Succinylation compared to acetylation supplies more benefits on functional properties. This is because of the electrostatic repulsive forces that result

from the enhanced negative charge. At the end those charges facilitate to a more extensive unfolding of the protein. The modifications due to succinylation were found to result in increased solubility, enhanced hydration, and emulsifying properties (Franzen & Kinsella, 1976).

Another chemical modification strategy is achieved by observing the effects of amidation and esterification on soy protein isolate. The reaction showed that amidation blocked 78% of the available carboxyl groups, producing a modified protein with an isoelectric point of 4.2 for native soy protein isolate. Esterification with methanol or ethanol produced derivatives with 83 and 55% of the carboxyl groups modified, yielding isoelectric point of 5.2. After reactions were completed the properties were examined. For example, emulsifying activity of amidated and esterified soy protein was slightly lower than that of native protein. On the other hand, stability of emulsions prepared with these modified proteins were significantly greater compared to the native protein (Muhammad, Saïd, & Thomas, 2012).

1.1.4.3. Modification by Glycation

Glycation is one of the non-enzymatic browning that is the reaction occurring between reducing sugars and free amino groups of protein molecules. Glycation has been widely used in the food industry since glycated proteins not only improve functional properties of proteins but also the appearance and taste of the final product (Sáenz-Suárez et al., 2016).

Glycation compared to other chemical modification techniques is more reliable in the food industry since it occurs under mild and safe conditions and it does not require any extraneous chemicals. There are several factors that affect the glycated proteins

functionality such as temperature, time, pH, water activity (a_w), type of sugars and the protein: sugar ratio (Garza, Ibarz, & Paga, 2000).

It has been documented that soy protein glycation brings important functional properties to the food formulations. In some of the studies, it was shown that properties like solubility, emulsion and foaming activity, antioxidant activity and gelation ability improved after soy protein is modified by different types of sugars. Furthermore, studies concluded that soy protein allergenicity that is considered as the most crucial disadvantage of soy protein could be eliminated by this safe modification technique. Therefore, among the other modifications, glycation of soy protein is superior (Liu, Ru, & Ding, 2012).

In one of the studies, soy protein was reacted with fructose as a reducing sugar. The glycation products were successfully produced in powder form. Those powders in solutions were examined with SDS-PAGE. The analysis showed that modification of the 7S and 11S fractions of soy protein took place. After that in the human body, antigenicity of soy proteins was measured by direct ELISA test and decrease on the allergenicity was observed for the glycated proteins (van de Lagemaat, Manuel Silván, Javier Moreno, Olano, & Dolores del Castillo, 2007b).

In another study, the functional properties of the soy protein after glycation with maltose were investigated (Xu & Zhao, 2019). In the experiment, glycated soy protein products showed enhancement with respect to emulsifying properties compared to non - glycated samples. In addition, glycation had positive effect on hydration behavior of the soy protein. Indeed, soy protein glycation increased the proteins' water absorption in a great extent.

Solubility is also one of the crucial problems of soy protein. In order to solve this problem modification of solubility by glycation was also studied in several researches. In the case of solubility, the attachment of hydrophilic groups increases the hydrophilic nature of the proteins, hence supplying enhancement in the solubility. However, the solubility phenomena is more complex than this and there are other parameters involving in the solubility (Tang, Sun, & Foegeding, 2011). Those parameters can be listed as; pH, monovalent ions such as NaCl, temperature and different protein subunit types of SPI (Lee et al., 2003a).

It has been proven that different types of sugars affect the glycation procedures differently (Kim, Park, & Kim, 2017). Since in glycation not only protein structure is modified but also sugar is affected from the reaction. Various reducing sugars showed different behavior on the glycation. There are many studies conducted about the glycation of soy protein with reducing sugars such as dextrose, fructose and maltose (Clemens et al., 2016).

1.2. Rare Sugar (D-Allulose/Psicose)

Recently, people try to reduce the consumption of sugars due to their high caloric values and the side effect of sugar consumption on people such as obesity, diabetes and coronary heart diseases. Although, people do not want to get the calorie from the sugars they want to feel the sweet taste of the sugar products. Because of those concerns in the society, researches on new monosaccharides that are actually found rarely in nature began to attract attention. D-Allulose/Psicose is one of them and got the attention in the public and in industry due to its health benefits and similar properties to sucrose.

D-Allulose/Psicose that is also known as rare sugar is one of the monosaccharides that is not widely available in nature. It is C-3 epimer of Fructose. When the sweetness value of the D-Psicose was examined it was found that it is equivalent to the 70% of the sweetness of sucrose. Furthermore, researches revealed that D-Psicose is metabolized in a lesser extent than other monosaccharides such as dextrose and fructose by the body. D-Psicose compared to monosaccharides has lower caloric value indeed 0.39 kcal/g (Oh, 2007). In addition, D-Psicose was announced as GRAS (Generally Recognized as Safe) by U.S. Food and Drug Administration (FDA) in 2012. Researches on D-Psicose increases day by day and experts try to develop this sugar as an alternative to sucrose.

The first production of D-Psicose was achieved in 1994 with the help of recombinant enzyme technology in Kagawa University using D-Tagatose 3 epimerase (DTE) enzyme specifically. However, in those times production of D-Psicose was quite expensive. That's why studies were tried to develop new methods to lower the cost of the production. Ken Izumori from Kagawa University has developed a strategy by immobilizing the DTE enzyme. This strategy brought the reusage of the enzyme thus facilitate a significant reduction in the cost. On the other hand, with the evolved technologies, Ken Izumori began taking measures to propagate the zuina tree in which D-Psicose is found naturally (Ogawa, Inoue, Hayakawa, O'Charoen, & Ogawa, 2017). Thus, the advantages of being a natural low calorie sugar source made D-Psicose one of the most researched sugars in the literature (Beerens, Desmet, & Soetaert, 2012; Chung, Oh, & Lee, 2012; Mu, Zhang, Feng, Jiang, & Zhou, 2012; O'Charoen, Hayakawa, Matsumoto, & Ogawa, 2014; Yagi & Matsuo, 2009).

1.2.1. Use of D-Psicose in Food Systems

Nowadays, D-Psicose is used in commercial food products such as beverages, yogurt, ice cream, baked products (Parkway & Estates, 2015). It is added to the formulations

as the low-calorie sweet sugar. Also, it was reported that D-Psicose provides great sweetness, smooth texture, desirable mouthfeel and proper stability to food products (Chattopadhyay, Raychaudhuri, & Chakraborty, 2014).

In an experiment, D-Psicose was added as sugar replacer in a cake formulation. The cakes made with D-Psicose was compared with the one made with sucrose. The ones prepared with D-Psicose showed a softer and fluffy texture. Another comparison was made on a yoghurt product. In the final yoghurt product, the flavor profile was less sour and the texture of the yogurt was thicker compared to the regular yoghurt products in the markets (Sweetness, 2019).

D-Psicose is considered as an ideal sucrose substitute for food formulations that require sugar sources. There are some advantages of D-Psicose examined throughout the researches. Those can be counted as high solubility, low glycemic response, desired sweet taste and being lower in calorie (Best, 2010). Furthermore, detailed studies on D-Psicose revealed that including D-psicose in the food products may enhance the gelling properties and antioxidant activity of the final products (Mu et al., 2012).

D-Psicose was also exposed to Maillard reaction with proteins like egg white and ovalbumin to observe the behavior throughout the reaction. According to the findings of the studies, D-Psicose contributed to good flavor in the product and also resulted in higher antioxidative Maillard products (Sun, Hayakawa, Puangmanee, & Izumori, 2006).

In the study of Sun, et. al. (2008), the effects of D-Psicose on foaming ability of egg white protein was analyzed. It was shown that compared to other sugar types like

dextrose and fructose, D-Psicose improved the foaming ability and stability of the egg white protein in a greater extent. In the next step of the research, D-Psicose was selected as sucrose replacer in the cookie formulation. When the cooked cookies were observed it was found that the ones with D-Psicose showed better crust color formation and higher antioxidant activity.

In a study by Oshima et al (2014), D-Psicose concentration was checked while caramelization and Maillard reaction were taking place to evaluate D-Psicose stability. The results showed that during caramelization and the Maillard browning reactions, the concentration of D-Psicose reduced when the temperature and the pH value of the reaction was increased. Therefore, it was emphasized that D-Psicose loss can be minimized by controlling the temperature and pH during those reactions (Oshima, Ozaki, Kitakubo, & Hayakawa, 2014).

1.2.2. Health Benefits of D-Psicose

D-Psicose has been shown to have numerous health benefits in the human body. D-Psicose has been shown to reduce weight loss and glycemic effect (Chung et al., 2012). Apart from these, in one study it was shown that D-Psicose has a huge impact on the treatment of obesity and reduction in type 2 diabetes, hyperglycemia, anemia, and hemophilia (Levin, 2003).

When diabetic patients were examined for the effect of different types of sugar ingestion like sucrose maltose and D-Psicose, D-psicose was found to be more useful for the protection postprandial hyperglycemia in diabetic patients compared to other sugars (Oh, 2007). As a support of this study, Baek & Park (2010) showed that D-Psicose was an effective sweetener in improving hyperglycemia and dyslipidemia as

well as facilitating weight loss. They pointed out that the mechanism underneath this was the enhancement of insulin sensitivity and improvement of the lipid composition.

A weight loss study was also performed using D-Psicose as the sugar source in the diet for 12-week period. The investigation showed that there was significant reduction in the body weight and fat percentage of the body in the group who was fed by D-Psicose rather than corn syrup. Also, this study showed that there was no side effects or abnormal blood parameters in the human body due to consumption of this rare sugar (Hayashi et al., 2014).

Researches on the metabolism of D-Psicose in the body was also examined in one study. According to the results, it was shown that D-Psicose was highly absorbed in the human intestine. Moreover, the study focused on whether the mechanism is different from fructose or not. Interestingly, even though both sugars showed differences in their metabolic behavior in the body, they were absorbed in the same way (Hishiike et al., 2013).

Researches on the formulation and absorption of D-Psicose, its effects on health and its functional usage in food industry still continue in Kagawa University.

1.3. Characterization of Glycated Soy Protein

1.3.1. Antioxidant Activity

There are several methods to determine the antioxidant activity of a substance. Most used methods can be listed as; CUPRAC, DPPH, ORAC, ABTS, FRAP, PFRAP, HORAC, TRAP and fluorimetry (Pisoschi & Negulescu, 2012). In this study, DPPH (2,2-di(4-tert-octyl-phenyl)-1-picrylhydrazyl) radical scavenging activity was used.

1.3.1.1. DPPH Radical Scavenging Method

DPPH is one of the rapid, simple, cheap, reliable and widely used method to determine the antioxidant activity of foods. The method uses free radical, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) to check the ability of components if they act as free radical scavengers and have antioxidant activity or not (Shekhar & Anju, 2014).

DPPH, a stable free radical at room temperature is in purple color and maximum absorption is obtained at 517 nm. The DPPH method starts with the reduction of DPPH in the prepared methanolic solution. The reduction occurs by H donor to DPPH molecule from the free radical scavengers like antioxidants. In other words, DPPH molecule reduces to DPPH-H. As a result of this reaction, color change occurs from purple to yellow with respect to the number of electrons attained. It was shown that more intense decolorization indicated more antioxidant activity of substances (Garcia et al., 2012).

There are some concerns to take into action while DPPH radical scavenging method is used. For instance, when the method is used, the temperature of the prepared solution should be in the range of ambient temperature to eliminate the degradation that may occur in the molecules. In addition, structural form of the antioxidant has a huge impact on the reaction. Some form of the antioxidant reacts faster while others are slower. This affects the decolorization intensity of the reaction and antioxidant activity at the same time (Marxen et al., 2007). The scheme of the reaction of DPPH with antioxidant follows as;

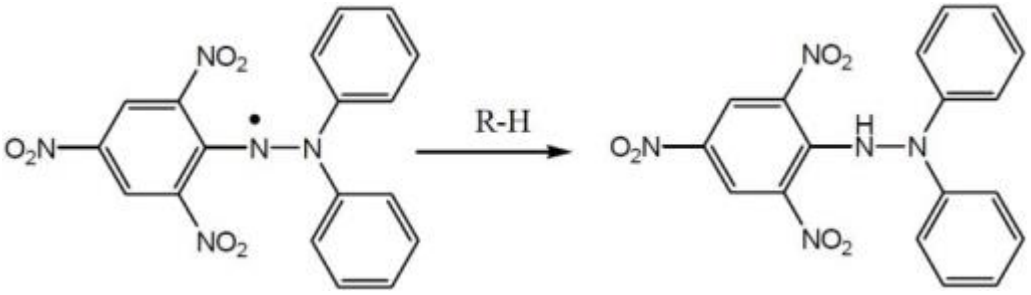

Figure 1.1 Reaction of DPPH with an antioxidant

1.3.2. Degree of Glycation

One of the changes occurred while glycation, a type of non-enzymatic browning is the formation of brown macromolecule substances. Generally, it is really hard to detect the brown substances in glycation process. However, the term browning index or degree of glycation could be used to monitor this browning reaction by optical observation. In order to monitor whether the brown pigments are formed or not, a UV spectrophotometer is usually used (Yu, Li, Yang, & Yu, 2017).

Absorbance values at 294 nm are used to determine the concentrations of the intermediate compounds. Those intermediate compounds are obtained before brown pigments formed on Maillard reaction and these exhibit fluorescent properties. On the other hand, for the absorbance value of 420 nm, brown compounds that are obtained at the last stage of the glycation could be monitored. Brown color is actually related with the accumulation of melanoidin pigment. According to the researches, the higher the absorbance values at 420 nm reveals more melanoidin pigments (Hong, Meng, & Lu, 2015).

1.3.3. Determination of Protein Solubility by Lowry Method

The determination of protein solubility in a solution by Lowry method was introduced by the Lowry in 1951 (Lowry, Rosebrough, Lewis, & Randal, 1951). From that year, this method is one of the much preferred one among the determination of protein solubility. Lowry method is a sensitive assay that does not require the full digestion of protein (Department of Biotechnology, 1996).

Lowry method determines the total concentration of the protein in a solution by colorimetric assay. Meaningly, color change is developed based on the protein concentration in the solution. In addition, the amount of color formed throughout the reaction is different in different proteins (Lowry., O, Nira J. Rosebrough, & Farr, 1994).

Lowry method relies on the reaction between a copper source, sodium carbonate, sodium potassium tartarate and Folin Ciocalteu's phenol reagent (Lowry et al., 1951). When copper ion is introduced, it reacts with the protein throughout the peptide bonds. After that, with the addition of Folin Ciocalteu's phenol reagent, Folin–protein binding takes place under alkaline pH conditions. In the final stage of that complex formation, the color changes from yellow to blue slowly (Kresge, Simoni, & Hill, 2005). The scheme below shows how the reaction occurs:

$$Cu^{2+} + \text{Protein (Peptide Bonds)} \longrightarrow (Cu^{1+}\text{-Peptide Bond Complex})$$

$$\text{Folin-Phenol Reagent} + (Cu^{1+}\text{-Peptide Bond Complex}) \longrightarrow \text{Reduced Folin Phenol Reagent}$$

After waiting for the formation of the complexes around 30 minutes, the absorbance values of the samples at 680 nm and 750 nm are recorded.

In the case of the total soluble protein determination in the solution, Lowry method gives accurate and reliable results. Moreover, its easy use at the lab scale and its sensitivity make that method as one of the most used method among the others. However, the possibility of the reaction of protein molecules with the other compounds in the solution may cause misleading results. Therefore, while using this method, those possibilities should be considered. On the other hand, according to some researches, the usage of the isolate form of the protein would eliminate these possible lack results (Glyk, Heinisch, Scheper, & Beutel, 2015).

1.3.4. Determination of Free Amino Groups by OPA Method

OPA is the used name of the term that is actually a chemical compound and its either iso-phthalaldehyde or ortho-phthalaldehyde. The formula of OPA is $C_6H_4(CHO)_2$. The OPA reagent is pale yellow solid that can be used for the determination of amino acids in a solution (Bill & Tarbell, 2003).

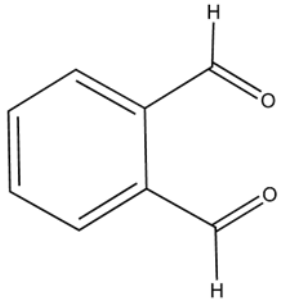

Figure 1.2 ortho-phthalaldehyde

It was shown that OPA dissolves in water and reacts with free amino acids under alkaline conditions (pH < 11.5). This reaction occurs with the presence of 2-mercaptoethanol and resulted in the formation of fluorescent compounds (Pravadali-Cekic et al., 2017). Free amino groups of the proteins by OPA method is obtained by the color change occurred during reaction and it is read by UV spectrophotometer at

340 nm. After absorbance values are read, the conversion was made by using the standard curve that is prepared by Bovine Serum Albumin (BSA) (Opa, 1987).

There are some crucial points that should be taken into account while OPA method is used. For instance, the reagent is so sensitive that it is easily affected by the exposure to light. Also, when the OPA solution is prepared, it should be used within 2 hours. It is also shown that when the OPA is added to the sample solution, 2 minutes of waiting time should be applied develop the color formation (Held, 2006). Therefore, those parameters should be done carefully to obtain accurate results.

According to the researches, OPA assay is highly accurate around 90–100% and it is so easy to perform and detect the free amino groups of proteins. Moreover, usage of OPA method has several crucial advantages. For example, modifications on the proteins such as surface charge change in the protein and glycation do not cause decrease in the accuracy of OPA assay. In addition, performing this method is simple and pH changes between pH 6.9 to 11.5 did not affect the accuracy of the final results. This means that OPA method can be easily applied as long as the pH values of the solutions are within this range (Diab et al., 2009).

Overall, the OPA assay is an accurate, sensitive, reproducible and simple to obtained free amino groups determination of proteins.

1.3.5. Reducing Sugar Amount Determination by DNS Method

3,5-Dinitrosalicylic acid (DNS) is an aromatic compound that can easily participate in a reaction with reducing sugars. The DNS method has also been used to determine enzyme activities of other carbohydrases, such as amylases, β-mannanases,

pectinases, and xyloglucanases. When this reaction is held in the alkaline solution, the formation of 3-amino-5-nitrosalicylic acid occurs with orange color (Miller, 1959). The Figure 1.3 shows the oxidation-reduction reaction mentioned.

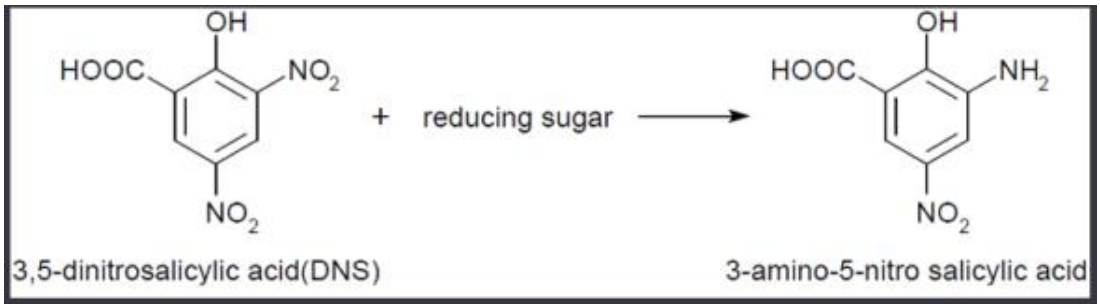

Figure 1.3 Conversion of reducing sugars by DNS

The DNS method works as a colorimetric method in that the yellow colored DNS is reduced to 3-amino-5-nitrosalicylic acid in the color of orange- brown. This reaction can be observed by UV spectrophotometry at 540 nm in which wavelength of maximum absorbance is obtained. Moreover, the concentration of the reducing sugar is proportional to the intensity of the color formation (Marsden, Gray, Nippard, & Quinlan, 2010).

According to a research that was focused on the comparison of two reducing sugar determination methods, Nelson-Somogyi (NS) assay and DNS assay was examined. The results show that even though NS assay is approximately 10 times more sensitive than the DNS assay, it did not supply accurate results with oligosaccharides. In addition, when monosaccharides such as dextrose and fructose were also compared, the same phenomena observed. Meaningly, the results of the DNS method were more reliable and accurate (Gusakov, Kondratyeva, & Sinitsyn, 2011).

Effect of some well-known salts was also studied to see how salts affected the DNS reagent. According to the results of the study, when the salt ions such as Na^{+}, $K^{+,}$ and especially Ca^{+2} and Ba^{+2} were added, the intensity of DNS color increased. On the other hand, with the addition of NH^{4+} and Mg^{+2} salts, the intensity of DNS color decreased (Sinegani & Emtiazi, 2011). Therefore, it was indicated that the buffer solutions that are prepared with salt ions have huge impact on the intensity of the color formation and DNS results as well.

Among the other reducing sugar determination methods, the dinitrosalicylic acid (DNS) method is quicker, simple and gives more reliable and accurate estimation of the sugar amount. However, there are some limits of the usage of DNS method. For instance, the pH of the solution so important. For example, citrate buffer (pH~5) causes false results in the experiment. Thus, the pH value of the solution should be in the alkaline range to use this method (MacDonald et al., 1983).

1.3.6. Fourier Transform Infrared (FTIR) Spectroscopy Analysis

FTIR Spectroscopy is a tool that is used to acquire the spectrum of absorption and emission. In fact, the working principle is that it measures the amount of light absorbed by a sample at each wavelength. The first commercially created FTIR spectroscopy was used with the help of microcomputers in 1960s. Over years, the expense of innovation of this tool decreased but accessibility of innovation expanded. Therefore, the FTIR spectra technology, which is the third era infrared spectrometer, has turned out to be broadly used with improved capacity and ability (Amir et al., 2013).

FTIR technology is based on both qualitative and quantitative analysis of organic compounds. Because of this, the usage of this tool is applied in most of the organic compounds such as proteins, enzymes, lipids, glycolipids, nucleic acids and

photobiological systems. Moreover, FTIR is an efficient, precise, nondestructive, sensitive and quick system that defines the functional groups by detecting both chemical composition and the physical state of the sample (LibreTexts, 2015).

FTIR Spectroscopy supplies the data of the all functional groups in terms of the infrared (IR) signatures or wavelengths. The identification of the specific residues in the chemical groups are done by several techniques such as isotope labeling, hydrogen/deuterium exchange and site-directed mutagenesis with the usage of the reaction-induced FTIR difference spectroscopy (Ulberth & Haider, 1992). FTIR record the absorption data of the functional groups according to their distinctive vibrations. For instance, polar bonds have strong infrared absorptions due to their permanent dipole strength. In addition, the atomic mass and the strength of the bond have huge impact on the vibration frequency. Namely, when a comparison is made on the number of bonds it is shown that triple bonds have highest vibration frequency followed by double bonds and lastly single bonds (Rahmah et al., 2016).

Common FTIR spectrophotometers contain a source, sample compartment, detector, amplifier, interferometer, computer, analog to digital (A/D) convertor and fixed mirror. The generation of the radiation occurs by the source and the generated radiation passes the sample through the interferometer and arrives the detector. Thereafter, the signal is amplified, and its conversion occurs to digital signal by the amplifier and the A/D converter respectively. At the end, transformation of the signal to the computer takes place in which Fourier transform is applied. Finally, the mapped information of the spectrum can be obtained (LibreTexts, 2015). The block diagram of the basic FTIR spectroscopy is given in the below in Figure 1.4.

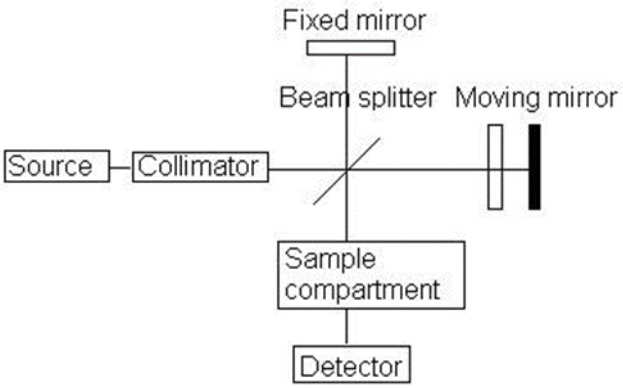

Figure 1.4 Block diagram of an FTIR spectroscopy

The graph is read by looking the range of the various regions. Particularly, the Infrared region is equivalent to 12800~10cm^{-1} and it is divided into three groups as near infrared region (12800~4000cm^{-1}), mid infrared region (4000~200cm^{-1}) and far infrared region (50~1000cm^{-1}) (Kulea, 2014). By looking at these ranges, the functional groups in the sample is determined with its amount that is absorbed by considering the peaks obtained.

Table 1.1 Infrared Spectroscopy frequency ranges of functional groups

Functional Group	**Frequency (cm-1)**	**Intensity**
Water OH Stretch	3700-3100	strong
Alcohol OH stretch	3600-3200	strong
Carboxylic acid OH stretch	3600-2500	strong
N-H stretch	3500-3350	strong
≡C-H stretch	~3300	strong
=C-H stretch	3100-3000	weak
-C-H stretch	2950-2840	weak
-C-H aldehydic	2900-2800	variable
C≡N stretch	~2250	strong
C≡C stretch	2260-2100	variable
C=O aldehyde	1740-1720	strong
C=O anhydride	1840-1800, 1780-1740	weak, strong
C=O ester	1750-1720	strong
C=O ketone	1745-1715	strong
C=O amide	1700-1500	strong
C=C alkene	1680-1600	weak
C=C aromatic	1600-1400	weak
CH_2 bend	1480-1440	medium
CH_3 bend	1465-1440, 1390-1365	medium
C-O-C stretch	1250-1050	strong
C-OH stretch	1200-1020	strong
NO_2 stretch	1600-1500 and 1400-1300	strong
C-F	1400-1000	strong
C-Cl	800-600	strong
C-Br	750-500	strong
C-I	~500	strong

It is obvious that chemical compounds that have several functional groups can be observed by FTIR spectroscopy easily. As can be understood, FTIR spectroscopy analysis gives detailed information about whether the sample contain that specific functional group or not by just looking at the peak in the specific range of the spectrum graph. For all this reasons, FTIR analysis is applied very commonly in all chemical

areas due to its reliable, accurate and sensitive information on the data (Naczk & Shahidi, 2004).

1.3.7. Hydration Behavior by Nuclear Magnetic Resonance (NMR) Relaxometry

The presence of nuclear magnetic resonance (NMR) was firstly discovered by a Dutch physicist, Gorter, in 1936. However, he could not achieve any results by using NMR spectroscopy with lithium fluoride in those times. After around 10 years, in 1948, two American researchers, Felix Bloch and Edward Purcell found and worked with NMR to understand the working principle of the NMR. These two researchers mutually granted for the Nobel Prize in Physics in 1952 thanks to their detailed studies with NMR spectroscopy. From that date, scientific experts and physicists worldwide have utilized uniform magnetic fields to study the molecular structure and dynamics of little homogeneous components (Princeton University, 2018).

Use of NMR spectroscopic methods to determine structures and behaviors of solids and fluids in the applications became more common in the researches. By the 21st century, with the help of developed technology, there were several studies with NMR spectroscopy that were granted for the Nobel Prize. In 2002, Kurt Wuthrich granted for the prize in Chemistry due to his achievements on the determination of protein in three-dimensional structure state in solids by using NMR spectroscopy. In addition, in 2003, Peter Mansfield were rewarded for Nobel Prize in Medicine for his discoveries related to Magnetic Resonance Imaging (MRI) (Reddy, 2004). The development of NMR has proceeded from its discovery to today's science world.

The working principle of NMR depends on the nuclear core's inherent magnetic dipole moment. It is created by a spin thanks to the finite angular momentum of most atomic nucleus in the ground states (Spectrum, n.d.).

When a sample is inserted in a magnetic field and simultaneously is exposed to radiofrequency (RF) energy at the suitable frequency, absorption of the energy by the nuclei in the sample can take place (Dsm & Spec, 2011). There are some crucial dependents of the frequency of the radiation. First of all, the type of the nucleus is important i.e., 1H or 13C. Then, the chemical environment of the nucleus plays an important role on the frequency of the radiation. For instance, the two different proton types of methanol that are the methyl and hydroxyl protons absorb the energy at different frequencies. Moreover, two different tryptophan protons of amide compounds in a native protein absorb the energy at different frequencies because of the fact that they are in different chemical environments (Press, 2019).

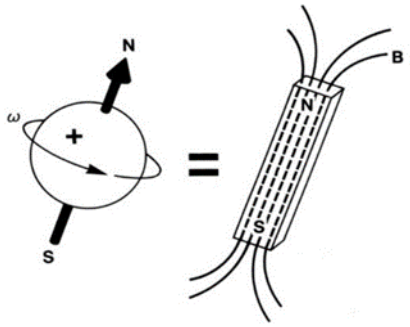

Figure 1.5 The charged nucleus rotating with angular frequency ω with the spin rotation axis

It is a fact that any charged and moving particle can produce magnetic fields. Nuclei also have positive charges and have ability to spin. Because of these features, a nucleus can be considered as a tiny magnet that is oriented along the spin rotation axis. It was shown that the most favorable orientation is the low-energy state and the less favorable orientation is the high-energy state. In NMR, the observation of the charged nuclei, like the hydrogen nucleus, which is a single, positively charged proton is determined.

Also, in order to have net magnetic field in the nucleus, there should be an odd number of protons meaningly there always exists one proton that is unpaired at the end. This is the most important feature of the usage of 1H or 13C in NMR spectroscopy with unpaired protons. After spinning of the nuclei, with the effect of external magnetc field, unpaired protons line up with that magnetic field. Then, if the specific frequency is sent into the sample, the alignment of the some spins changes because of this new magnetic field. At the end, while they are returning to their original alignment signal will be generated. This signal is actually the NMR signal that is measured and provide the NMR the data (Hans J. Reich, 2017).

In NMR experiments, two relaxation terms are commented by considering their values. These two relaxation mechanisms are T_1 relaxation (spin-lattice relaxation or longitudinal relaxation) and T_2 relaxation (spin-spin relaxation or transverse relaxation) times. Moreover, since these two times are inherent properties of the sample tissues, they are fixed and specific to the sample itself. T_1 relaxation time is observed because of the energy exchange between surrounding medium conditions of spin. T_1 also shows a strong magnetic field dependency and as the magnetic field strength increases T_1 increases. On the other hand, T_2 relaxation time is observed because of the energy exchange between distinct nuclear spins (Castaño & Maurer, 2015). In other words, spin–lattice relaxation time (T_1) is actually the time that requires for the spins to realign along the external magnetic field axis, and it is found from the recovery curve. Spin-spin relaxation time (T_2) is the time which requires for the transverse magnetization to decay till the equilibrium value of zero (Kirtil & Oztop, 2016). When these two relaxation times were compared it was pointed out that T_1 relaxation time can be 5 to 10 times longer than T_2 relaxation time. Indeed, the time range of T_1 is changing from the tens of milliseconds to several seconds according to intensity of the proton in the sample. For example, when the pure water of the T_1 value was measured, it is found that time is around 2.7 seconds whereas the T_1 time of the solids (as long as they are not crystalline) that include little amount of water are much

shorter compared to pure water. On the other hand, the range of T_2 changes from tens of microseconds to hundreds of milliseconds (Hoffmann et al., 2012).

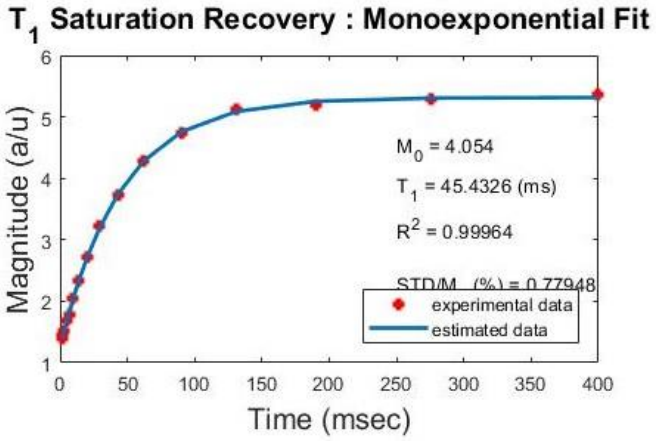

Figure 1.6 Example for T1 Recovery Curve in NMR Relaxometry

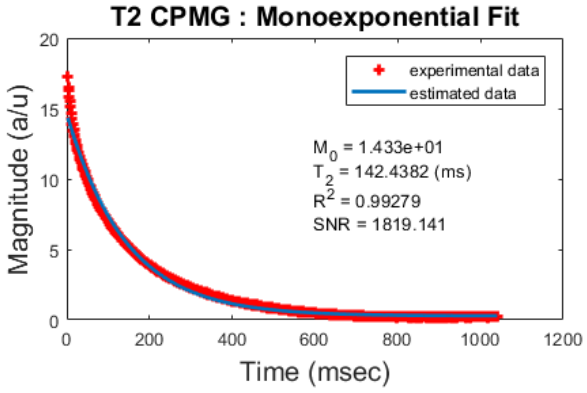

Figure 1.7 Example for T2 Recovery Curve in NMR Relaxometry

While an NMR experiments is carried out, there are some important parameters to be controlled and applied by the operator. These are TR (Repetition Time) and TE (Echo Delay Time or Time to Echo). TR is defined as the time interval between two applied RF (Radio Frequency) pulse. The time for TR should be chosen enough in order to get the best signal. If the time is not sufficient, recovering of some magnetization along the axis will not be observed fully so the data that taken would be biased. The time TE is defined as the waiting time between the RF pulses. Moreover, it was pointed out that choosing long TR and short TE are usually used while determining the T_2. For instance, the TR values while T_2 time is determined is chosen as 3 to 5 times of T_1 value of the sample that is used. On the other hand, while T_1 time is determined, short TR and short TE values are selected (Manuscript & Agent, 2015). To sum up, those two parameters should be decided very carefully to get the best signal from the output of the NMR relaxometry.

The determination of the spin-spin relaxation time (T_2) is generally done by the Carr-Purcell-Meiboom-Gill (CPMG) sequence. CPMG provides many advantages due to the fact that it even evaluates the small deviations so increases refocusing efficiency (Pell et al., 2006). In a CPMG sequence, at first, 90° RF pulse is sent, and this is followed by an echo train that is induced by 180° pulses (90°→180°-echo1→180°-echo2 …). Between the 90° RF pulse and 180° pulses, there was a phase shift in the rotating frame to decrease the accumulating of imperfections in the 180° pulses (Hnilicová, Bittšanský, & Dobrota, 2014). Moreover, CPMG sequence enables to have greater echo signals and efficient diffusion constant by increasing the signal/noise ratio. Higher the signal/noise ratio means higher the quality of the signal. CPMG sequence also help to eliminate the inhomogeneities due to the magnetic field (Kruk et al., 2014).

In industrial applications, high field NMR (HF-NMR) relaxometry is preferred because of the high signal to noise ratio. Higher sensitivity and resolution are also obtained in the signal. However, HF-NMR is not practical, requires large spaces and has high cost value. On the other hand, low field NMR (LF-NMR) has been progressively well known as a systematic tool for designing engineering research. LF-NMR is reasonable, informative, applicable, low in cost and does not require large space areas (Barbosa et al., 2013). The operated frequency range of LF-NMR changes between 10 MHz and 50 MHz. Also, the magnetic field is less than 1 Tesla. However, HF-NMR has the magnetic field value more than 1 Tesla (Hausser & Kalbitzer, 2011).

NMR relaxometry is characterized as an accurate, reliable, sensitive, harmless, elucidative technique that utilize the information from mobile protons. Also, it is a critical instrument that determines pore measurement of permeable media, water uptake, water content and water distribution of the substances (Williams, Oztop, Mccarthy, Mccarthy, & Lo, 2011). The demand of the accurate information from NMR relaxometry is increasing day by day. There are several areas that NMR relaxometry is used. Medicine, food, chemistry and biochemistry applications are getting very common (Appl & Nmr, 2019).

NMR relaxometry is an important analytical tool that may be applied to materials that are in solid or liquid state. There are several food areas that NMR relaxometry is applied such as beverages, oils and lipids, vegetables, meat, and dairy products (Hatzakis, 2011). There are several purposes of using NMR relaxometry in foods. These are included as classification, quality control, distribution of the compounds, sensory analysis, structural characterization as well as stability and durability of the food samples (Cheumani, Ndikontar, De Jéso, & Sébe, 2011). In food industry, NMR relaxometry can give information about the observation of water and oil distribution in a sample (Li et al., 2016). In addition, NMR tool gives idea about the gel systems

(W-M Fan & Lane, 2016), release or uptake of the water after modifications on proteins and the cross-linking mechanism on gel system (Kilercioğlu, Özel, Karaçam, Poçan, & Öztop, 2015).

One of the most important area where NMR relaxometry can be used is the hydration behavior of the proteins. NMR relaxometry provides useful information about the interactions between water and proteins. Hydration of the proteins is defined as binding water up to a maximum value and it differs for different proteins. Indeed, it was shown that when dry protein is exposed to high water content, it absorbs 10 to 20 weight percent of the protein (Prev, 2019). Furthermore, modifications on proteins affect the hydration behavior of the proteins. Therefore, NMR relaxometry can be a good tool to understand how changes affect the hydration of the proteins. By looking at the spin-spin (T_2) and spin-lattice (T_1) relaxation times this information can be obtained. For instance, if the value of T_2 time decreases in proteins, this means that the free water in the environment is absorbed by the protein. Since water in free form decreases, the mobile proton in the environment also decreases thus T_2 value will be shorter (Wang, Chen, Fulcher, & Pesheck, 2019). In addition, mobility of the water and the protein structure is affected with the applications such as heat exposure, pH and the type of modification on proteins. Moreover, it is pointed out that relaxation times are also affected by the heat treatment on the samples. There is a possible change in the protein conformation due to denaturation by heat. Denaturation of the protein is so crucial that since after the structure of the protein is disrupted, some changes occurs in proteins. It was shown in several researches that while some proteins are releasing water after denaturation, some of them absorb water from the environment. Thus, with the help of NMR relaxometry, those behavior can be easily determined by just checking the relaxation times (Bennett, 1991).

1.4. Objectives

With the increasing number of populations every day, the demand for the protein rich foods attract the attention from all over the world. Moreover, animal-based proteins such as meat, fish, poultry, eggs and dairy are not sufficient to fulfill people's needs. Apart from this, compared to vegetable proteins, animal protein sources are considered less healthy due to their high saturated fat and cholesterol content. In that regard, vegetable protein sources are cultivated more to supply the protein needs of humans. Soy protein includes all of the essential amino acids which are required for human being's metabolism. It can be considered as a full protein source and the vegetable analogue of egg in terms of amino acid content. However, it has been shown that some people are allergic to soy protein. In order to eliminate this drawback, some modifications on soy protein can be applied. One of the most suitable modification techniques is the glycation of the proteins. Glycation is the reaction of reducing sugars with a protein source. Glycation not only enhances the properties of proteins but can also help to eliminate the allergenicity of the protein

In this thesis, main objective is to observe the effect of glycation on the physicochemical properties of soy protein. Effects of different reducing sugars such as dextrose, fructose and D-Psicose, glycation techniques like freeze drying and spray drying, pH and sugar to protein ratios are evaluated. On the glycated proteins, emulsification activity, hydration behavior by NMR Relaxometry, free amino groups, protein solubility, glycation degree, reducing sugar content and antioxidant activity were all determined.

The hypothesis of the study can be described as follows:
If soy protein is glycated with reducing sugars such as dextrose, fructose and D-Psicose, physicochemical properties like solubility, antioxidant activity, emulsification activities are going to improve.

To our knowledge there has not been any studies in the literature that examined the use of D-Psicose with soy protein for glycation. Moreover, comparison of the different glycation techniques on soy protein was also not studied in such an extent. Therefore, the study will be a novel contribution to the literature.

CHAPTER 2

MATERIALS AND METHODS

2.1. Materials

Soy protein isolate with 90 % protein content was purchased from Alfasol (Turkey). Reducing sugars that are D-Psicose, fructose and dextrose were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

In order to determine the properties of glycated soy proteins several chemicals were used. 3,5-Dinitrosalicylic acid (DNS) reagent, DPPH (2,2-diphenyl-1-picrylhydrazyl) reagent, Copper(II) sulfate pentahydrate ($CuSO_4.5H_2O$), sodium potassium tartrate tetrahydrate ($KNaC_4H_4O_6.4H_2O$), sodium hydroxide (NaOH), Folin-Ciocalteau's phenol reagent, trolox (TR), ortho-phthalaldehyde (OPA) reagent, Bovine Serum Albumin (BSA), sodium dodecyl sulfate (SDS), sodium carbonate (Na_2CO_3), neocuproine ($C_{14}H_{12}N_2$), ethanol (C_2H_5OH), ammonium acetate ($C_2H_7NO_2$), copper chloride ($CuCl_2$) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). In addition, Bizim brand corn oil was used for emulsification determination.

2.2. Methods

2.2.1. Preparation of Glycated Soy Proteins

First of all, soy protein and different sugars were mixed in petri dishes with soy protein to sugar ratio at five different ratios as 1:1, 2:1, 3:1, 5:1 and 10:1. Then, buffer solutions at pH 7 and pH 10 were added to those solid mixtures. Total solid concentration in the

solutions was kept as 5% (w/v) for freze dryer and 10 % for spray dryer samples. Two different glycation methods were used in the study.

2.2.1.1. Glycation with Freeze Dryer

Protein and sugar solutions were put to a freeze dryer (FD) for 48 hours. Following FD, samples were put to the incubator that was set to 50 ^{o}C and 55% RH (Relative Humidity) for 24 hours. Finally, the glycated samples were ready to be examined for characterization.

2.2.1.2. Glycation with Spray Dryer

For the spray dryer (SD), samples were prepared as %10 g/ml solid to buffer solutions ratios. Since the spray dryer chamber was very large, 5% was very inefficient. That's why total solid concentration increased for SD samples. The samples were spray dried at air inlet of 130 °C and air outlet temperatures of 50 °C. During the process, the speed of the atomizer was constant (air pressure of 296 kPa), and the rate of feed flow was adjusted to keep the outlet temperature at the desired level.

2.2.2. Characterization of Glycated Soy Proteins

2.2.2.1. Determination of Antioxidant Activity

2.2.2.1.1. DPPH Radical Scavenging Method

For the antioxidant determination, DPPH method of Wong & Nyam (2014) was modified and used. The first step of the procedure was the preparation of the solvent which included ethanol:distilled water:acetic acid mixture at ratios of 50:42:8. In another beaker, 2.5 mg DPPH (2,2-diphenyl-1-picrylhydrazyl) was dissolved in 100 mL methanol. Then, 1 mL of solvent and 0.1 g of 1% sample solution was mixed, vortexed for 30 seconds and filtered by 0.45 µm filters. After filtration process and

necessary dilutions, 3.9 ml DPPH solution was mixed with 0.1 ml filtrate and kept in a dark environment for one hour to be sure about the complete extraction of antioxidants in the samples. In addition to the samples, blank sample was used as methanol and DPPH mixture in same amounts. After one hour waiting, absorbance values were read by using Optizen POP Nano Bio UV Spectrophotometer (Mecasys Co. Ltd., Korea) at 517 nm. Finally, the values were read from the calibration curve.

The calibration curve (y=-0.0005x + 0.8577) was prepared as the inhibition percentage vs ppm trolox samples. The curve was drawn by trolox solution (Trolox + distilled water) at 1600 ppm, 800 ppm, 400 ppm, 200 ppm, 100 ppm, 50 ppm and 25 ppm. At the end, the values from the spectrophotometers were converted to mg Trolox/g sample by using that standard curve (Appendix A).

2.2.2.2. Determination of Degree of Glycation

Degree of glycation was determined by observing the color changes occurred as a result of the glycation. In the experiment, glycated soy proteins were prepared at 1% (w/v) at pH 7 and pH 10 buffers. Degree of glycation was measured at an absorbance value of 420 nm by using Optizen POP Nano Bio UV Spectrophotometer. The results were given as absorbance values in nm.

2.2.2.3. Determination of Emulsification Activity

Emulsification activity experiments were conducted by following the methods of Pearce and Kinsella (Pearce and Kinsella, 1978). The samples were prepared with buffer solutions at pH 7 and pH 10, containing glucose, fructose and D-psicose (rare sugar) as sugar types and having soy protein-sugar ratios of 1: 1, 2: 1, 3: 1, 5: 1 and 10: 1 respectively. For the experiment, 1 ml of glycated soy proteins that were 1% in 10 mM buffer solution were mixed with 0.5 ml of corn oil at 10,000 rpm using silent

crusher (Heidolph Instruments GmbH & Co. KG). After the emulsions were obtained, the samples were centrifuged at 10,000 rpm for 1 minute (ModelTD10). The height of oil fraction which was remained at the top after centrifugation was measured as the non-emulsified fraction. The ratio of the remaining mixture height to the total height of before centrifugation was calculated as the emulsification activity and the results were reported in percentage.

2.2.2.4. Protein Solubility Determination by Lowry Method

Lowry method determines the solubility of the proteins by the reaction of the proteins with copper ions in an alkali environment. The determination of the glycated soy protein solubility was conducted by modifying the method of Sun & Izumori (2004). At first, reagents that are named as Reagent 1, Reagent 2 and Reagent A were prepared. Then, to prepare Lowry ACR reagent, Reagent A, Reagent 2 and Reagent 1 were mixed at the ratios of 100:1:1 respectively. Folin-Phenol Reagent preparation was made by diluting 2N stock (commercial) as a 1:1 ratio with distilled water. After preparing the reagents, calibration curve was made by BSA (Bovine Serum Albumin) stock solution with series dilutions from 1 mg/ml to 0.03125 mg/ml to determine the solubility of the proteins. Reagents that used for Lowry experiment are given in Table 2.1;

Table 2.1 Reagents of Lowry Method

Reagent A:	2% Na2CO3 dissolved in 0.1 N NaOH
Reagent 1:	2% CuSO4.5H2O, Copper source
Reagent 2:	2% Na-K Tartarate
Lowry Reagent:	Mix of Reagent A:1:2 with a ratio of 100:1:1
Folin and Ciocalteu's Phenol Reagent:	Diluted 2N stock solution as a ratio of 1:1 with distilled water
Bovine Serum Albumin:	20 ml 1 mg/ml BSA stock solution

The experiment was conducted to the glycated soy proteins that were prepared at 1% (w/v) at pH 7 and pH 10 buffers. The blank sample was prepared by the addition of 0.5 ml distilled water instead of the protein sample. Afterwards, 2.5 ml Lowry ACR reagent was added. Finally, 0.25 ml Folin Reagent was added. The tubes were vortexed for 8 seconds and kept at room temperature for 30 minutes. The absorbance values were read at 750 nm by using Optizen POP Nano Bio UV spectrophotometer. Calibration curve was constructed as absorbance values vs mg/ml BSA solution (y=1.685x + 0.1289) and the solubility of the glycated soy proteins were calculated using this curve (Appendix A).

2.2.2.5. Free Amino Group Determination by OPA Method

Determination of the free amino groups of glycated samples were determined by making some modifications on the method of Diab et al., (2009). OPA reagent was prepared by using OPA (o-Phthalaldehyde), ethanol, borax buffer, β-mercaptoethanol, and SDS (Sodium Dodecyl Sulfate) solution. For the preparation, 80 mg of OPA reagent was dissolved in 2 ml 95% ethanol solution. After complete dissolution, 50 mL of 100 mM borax buffer (at pH 9.75) was added into the solution. The reagent preparation was completed by the addition of 200 μL β-mercaptoethanol and 5 mL 20% SDS solution. At the end, the volume of the mixture was brought to 100 mL in total. After the preparation of the mixture, 0.5 mL of glycated soy protein samples were mixed with 1.5 mL of the prepared OPA reagent for 2 minutes. Then, the absorbance values were measured at 340 nm by using Optizen POP Nano Bio UV Spectrophotometer.

The standard curve preparation was prepared by using glycine. The glycine solution was prepared and diluted at 1%, 0.8%, 0.4%, 0.2%, 0.1%, and 0.05% respectively. 0.5 ml glycine was mixed with 1.5 ml OPA reagent and absorbance values were recorded. At the end, the standard curve was constructed as absorbance values vs concentration

(g/100ml) (y=0.588x + 0.0358) and the free amino groups of the glycated soy proteins were determined using this curve (Appendix A).

2.2.2.6. DNS Method for Reducing Sugar Determination

DNS method as described in Saqib & Whitney, (2011) was followed. In order to prepare the DNS reagent, NaOH (sodium hydroxide), DNS (dinitrosalicylic acid), Rochelle salt (sodium potassium tartrate), sodium sulfate was used. Firstly, 1 g of NaOH was dissolved in 50 ml distilled water. At the same time, 4 g of NaOH was dissolved in 250 ml distilled water in another beaker. 5 g DNS was added into the beaker that contains 1 g of NaOH in it and mixed. In addition, 181g Rochelle salt was added to second beaker while the temperature was kept constant at 50ºC. After all dissolution of reagents in both beakers were completed, those two solutions in the beakers were brought together in one beaker and mixed. 0.25 g sodium sulfate was added to the final mixture. After preparation of DNS reagent, 1 ml, 1% glycated sample solutions were mixed with 1.5 ml of DNS reagent. Those mixtures were held in water bath for 10 min at 90 ºC, and then cooled to room temperature. Finally, absorbance values were recorded at 540 nm by using Optizen POP Nano Bio UV Spectrophotometer.

The calibration curve (y=4.9086x – 0.1247) was prepared as the absorbance values vs dextrose concentration (g/l) at 6 different concentrations (0.06, 0.09, 0.12, 0.15, 0.18, 0.21 g/l). (Appendix A).

2.2.2.7. Fourier Transform Infrared (FTIR) Spectroscopy Analysis

Glycated soy protein samples were examined with an IR Affinity-1 Spectrometer with Attenuated Total Reflectance (ATR) attachment (Shimadzu Corporation, Kyoto, Japan). The samples were analyzed in the region of 4000-500 cm^{-1} at a resolution of 4

cm^{-1} for 32 scans. The obtained spectra were analyzed by making comparison with the literature.

2.2.2.8. Determination of Hydration Behavior by Nuclear Magnetic Resonance (NMR) Relaxometry

Determination the hydration behavior of the glycated soy proteins was observed by the help of NMR relaxometry. Samples were prepared at 1% by weight by preparing solutions with distilled water in the tubes. Experiments were performed using 0.5 T system operating at a frequency of 20.34 MHz (Spin Track, Russia). T_2 (spin-spin relaxation) times were measured. The data were acquired using CPMG (Carr-Purcell-Meiboom_Gill) pulse sequence with an echo time of 2,000 ms, 4s repetition time, 16 scans and number of echoes changed between 2000 - 4000. Analysis was done using MATLAB (The MathWorks Inc., USA) considering mono-exponential relaxation behavior.

2.2.2.9. Statistical Analysis

For all the experiments conducted statistical analysis was conducted using MINITAB (Version 16.2.0.0, Minitab Inc., Coventry, UK). In order to check the effects of the parameters on the functional properties, in all steps multi factor analysis of variance (ANOVA) was used. In addition, Tukey's comparison test was conducted at 95% confidence interval as the multiple comparison test. In all experiments data were taken as three replicates and coefficient of variation less than 10% was the criteria for analysis. Assumptions of ANOVA (Normality and Test of Equal Variances) were checked on the standardized residuals; outliers were excluded, and transformations were applied when necessary.

2.3. Experimental Design Summary

Table 2.2 Parameters of the experimental design

Experiments	Factors	Levels
Antioxidant Activity Determination by DPPH Method	Sugar Type	Dextrose Fructose D-Psicose
Determination of Degree of Glycation	pH Type	7 10
Emulsification Activity Determination		
Protein Solubility Determination by Lowry Method	Glycation Type	Freeze Drying (FD) Spray Drying (SD)
Free Amino Group Determination by OPA Method		
DNS Method for Reducing Sugar Determination		
Fourier Transform Infrared (FTIR) Spectroscopy Analysis Determination of Hydration Behavior by Nuclear Magnetic Resonance (NMR) Relaxometry	Concentration	1:1 2:1 3:1 5:1 10:1

CHAPTER 3

RESULTS AND DISCUSSION

Glycated soy proteins were analyzed and observed by considering the different parameters that were listed in the table mentioned in the previous section. As will be seen, the samples that were glycated in the freeze dryer were named as 'FD' samples and the ones glycated in the spray dryer were named as 'SD' samples throughout the text. In addition, in the results that were explained with the statistical analysis, it was mostly based on analysis of multiple factor ANOVA. That's why the letters used to show the statistical differences could not be given on the graphs. However, the detailed statistical results can be found Appendix section. Results were interpreted according to the results of Multiple Factor Analysis of Variance in all statistical results.

3.1. Determination of Antioxidant Activity

3.1.1. DPPH Radical Scavenging Method

For all the results that were obtained by the DPPPH Method, multiple variance analysis (ANOVA) was performed. The results are given in detail in the Appendix. Effects of sugar type (dextrose, fructose and D-Psicose), glycation type (FD / SD) and protein : sugar ratio and pH (7, 10) were investigated. According to the results, among three different sugar types, D-Psicose gave the highest antioxidant activity followed by fructose and dextrose ($p<0.05$). In addition, pH 10 samples dominated the pH 7 ones and FD samples resulted in higher antioxidant values than the SD ones ($p<0.05$). Moreover, interaction of the pH with the results on antioxidant activity was found to be significant ($p<0.05$).

When the glycated samples with dextrose being the sugar source were compared, it was observed that the highest antioxidant activity was seen for the pH10/FD. In

addition, the lowest values were determined for pH7/SD samples. These results showed that that the alkaline pH had an unignorable effect on the antioxidant activity of the samples with dextrose. Also, the glycation type affected the antioxidant activity significantly ($p<0.05$). In other words, the samples that were obtained from the freeze dryer showed higher antioxidant activity than the spray dried ones in the dextrose samples. As stated before when all factors were considered, the soy protein : sugar ratio also affected the antioxidant activity ($p<0.05$).

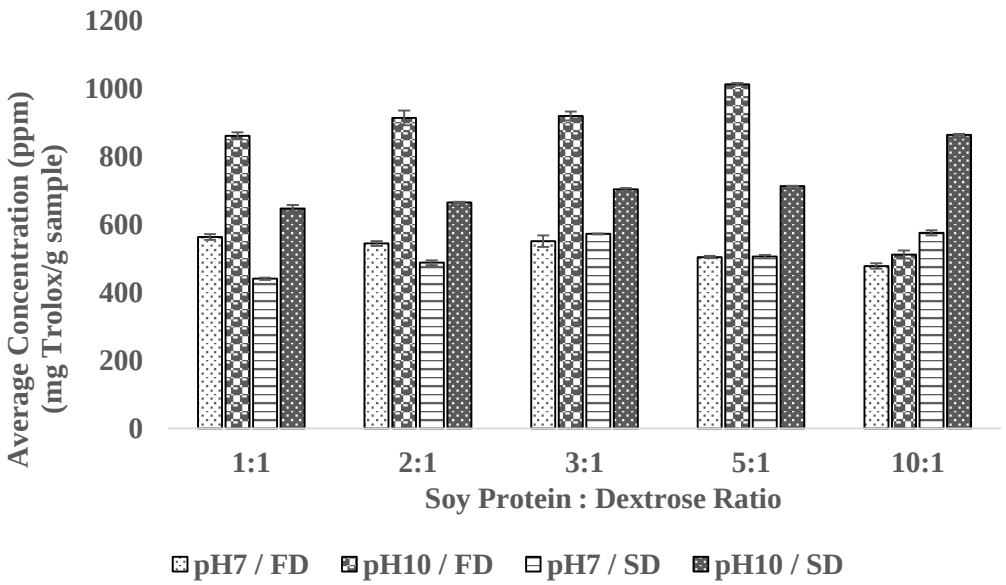

Figure 3.1 Antioxidant activity values of glycated samples by DPPH Method with different soy protein: dextrose ratios.

Fig 3.2 shows the results for fructose as the glycation sugar source. When the pH was increased to 10, antioxidant activities increased significantly ($pH<0.05$). Like the dextrose samples, in the fructose samples, the effect of glycation technique was important to be considered. Glycation was found to statistically significant ($p<0.05$). For 10:1/pH10/ FD samples, the antioxidant activity was lower than the other ratios.

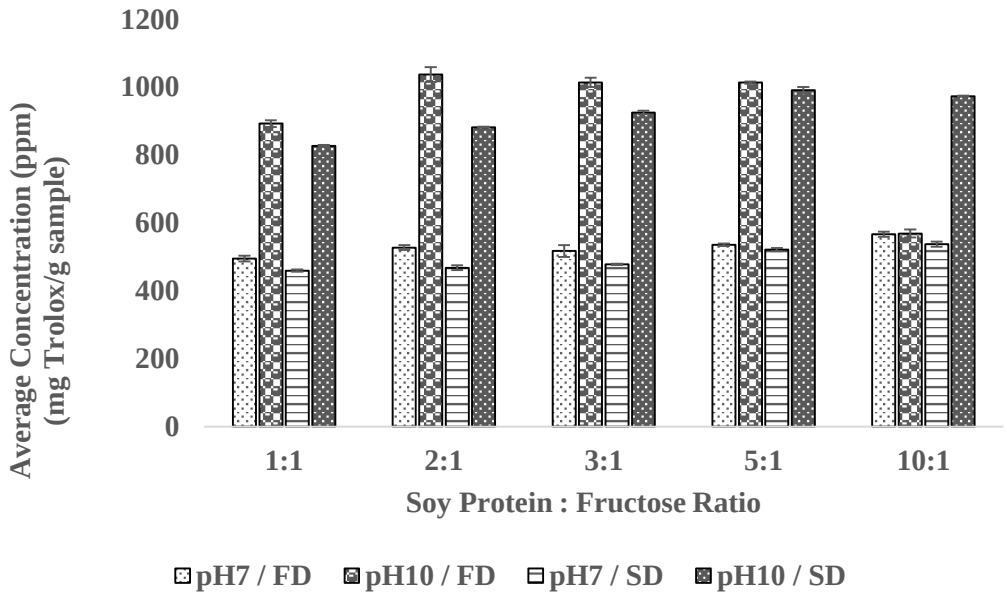

Figure 3.2 Antioxidant activity values of glycated samples by DPPH Method with different soy protein: fructose ratios.

The antioxidant activity results of the D-Psicose samples showed nearly the same pattern as the dextrose and fructose samples. The alkali pH again increased the antioxidant activity. Apart from those, when sugar types were considered among the three sugars, D-Psicose was the one that gave the highest results ($p<0.05$).

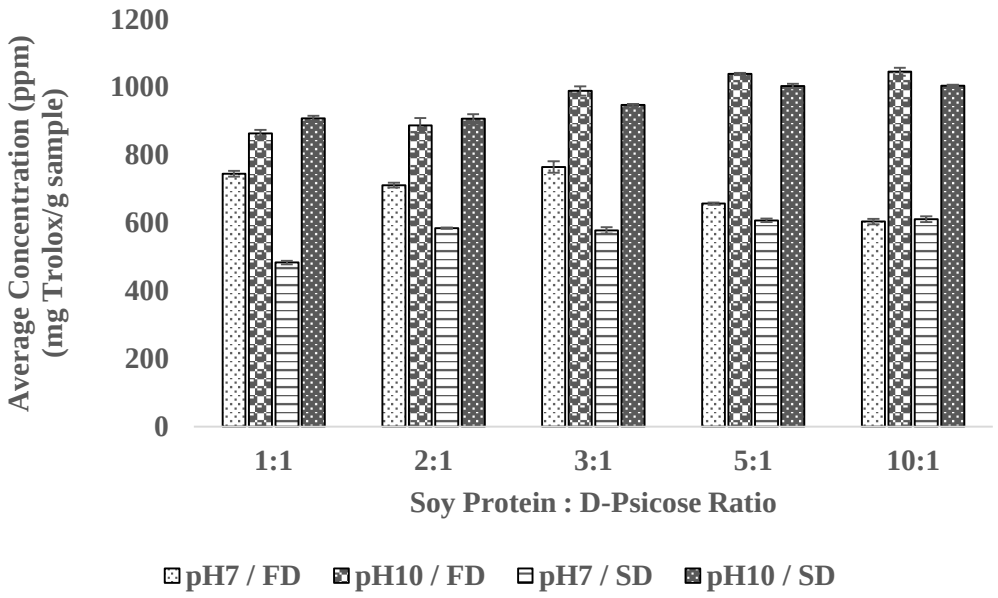

Figure 3.3 Antioxidant activity values of glycated samples by DPPH Method with different soy protein: D-Psicose ratios.

In the all results, it was shown that alkali pH increased the antioxidant activity in DPPH method. This results were also consistent with the results of the one study in which tea infusion showed higher antioxidant activity in less acidic media (Pekal & Pyrzynska, 2015). It was also concluded that alkali pH increased the antioxidant activity by using DPPH assay. Indeed, the idea of this was explained by Dawidowicz & Olszowy, (2014) that scavenging process may be changed because of the pH of the medium changing the hydrogen ion concentration. Thus, pH has the huge impact on the results obtained in DPPH assay.

According to the researches, it has been shown that glycated D-Psicose exhibited a higher free radical scavenging and antioxidant activity than the glycated proteins with other reducing sugars (Sun et al., 2004a). In addition, D-Psicose has an ability to keep a high level in scavenging radical effect (Sun, Hayakawa, Ogawa, & Izumori, 2007). Therefore, highest antioxidant activity of D-Psicose compared to fructose and dextrose sugars was expected and the results also showed the expectation in this study.

3.2. Determination of the Degree of Glycation

Glycation, the 1st step of Maillard reaction occurs between reducing sugars and proteins. In the food chemistry, this reaction occupies a wide range on the researches because there is a correlation with food processing, browning by storage, and change in nutritive values (Báez, Shah, Felipe, Maynard, & Chalew, 2015). In that regard, browning could be considered as the parameter that can give an idea about the glycation degree of the samples. In order to monitor this phenomena and to be sure the reaction did not proceed to further stages, browning degree was usually monitored at 420 nm absorbance value (Yu et al., 2017).

Table 3.1 Experimental results of degree of glycation of all samples

	Soy Protein : Sugar Ratio	Absorbance (nm) [pH 7/FD]	Absorbance (nm) [pH 10/FD]	Absorbance (nm) [pH 7/SD]	Absorbance (nm) [pH 10/SD]
Dextrose	1:1	0.034±0.002	0.094±0.004	0.082±0.002	0.236±0.002
	2:1	0.032±0.004	0.042±0.003	0.069±0.003	0.279±0.003
	3:1	0.033±0.001	0.036±0.001	0.079±0.002	0.295±0.004
	5:1	0.024±0.002	0.038±0.003	0.083±0.004	0.307±0.002
	10:1	0.025±0.001	0.029±0.002	0.106±0.003	0.321±0.001
Fructose	1:1	0.033±0.001	0.107±0.004	0.089±0.002	0.151±0.003
	2:1	0.023±0.002	0.043±0.003	0.098±0.003	0.207±0.004
	3:1	0.022±0.003	0.032±0.001	0.122±0.004	0.228±0.005
	5:1	0.020±0.004	0.029±0.002	0.139±0.002	0.256±0.004
	10:1	0.018±0.001	0.021±0.001	0.204±0.003	0.288±0.002
D-Psicose	1:1	0.083±0.003	0.127±0.004	0.120±0.004	0.161±0.002
	2:1	0.058±0.003	0.049±0.003	0.143±0.003	0.192±0.004
	3:1	0.042±0.002	0.038±0.001	0.205±0.004	0.218±0.005
	5:1	0.038±0.004	0.031±0.002	0.228±0.002	0.258±0.004
	10:1	0.033±0.002	0.025±0.001	0.305±0.006	0.309±0.003

When Table 3.1. was investigated, for all three different sugars, it was concluded that the highest browning value was seen for pH 10/ SD samples. It is a fact that when the temperature increases, the Maillard reaction rate increases. In addition to that, it is also known that alkali pH has an increasing effect on the Maillard browning (Tessier, 2010). Therefore, the high results of the pH 10 and SD samples were expected due to the fact that Maillard browning rate increases both in high temperature and alkali pH values. Apart from this, when the soy protein : sugar ratio was examined, it was observed that while the soy protein concentration increased, degree of glycation has decreased in FD samples. On the other hand, in the SD samples, the opposite results were observed. This can be explained by the fact that two mechanisms work differently from each other. In the freeze dryer, while drying occurs in the samples, the structural change of the sugars and proteins may lead an important effect on the brown color formation in the incubator afterwards. It has been shown that sugars become amorphous after freeze drying and in this form they absorb more water and consequently Maillard reaction rate could be affected (Grunin, Oztop, Guner, & Baltaci, 2019). Moreover, in spray dryer, the temperature rise might lead to the exposure of the amino groups of hydrophilic residues and may lead to help to participate in Maillard browning and contributes to brown color formation. In one of the study, whey protein isolate in the spray dryer was studied at different ascorbic acid to protein concentrations (1:100, 1:20, 1:10, 1:5 and 1:2) and it was observed that the increase in the protein concentration resulted in more intense brown color formation in the samples (Zhong, Tan, & Langrish, 2019).

When the all samples were analyzed together with statistical results, it was shown that among the sugar types, dextrose samples were the highest in the case of degree of glycation followed by fructose and D-Psicose. This was an interesting result since compared to fructose and D-Psicose (ketoses) glucose as being an aldose has lower tendency for glycation (Kawasaki et al., 1998). At that stage another important finding comes out. As also will be discussed later, during FD or SD it is not only Maillard but

sugar degradation reactions (caramelization) might also have taken place and ketoses having more tendency to Maillard; dextrose might have degraded, and degradation products could give more brown color.

In addition, increasing the soy protein : sugar ratio increased the degree of glycation. As mentioned above, between the glycation types spray dryer was the dominant compared to the freeze dryer. Also, the samples pH 10 samples were browner than the pH 7 samples.

When a general consideration was made, it was found that all the factors studied had an effect on glycation. However, it was concluded that this method was not sufficient to determine the degree of glycation when it was considered that there may be reaction products which could show more brown color even at low concentrations and also contribution of sugar degradation reaction may overestimate the results.

3.3. Emulsification Activity Determination

It is known that many chemical and physical factors affect the formation, stability and activity of the emulsions. Some of those factors are the type of protein and its concentration, pH, temperature and the ionic strength. In addition, emulsification behavior of globular proteins are affected by the solubility, surface hydrophobicity, and molecular flexibility of the proteins (Combrinck, Otto, & Plessis, 2014).

Glycation of proteins are known to affect their emulsification activity, and, in this study, emulsification properties were also determined as explained in Chapter 2.

In Figure 3.4., the instant emulsion activity of the dextrose samples is given. According to the figure, pH 7 / FD samples showed the lowest emulsification activity ($p<0.05$). In addition, it was observed that the ratio of soy protein to sugar did not affect the emulsification activity in these samples. On the other hand, considering the effect of pH, it was observed that FD samples prepared at pH 10 exhibited higher emulsion activity than pH 7 ones ($p<0.05$). Meaningly, when the pH value increased to alkaline pH the emulsification activity increased in dextrose samples ($p<0.05$).

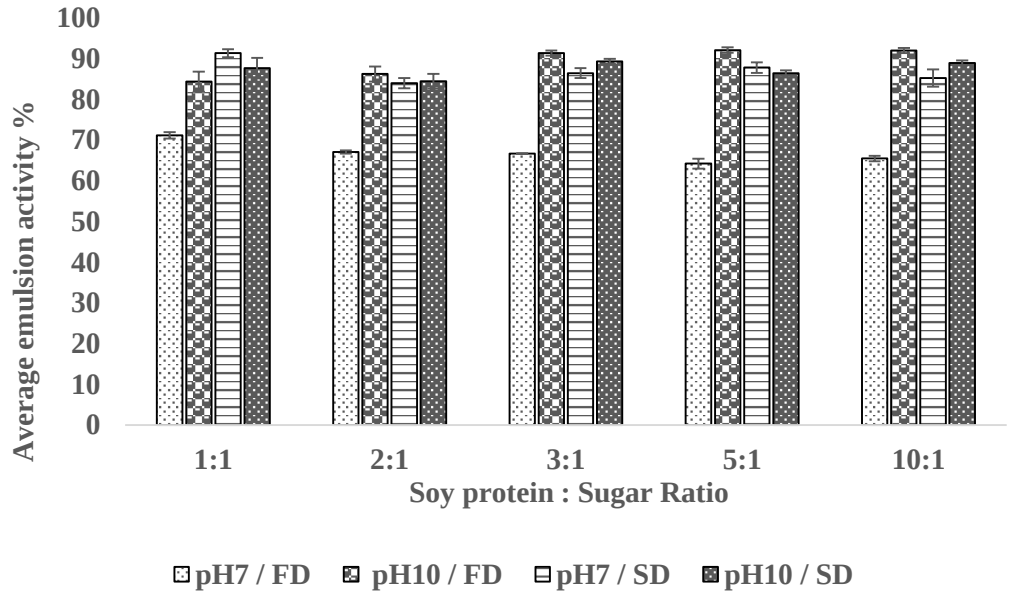

Figure 3.4 Average Emulsion Activity % values of glycated samples with different soy protein : dextrose ratios.

Figure 3.5. shows the instant emulsion activity percentage results of glycated soy proteins with fructose. In the samples, the highest combination of the activity was observed in the 2:1 (soy protein : sugar ratio) at pH 10 as the 89.83% in the FD sample. Apart from the samples with dextrose, the emulsion activities of all combinations in fructose samples were observed to be very close to each other and varied between

79.19% and 89.83% values. Thus, it can be concluded that glycated fructose samples exhibited statistically same results in the case of emulsion activities in all different parameters.

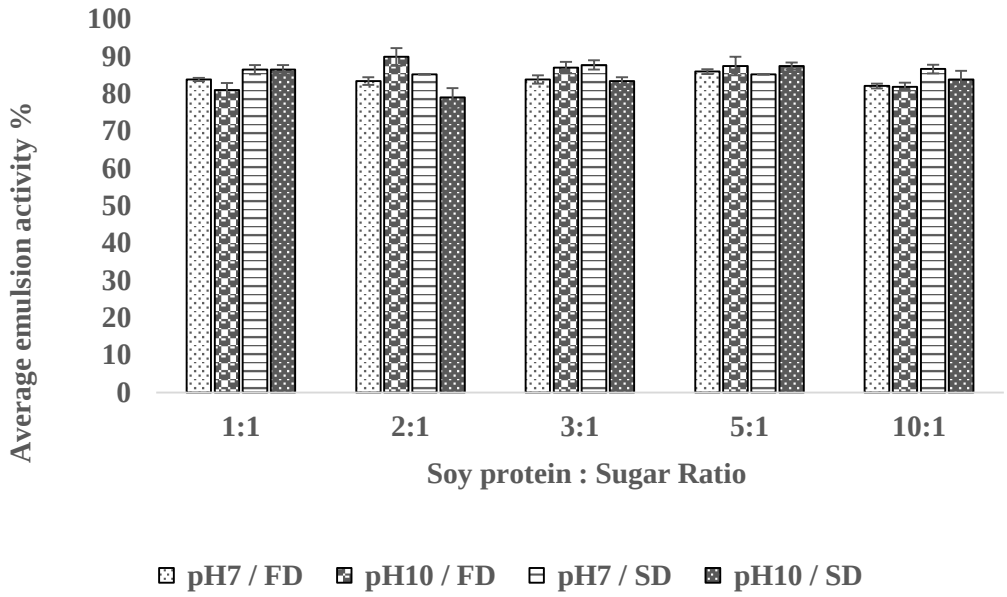

Figure 3.5 Average Emulsion Activity % values of glycated samples with different soy protein : fructose ratios.

In Figure 3.6., the instant emulsion activity of the samples prepared with D-Psicose as sugar component was examined. In this experiment, pH 7 / FD samples showed slightly lower emulsion activity than other formulations, indicating that pH had a significant effect on emulsion activity ($p<0.05$). It was concluded that glycation of soy protein with D-Psicose had a significant effect on the emulsification activity.

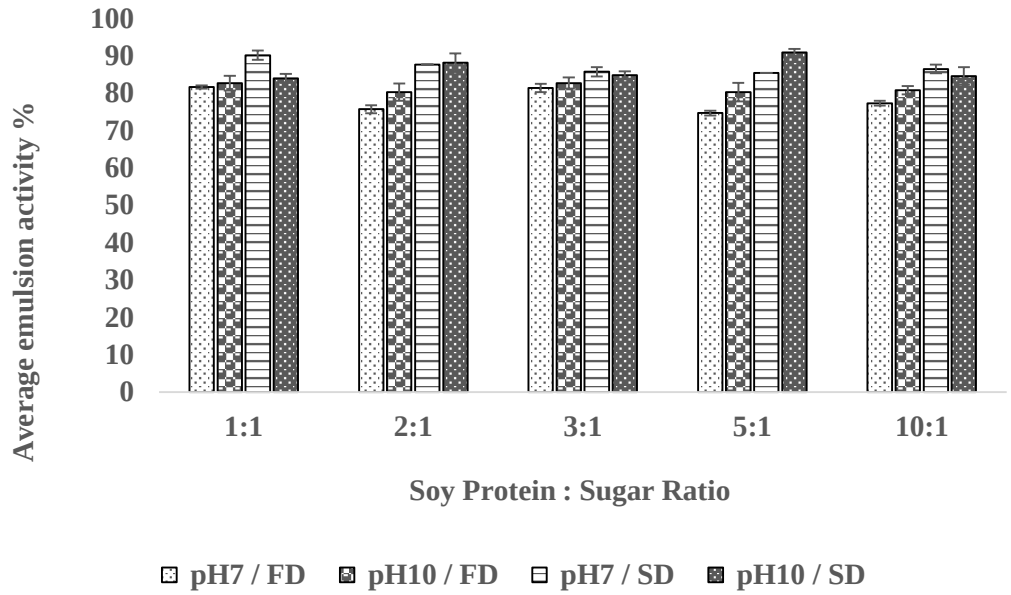

Figure 3.6 Average Emulsion Activity % values of glycated samples with different soy protein : D-Psicose ratios.

When the ANOVA results in the Appendix were examined, dextrose and D-Psicose showed very similar trend, while the results of the samples prepared with fructose was higher ($p<0.05$). Moreover, soy protein : sugar ratio did not have effect on the emulsion activity.

Despite the high antioxidant activity of D-Psicose samples, the fact that emulsification activity did not increase too much could be associated with the ability of the D-Psicose to reduce hydration (Matsuo et al., 2003). On the other hand, SD samples in general had higher activity ($p<0.05$). The higher emulsification activity in the SD samples can be explained by the denaturation of proteins at high temperature. Hence, better adhesion is observed with the oil droplets as a result of denaturation (Ibanoglu & Erçelebi, 2007). Correlation analysis was also made between the parameters and the emulsion activity results and both glycation type (FD / SD) and pH were found to be

correlated to the emulsion activity results with the correlation coefficients as 0.829 and 0.718 respectively ($p < 0.05$).

3.4. Protein Solubility Determination by Lowry Method

As mentioned in the introduction part, one of the biggest drawbacks of soy protein is that the solubility of soy protein is not that high in the solutions. Therefore, how modifications on the soy protein like glycation would affect the total solubility afterwards is quite important. In that regard, Lowry method was used to determine the solubility. Results are shown in the Fig 3.7-3.9. Results were evaluated as comparing the total protein amount before and after the glycation and shown as percentages.

Experimental results of the glycated soy protein with dextrose are shown in the Figure 3.7. According to this figure, it can be seen that as soy protein : sugar ratio increases, solubility of the proteins decrease. Apart from that, there was no obvious parameter that had a dominant effect. By saying no dominant parameter, it was meant that there was no generalized trend. For instance, in the FD samples, higher solubility values were obtained in the pH 10 values however it was just the opposite in SD samples.

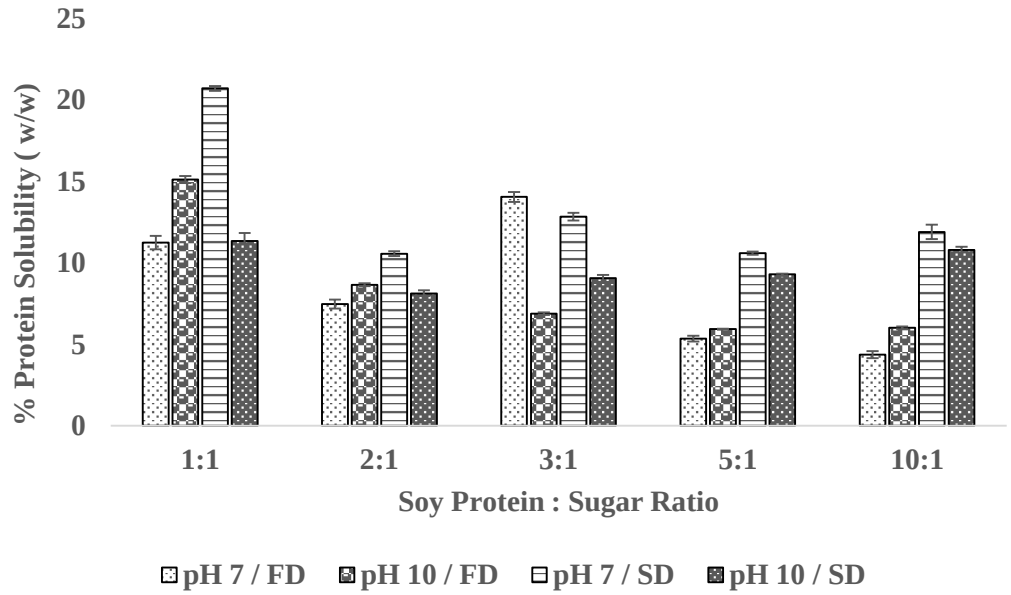

Figure 3.7 % Protein Solubility (w/w) values of glycated samples by Lowry Method with different soy protein : dextose ratios.

Experimental results of the glycated soy protein with fructose sugar are shown in the Figure 3.8. Again like in the dextrose samples, in fructose samples, highest solubility values were obtained in the 1:1 soy protein : sugar ratio and as this ratio increased, the solubility decreased accordingly. Apart from this, it can be seen that for all ratios, the combination of pH 7 / FD was the highest. Moreover, when the glycation types were compared, FD samples were dominant over the SD samples up to ratio of 3:1. Then, the effect of pH was dominating the results from 3:1 to 10:1.

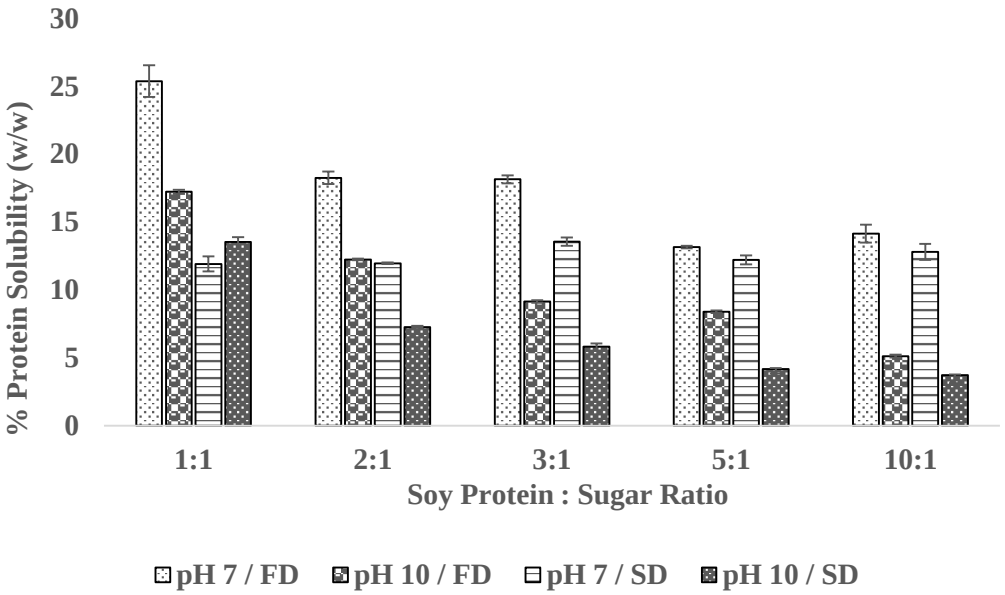

Figure 3.8 % Protein Solubility (w/w) values of glycated samples by Lowry Method with different soy protein : fructose ratios

Experimental results of the glycated soy protein with D-Psicose sugar are shown in the Figure 3.9. According to the results, glycated soy proteins with D-Psicose of 1:1 ratio showed highest total solubility values again. Furthermore, it can be commented about that the effect of pH even dominated the glycation type. For all combinations, pH 7 showed higher solubility values. Also, this effect was observed even at all soy protein : sugar ratios.

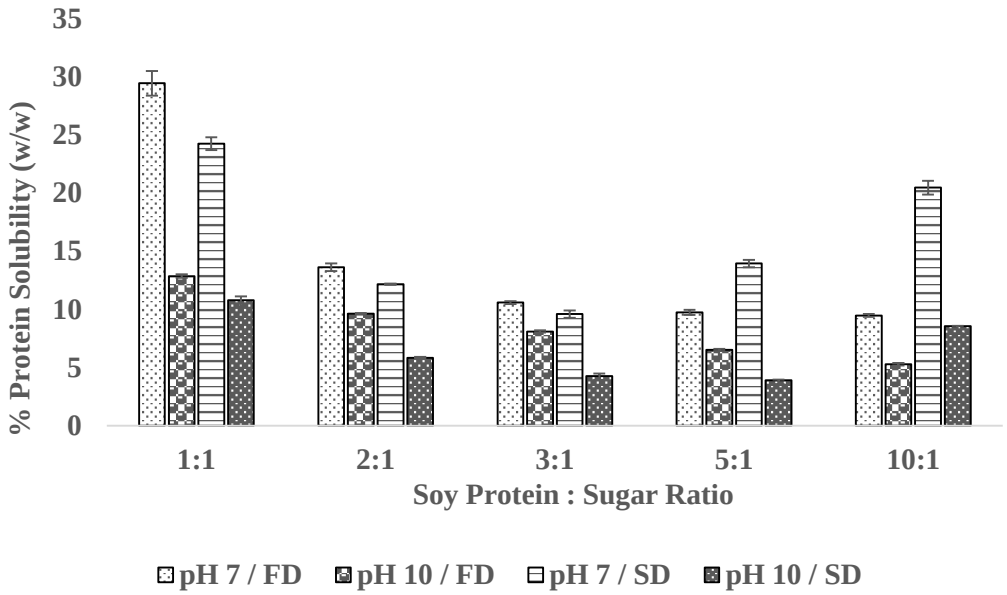

Figure 3.9 % Protein Solubility (w/w) values of glycated samples by Lowry Method with different soy protein : D-Psicose ratios.

According to the results on sugar type, the solubility was the highest in fructose followed by D-Psicose and dextrose. Actually, mean values of the fructose and D-Psicose different from each other ($p<0.05$). When the results of the soy protein to sugar ratios were examined, samples with 1:1 ratio were solubilized more than the other ratios and the solubility decreased as the ratio increased.

According to the results obtained by the Lowry method, protein solubility was higher at low pH (Appendix: pH 7 > pH 10). These results were unexpected since degree of glycation increased when pH increased to alkali pH and glycation was expected to increase the protein solubility. One important finding that came to mind here is that the Lowry method was highly affected by the pH of the solution. As a matter of fact, Waterborg (2002) stated that Lowry method could give accurate results between pH 10-10.5 but in buffer solutions that control pH carefully.

It is known that proteins have buffer effects in the solutions that they have put. In that regard, the buffer effect of the soy protein should also be considered. For instance, isoelectric point of soy protein is nearly 4.5 and at that pH values, soy protein is coagulated rather than dispersed (Hefnawy & Ramadan, 2011). Around this pH, soy protein does not unfold so solubility of the protein will be lowest (Hefnawy & Ramadan, 2011). Unfolding does not necessarily increase the solubility though. It is a balance between the hydrophobic and hydrophilic residues present in the protein. In one of the study, soy protein isolate films were examined at pH values of 2, 8 and 11. It was pointed out that extreme pH values (below 2 and over 11) disrupted the protein structure and even these buffer solutions were chosen, the final pH of the solution would be different because of the buffer effect of the soy protein. The results of the study showed that the highest solubility was observed at the pH value of 8. They explained why pH 11 sample had lower solubility than pH 8. Around pH 8, the protein kept its native form and denaturation of soy protein occurred at pH 11 with the exposure of insoluble aggregates. These insoluble compounds caused a decrease in the solubility of the protein at the end (Veliyulin, Mastikhin, Marble, & Balcom, 2008).

To conclude, there are many information in the literature about the pH effect on the Lowry Method. It can be said that special proteins have characteristic behaviors that require different pH while determining total solubility of the protein. Although, it was expected an increase in the solubility at pH 10 with glycation, soy protein did not show that trend due to its characteristic behavior. Although, it might have been glycated more unfolding could have caused a decrease in the solubility. In that case, it might be remarked that pH 7 would be better choice than pH 10 to have higher solubility for the glycated proteins.

3.5. Free Amino Group Determination by OPA Method

Free amino group determination of the proteins by OPA Method is a common technique. OPA measures the free amino groups in a protein and since in glycation amino groups are consumed, the decrease in free amino groups could be a direct way to quantify glycation. One of the distinctive features of the OPA is that it is water soluble and stable. There are several factors that can influence the reactivity of OPA with proteins. One of them is the use of a buffer. According to the studies, it was found that optimal pH to get greater results with OPA is around pH 9. On the other hand, pH range from 6-10 provide quite acceptable results (Held, 2006). In that regard, pH 7 and pH 10 which can be counted as around optimal pH would be good choices to get results with OPA. This method was considered as simple due to the fact that addition of one reagent with a short incubation period of time allows rapid determination on the samples.

OPA results are given in Fig 3.10-3.12. The lower the free amino group the more protein – sugar interaction occurred in the glycation. In other words, if there is less amino group in the solution, more amino groups were bounded to sugars to conduct glycation reaction. Therefore, there is an inverse relationship between the free amino groups and the glycation rate with OPA method. Results were expressed by dividing the total free amino group in the solution to total dry protein amount used

When Figure 3.10. was investigated, the huge difference in the glycation type can be easily seen. While FD samples had very low amount of free amino groups after glycation, SD ones had higher amounts ($p<0.05$). Apart from this, there was no parameter that can give generalized trend in the glycated soy proteins with dextrose as the sugar source.

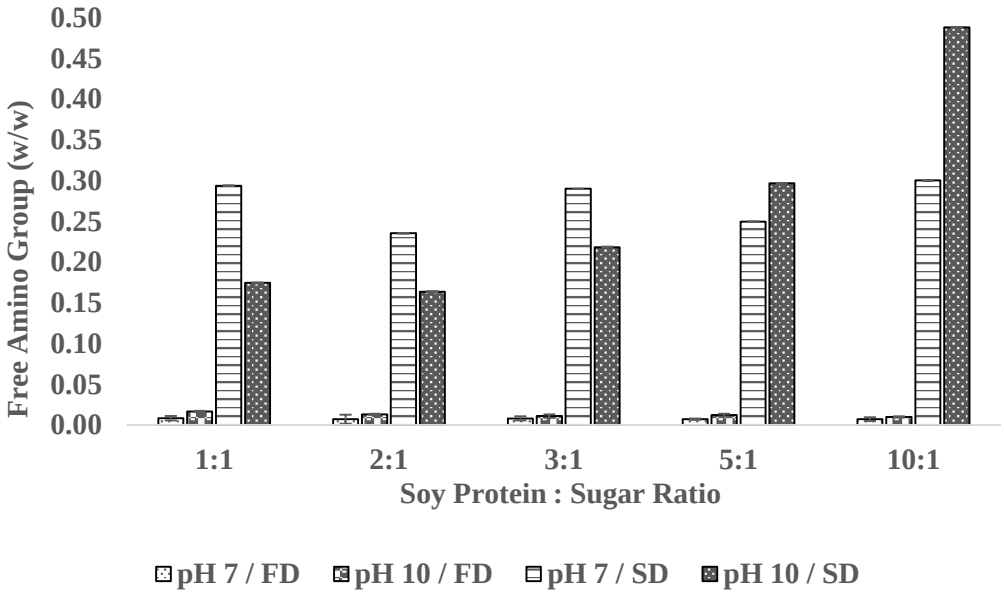

Figure 3.10 Free amino group determination of glycated samples by OPA Method with different soy protein : dextrose ratios.

Figure 3.11. below shows the OPA results of the glycated soy protein with fructose as sugar source. In these samples, the big difference between FD samples and SD samples were observed like in dextrose samples ($p<0.05$). As dextrose samples, other parameters did not show that much effect on the free amino group like glycation type did.

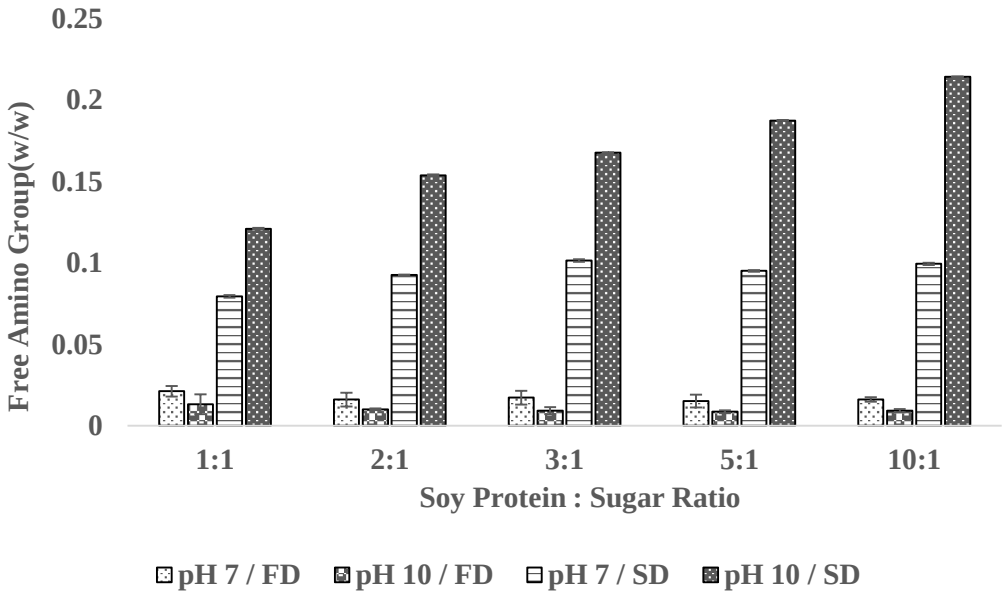

Figure 3.11 Free amino group determination of glycated samples by OPA Method with different soy protein : fructose ratios.

Figure 3.12. below shows the OPA results of the glycated soy protein with D-Psicose as the sugar source. In the samples, same trend was observed which both fructose and dextrose. Thus, it can be said that all three sugars showed the same trend in which glycation type was an important contributing factor.

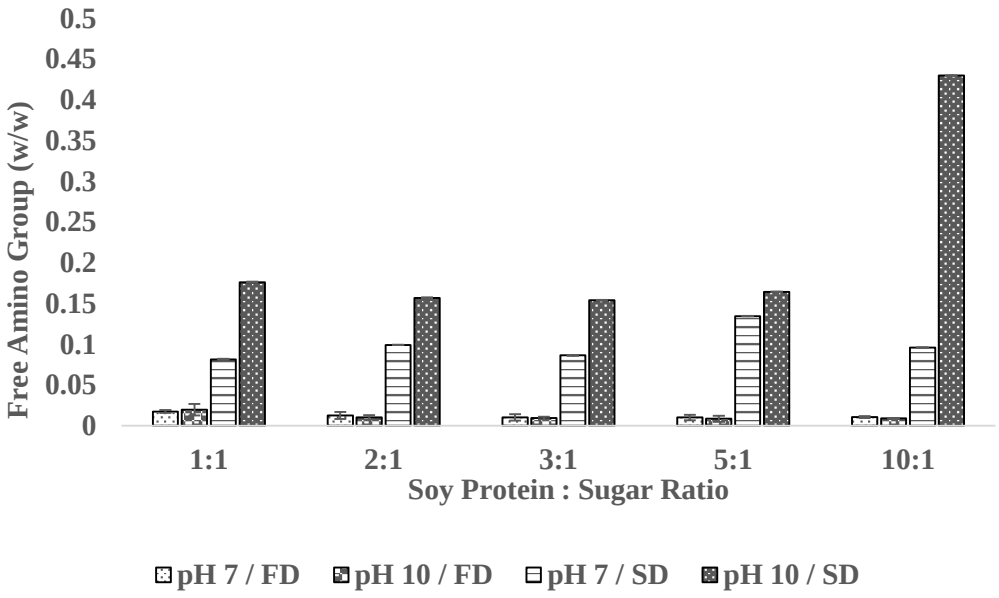

Figure 3.12 Free amino group determination of glycated samples by OPA Method with different soy protein : D-Psicose ratios.

When all the figures above were taken into consideration, it was seen that as the protein: sugar ratio increased, the amount of free amino group increased. Actually, it was an expected result due to the fact that the free amino group increases as the amount of protein increases. In addition, the obtained data showed that the amount of sugar used in the selected protein sugar ratios was the limiting factor. By keeping the amount of sugar constant and increasing the amount of protein, it may be possible to determine the amount of glycated protein corresponding to a particular sugar concentration. However, as soy protein is creating trouble in terms of solubility, it did not give the expected results in the preliminary studies Therefore, as in the literature, sugar protein ratio was chosen as a factor (Diab et al., 2009).

By looking at the ANOVA results obtained, as a general comparison, the amount of free amino group increased as the protein: sugar ratio increased from 1:1 to 10:1.

When the sugar types were compared with each other, the highest amino groups were observed in dextrose, followed by D-Psicose and fructose. From that results it can easily be said that the lowest glycation occurs with dextrose as was expected compared to D-Psicose and fructose. When the effects of pH buffers were compared, it was seen that glycation was mostly observed in pH 7 samples. Moreover, as seen in all figures above, among the glycation types freeze drying was a better option for glycation with respect to free amino groups.

The amount of free amino group also showed its effect at different pH values. When the comparison was just made in terms of glycation, it indicates that the higher the free amino groups, the less glycation occured. As can be seen from the ANOVA results, there was a significant difference between SD samples and FD samples. Spray drying also caused non-glycated amino groups to be released by denaturation because of the temperature rise.

Nevertheless, it is important to note here that according to the ANOVA results, higher results obtained at pH 10 should not be interpreted as less glycation occurs in this pH. At that point, the results that were obtained in the Lowry Method should also be considered. As can be remembered, the samples with pH 10 buffer showed lower total protein solubility compared to pH 7 buffered ones. Moreover, in OPA results, free amino groups were higher in pH 10 buffered samples compared to pH 7 ones. This can be explained by the denaturation of the soy protein in the pH 10. With denaturation, some free amino groups that did not participate in the glycation and may have been exposed to the solution and showed higher results than the pH 7. If both OPA and Lowry results were compared results were found to be complementing each other. Thus, a conclusion of *pH 7 was better in the case of glycation compared to pH 10* can be made.

Correlation analysis was also conducted between the OPA results and different types of parameters. According to the analysis, soy protein : sugar concentration was found to be significant in the OPA results with correlation coefficient of 0.789 ($p < 0.05$).

3.6. DNS Method for Reducing Sugar Determination

DNS method is based on testing the presence of free carbonyl group that is coming from reducing sugar such as dextrose, fructose and D-Psicose. The reaction is reduced to 3,5-dinitrosalicylic acid (DNS) under alkaline conditions (Miller, 1959). This method was considered as a proper way to give an idea about the sugar that was not bounded with the protein as a result of glycation. In other words, it was thought that DNS would give information about the binding of the reducing sugars with proteins since binding increase as glycation increases. In that regard, DNS could be thought as a complementary method to OPA. Thus, the best combination which gives the highest glycation in the case of different parameters is expected to be determined by considering the reducing sugar amount before and after the reaction with DNS reagent.

In this thesis, to determine the amount of reducing sugar in glycated samples, the method of Saqib & Whitney (2011) was followed. The results are shown in Table 3.2. The results are given as the initial sugar amount; that was the sugar amount before the glycation in one column and the total sugar amount found after the glycation in the other column. As can be seen from the results, the final sugar amount that was bounded seemed higher than the initial data for certain samples. In other words, there were unexpected increases in the final results. Well, there are some reasons of having that conflicted values. One of them is that after the glycation, some of the newly formed compound that contributed to the color produced by the Maillard reaction could show a reducing potential with the DNS reagent. Generally, DNS reaction shows that one mole of sugar reacts with one mole of 3,5-dinitrosalicylic acid. However, it is thought that there are many side reactions, and the actual reaction stoichiometry is more

complicated. In that regard, the type of side reaction is affected by the nature of the reducing sugars (Miller, 1959). Different reducing sugars generally yield different color intensities (Hide & Horrocks, 1994). Furthermore, other side reactions such as the decomposition of sugar also competes to react with 3,5-dinitrosalicylic acid.

Table 3.2 Reducing sugar amount results by DNS Method

	Initial Sugar Amount(g/L)	Bounded Sugar Amount (g/L) [pH 7/SD]	Bounded Sugar Amount (g/L) [pH 10/SD]	Bounded Sugar Amount (g/L) [pH 7/FD]	Bounded Sugar Amount (g/L) [pH 10/FD]
Dextrose	5	5.13±0.1	4.20±0.1	3.21±0.05	2.48±0.05
	3.33	3.58±0.02	2.66±0.04	1.99±0.01	3.66±0.12
	2.5	2.92±0.03	0.49±0.02	1.81±0.05	1.11±0.05
	1.67	2.42±0.02	0.12±0.02	0.67±0.02	0.64±0.03
	0.91	1.06±0.01	0.05±0.001	0.19±0.01	0.26±0.01
Fructose	5	4.57±0.03	4.30±0.06	3.00±0.1	2.04±0.02
	3.33	4.15±0.05	2.65±0.05	1.60±0.09	1.23±0.02
	2.5	2.75±0.03	1.80±0.02	1.44±0.04	0.80±0.03
	1.67	2.09±0.02	0.45±0.01	0.86±0.03	0.64±0.01
	0.91	1.12±0.01	0.05±0.002	0.31±0.01	0.35±0.01
D-Psicose	5	5.72±0.01	4.77±0.02	3.84±0.04	2.23±0.09
	3.33	3.72±0.01	2.90±0.02	2.27±0.07	1.39±0.01
	2.5	2.38±0.02	1.99±0.04	1.80±0.06	1.13±0.01
	1.67	2.01±0.003	1.14±0.01	1.11±0.01	0.62±0.01
	0.91	1.04±0.01	0.40±0.01	0.44±0.01	0.32±0.01

When DNS method was explored more on the literature, it was observed that several problems were experienced with this method in different studies. Indeed, it was pointed out that the results obtained by the method could be considered as inaccurate compared to other methods like NS (The Nelson-Somogyi) reducing sugar method and HPLC (Mccleary & Mcgeough, 2015). In another detailed research on the limitation of the DNS method, the conclusion was made as that hydrolysis of compounds due to chemical reactions may result in color interferences with the DNS assay. In addition, more hydrolyzed compounds lead to have greater degree of color interferences. It was also shown that DNS reagent is easily affected by high temperatures, pressures and pH of the environment and may lead to false results at the end (Rivers, Gracheck, Woodford, & Emert, 1983).

In this study it was also hypothesized that, DNS could also react with the other carbonyl groups present in the system. As a result of glycation, carbonyl containing groups could be formed. Therefore, although only reducing sugar amounts were considered before the reaction, there may be increase in the carbonyl group afterwards of the reaction like it happened in the glycated soy protein samples.

Since it was not possible to predict how much of the DNS results was really coming from the unused sugar, it was concluded that this method cannot be used directly to determine the degree of glycation. In that regard, other methods to determine the reducing sugar amount can be considered. One of those method is HPLC for exact sugar amount determination. In order to resolve the problem coming from the DNS method, HPLC experiments to measure the degree of sugar binding can be considered in the future of the work on glycation of soy proteins.

3.7. Fourier Transform Infrared (FTIR) Spectroscopy Analysis

Glycated soy proteins with several parameters were examined by using FTIR spectroscopy. For this purpose, the glycated samples with their FTIR spectra (500 cm^{-1} to 4000 cm^{-1}) were recorded and spectra were drawn by also including the results of native form of dextrose, fructose, D-Psicose and SPI respectively.

Table 3.3 Components observed in the FTIR spectra glycated soy protein

Compounds	Groups	Frequency (cm-1)
alcohols, a broad, strong band	O-H stretch	3200-3500
1^0, 2^0 amines and amides	N-H bend	1300-2000
Alcohols, Carboxylic acids, Esters, Ethers	C-O stretch	1000-1100
Aliphatic amines	C-N stretch	1070-1150

In order to visualize the differences carefully, the results of the samples have been demonstrated in detailed and separated graphs. In one plot, only one type of pH, glycation and sugar parameters were shown. In Figure 3.13. below, the results of the glycated soy protein with D-Psicose at pH 7 / FD can be seen. The other spectra were included in the Appendix section.

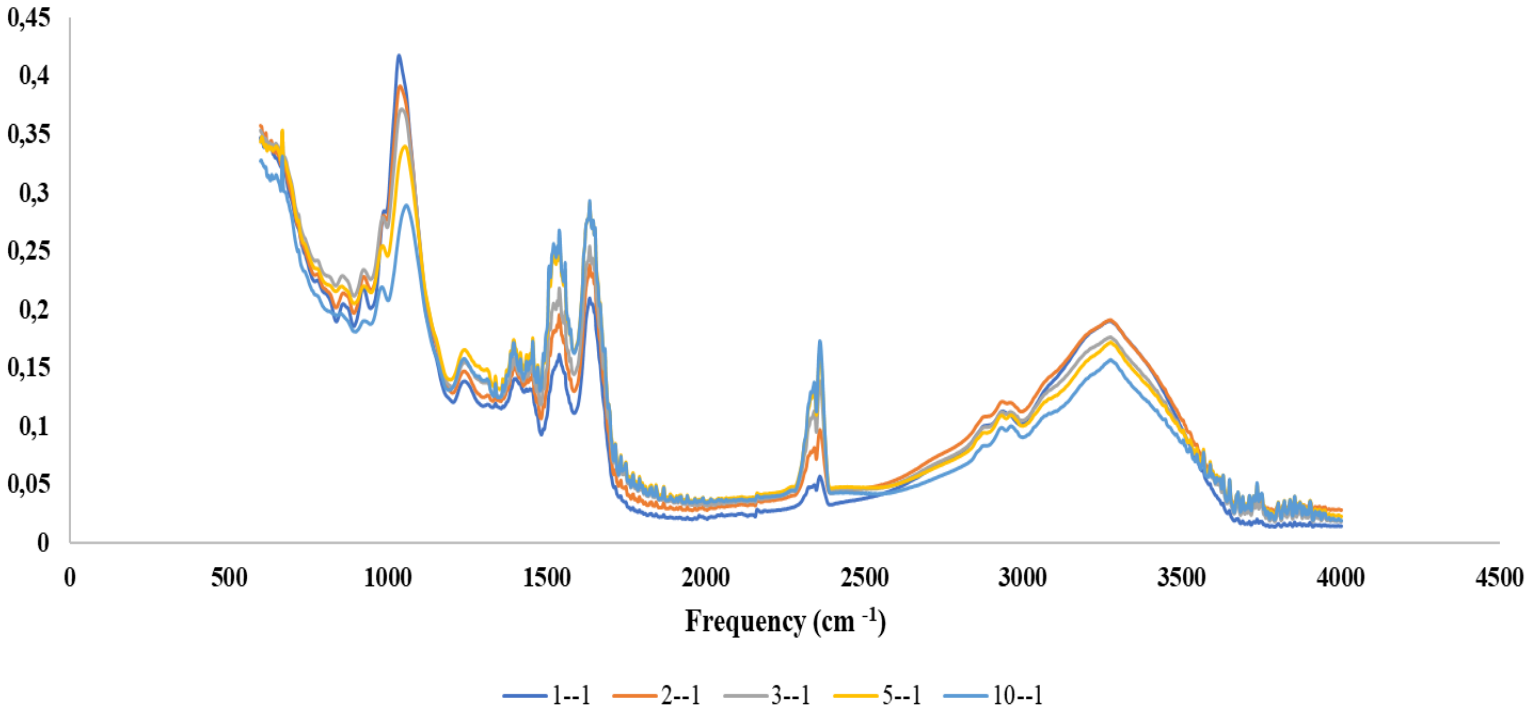

Figure 3.13 Compounds of glycated soy protein with D-Psicose at pH 7 / FD conditions

When the spectra were investigated, it was seen that peaks at 1637 cm^{-1} and 1543 cm^{-1} corresponded to the structure of Amide I, Amide II respectively. The Amide I band is obtained by C=O stretching of the peptide bonds of proteins, while the Amide II band is obtained from both C-N stretching and N-H bending (Oliver, Kher, McNaughton, & Augustin, 2009). Those peaks were observed due to the amino acids in the soy protein. When those two peaks were examined, it was observed the values of the peaks increased as soy protein : sugar ratio increases. As glycation occured, it was expected to see a decrease in both peaks due to the fact that there would be the loss of NH_2 groups of the protein that was bounded to sugar (Mao, Pan, Hou, Yuan, & Gao, 2018). As can be seen from the graph of just SPI from Appendix, the peaks were higher compared to glycated ones. Thus, by looking at the figure, it can be concluded that more loss of the NH_2 groups were lost at 1:1 ratio. Meaningly, there was more protein-sugar interaction occurred. As a result, higher glycation rate was obtained in 1:1 ratio. This finding was also matched with the results obtained by both OPA and Lowry methods.

There is also one important peak that was observed in the glycated samples. Indeed, glycated proteins show higher absorption near the peak located at 3400 cm^{-1}. According to the literature, when glycation is achieved, there is a shoulder observed near the peak at 3400 cm^{-1} (Otero de Joshi et al., 2003). Moreover, it was pointed out that the higher the shoulder values, higher the glycation rate (Otero de Joshi et al., 2003). When the results obtained from the Figure 3.13. was investigated, the shoulder appeared near the 3400 cm^{-1}. Also, shoulder intensity decreased as soy protein : sugar ratio increased. This also supported the hypothesis that 1:1 ratio was the best choice for glycation among the others.

When all the other spectra were investigated, same trend was observed. In other words, decrease in Amide I and Amide II peaks and a higher intensity shoulder was observed near 3400 cm^{-1} in all cases. These two features certainly indicated the glycation between soy protein and the reducing sugars. In addition, the conclusion of *increase of soy protein : sugar ratio decreased the glycation rate* could be stated for all cases with different parameters.

3.8. Determination of Hydration Behavior by Nuclear Magnetic Resonance (NMR) Relaxometry

One of the most important choice of using NMR relaxometry was to observe hydration behavior of the glycated soy proteins due to the fact that relaxation times are good indicators of the mobile protons inside the samples (Mowery, Assink, & Celina, 2005). Therefore, effect of glycation on the proteins was aimed to be explored by the help of these relaxation times.

Between two relaxation times (T_2 and T_1), T_2 relaxation time was taken into consideration due to the fact that T_1 measurements take longer times because of the nature of pulse sequence used to measure it. Moreover, T_2 times could be more exploratory since it can give information about the proton pools in a much better extent. In T_2 measurements, in addition to soy protein and glycated samples, the samples that were not exposed to any glycation procedure (named as NFD) were also prepared in the same way of glycated ones. These were the just the mixtures of protein and sugar at the ratios used in the study since sugar in their free form could also contribute to the hydration to see the effect of glycation. Table 3.4. shows all the results of T_2 relaxation time (ms) obtained through the NMR Relaxometry experiments.

Table 3.4 Experimental results of T_2 (ms) results of the all samples obtained by NMR Relaxometry

	Soy Protein : Sugar Ratio	T2 Results (ms) [pH 7/FD]	T2 Results (ms) [pH 7/SD]	T2 Results (ms) [pH 10/FD]	T2 Results (ms) [pH 10/SD]	T2 Results (ms) [NFD]
Dextrose	1:1	209.572±2.369	269.233±3.545	198.057±1.569	91.192±0.888	162.865±1.223
	2:1	210.095±1.805	177.543±2.431	134.204±3.443	64.339±0.912	108.432±0.884
	3:1	156.347±3.936	158.581±2.147	142.040±1.348	59.548±0.864	86.664±0.824
	5:1	120.517±2.631	113.043±1.649	120.650±2.466	51.019±0.369	77.017±0.442
	10:1	92.345±2.288	96.729±1.044	125.744±2.224	43.442±0.486	73.103±0.322
Fructose	1:1	386.743±3.489	264.622±2.889	439.090±3.664	196.633±1.042	137.95±1.007
	2:1	250.473±3.201	153.145±0.987	265.006±2.888	113.285±0.906	112.985±0.732
	3:1	224.647±2.756	123.146±1.126	276.011±2.663	92.046±0.882	103.120±0.665
	5:1	174.935±2.109	108.653±1.423	213.621±2.669	87.188±0.712	79.623±0.406
	10:1	173.695±1.988	97.466±0.902	133.448±1.442	77.439±0.624	81.237±0.342
D-Psicose	1:1	351.128±4.757	428.329±2.487	418.093±3.882	212.845±1.112	136.291±0.864
	2:1	254.921±2.887	208.711±2.105	435.268±4.102	126.042±0.554	99.100±0.685
	3:1	246.756±3.212	133.614±1.481	271.481±2.987	97.459±0.356	84.672±0.452
	5:1	244.289±1.786	118.691±1.413	255.767±2.144	92.255±0.408	75.221±0.268
	10:1	122.646±1.654	131.341±1.324	159.118±1.756	80.228±0.413	72.621±0.216

When the Pearson correlation was applied for the ANOVA results given in Appendix, it was observed that sugar type, soy protein : sugar ratios, glycation types and pH had a significant effect on T_2 values ($p < 0.05$).

The detailed examination showed that T_2 values decreased as soy protein : sugar concentration increased. Moreover, the highest T_2 values were seen in the glycated samples with D-Psicose as the sugar type. Apart from that, among the three-comparison made for FD, SD and NFD, it was observed that lowest T_2 values were obtained in NFD samples followed by SD and FD. Thus, looking at this result, it can be said that glycation caused an increase in T_2 values, hence a decrease on the hydration of the samples. There is no study examining the hydration of the glycated proteins with relaxation times in the literature, so comments were made considering general NMR studies.

The decrease in T_2 value means that free water is reduced in the system (Counsell et al., 2003). Since T_2 value is associated with the release of free protons, free water proton prolongs relaxation times (Kirtil & Oztop, 2016). Apart from this, the abundance of free water protons also means that water retention capacity as well as hydration is less. The lowest T_2 values were observed in NFD samples which were just prepared as the mixture of soy protein and sugar in same ratios and not exposed any reaction. Therefore, it can be concluded that glycation, whether it is SD or FD, decreased the water holding capacity and so hydration rate. In addition, the conclusion of more glycation resulted in longer T_2 values can be made and thus by looking at the T_2 values between FD and SD types, FD samples were found to result in more glycation rate with longer T_2 values.

The effect of pH, sugar type and soy protein : sugar ratio were examined by considering the outcome of the results that glycation caused increase in T_2 values. According to the average T_2 values in the ANOVA results, it was observed that pH 7 had longer T_2 values compared to pH 10 ones. This indicated that glycation was higher in the samples prepared at pH 7. Moreover, FD and 1:1 ratio was the highest option to have more glycation again like the results of other experiments such as Lowry, OPA and FTIR. In addition, among the sugar types, longest T_2 values were obtained in D-Psicose followed by fructose and dextrose. This outcome was different from the other experiments that fructose was better in the OPA and Lowry results although D-Psicose was so close to fructose.

Nevertheless, D-Psicose is known to have a higher ability to enter Maillard reactions than other sugars (Sun et al., 2004) Therefore, this result was comprehensible. At that point, the hydration behavior of different sugar types being different is an important point. According to one study (Maugeri et al., 2017), it was pointed out that, average hydrogen bond length to sugar was the main reason of having different hydration behavior of sugars. It was explained that sugar molecules form H-bonds of different length and strength that correlate with their hydration behaviour. In the study, fructose and dextrose sugars were compared and found out that hydrogen bond length of fructose was shorter than dextrose; thus, water holding capacity was lower due to shorter length (Maugeri et al., 2017). D-Psicose was also found to let more free water (Ikeda, Gohtani, Fukada, & Amo, 2011). Thus, the unglycated amount of D-psicose in the samples could cause longer T_2 times.

To conclude, as the T_2 value increases, it was seen that the water holding capacity decreased and glycation was observed to reduce water retention. Thus, samples with high T_2 values can be considered as glycated samples. As a result, it can be stated that

D-Psicose sugar, pH 7, FD and soy protein : sugar ratio of 1:1 can be considered as the best option for glycation by looking at the T_2 results.

CHAPTER 4

CONCLUSION AND RECOMMENDATIONS

In the current study, the influences of different parameters that are glycation type (FD, SD), pH (7,10), sugar type (dextrose, fructose and D-Psicose) and protein : sugar ratio (1:1, 2:1, 3:1, 5:1 and 10:1) were investigated on the glycation of soy protein. After the samples were at these conditions a detailed physicochemical characterization was performed.

Some chemical experiments such as antioxidant activities by DPPH method, degree of glycation, solubility by Lowry method, free amino group by OPA method, reducing sugar amount by DNS method and FTIR analysis were performed. In addition to that physical experiments like hydration behavior by NMR Relaxometry and emulsification activity determinations were made.

It was seen that when the soy protein was glycated, the antioxidant activities increased in D. However, these two assays were based on different mechanism. That's why, highest antioxidant activities were different in the case of pH for those methods. Apart from pH, all other parameters were correlated in both methods for antioxidant activity determination.

Degree of glycation was not that reliable because of the fact that some compounds that may be formed and gave brown color after glycation reaction. It was thought that those compounds showed brown color formation at 420 nm and the results obtained by this method did not match with the other experiments.

Like in the case of degree of glycation, brown color formation of the exposed compounds throughout the glycation caused unexpected results in DNS Method. Apart from brown color formation, the carbonyl group containing compounds formed as a result of the reaction might have caused false results. Therefore, although only reducing sugar amounts were determined before the reaction, there might be an increase in the carbonyl group afterwards due to the formation of new compounds

According to the researches conducted on soy proteins, one of the most drawback was found as the low solubility. That's why, the effect of glycation on the soy protein solubility was crucial part of this study. Obtained results of Lowry Method showed that protein solubility slightly increased thanks to glycation. With glycation, emulsification activity of the soy protein was improved. In addition, as stated in the literature that D-Psicose has an ability to improve emulsification activity, samples containing D-Psicose as sugar showed high emulsification activity.

In OPA results, the aim was to see the remained amount of free amino groups (FAG) after glycation reaction. In the experiment, lower amount of FAG indicated higher glycation rates. It was seen that glycation was successful in both freeze dryer and spray dryer. FTIR analysis was also conducted on the glycated samples. FTIR spectra showed that with glycation decrease in Amide I and Amide II peaks were observed (1600-2000 cm-1) and a higher intensity shoulder near 3400 cm^{-1} indicated the occurrence glycation between soy protein and reducing sugars

One of the novelest technique that was applied in this study was the use of NMR Relaxometry on the glycated soy protein. Since there is no literature study on the glycation before, this trial was crucial. The hydration behavior of the glycated soy proteins was examined by also including non-glycated samples to understand the

effect of glycation. The comparison was made by considering T_2 values. According to the results, higher T_2 values indicated higher the glycation rate. Therefore, it was concluded that glycation, whether it is SD or FD, decreased the water holding capacity and so hydration rate.

When all the parameters were considered in the statistical analysis, the best combination to have the highest glycation rate was thought to be samples with pH 7 buffer, 1:1 soy protein to sugar ratio, FD as the glycation type. Among the sugar types, the lowest glycation was observed in dextrose samples for sure except degree of glycation results which may interrupted by the compounds that may give brown color after glycation. In addition, D-Psicose was considered to be a good alternative sugar source in glycation reactions.

This study proved that application of glycation on soy protein improved its functional properties. Indeed, developed properties of D-Psicose helped to improve the functional properties of the soy protein. In addition, it was seen that NMR Relaxometry can be used to determine the rate of glycation by considering the hydration behavior.

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APPENDICES

A. CALIBRATION CURVES

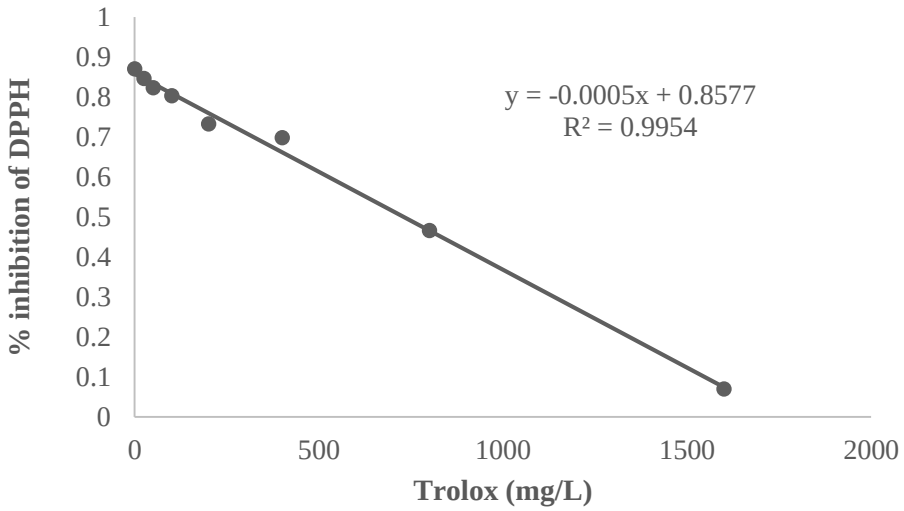

Figure A.1 Calibration curve for DPPH assay prepared by Trolox to determine antioxidant activity in glycated soy protein

Absorbance (at 517 nm) = -0.0005 * (mg trolox/L) + 0.8577 where R^2 = 0.9954

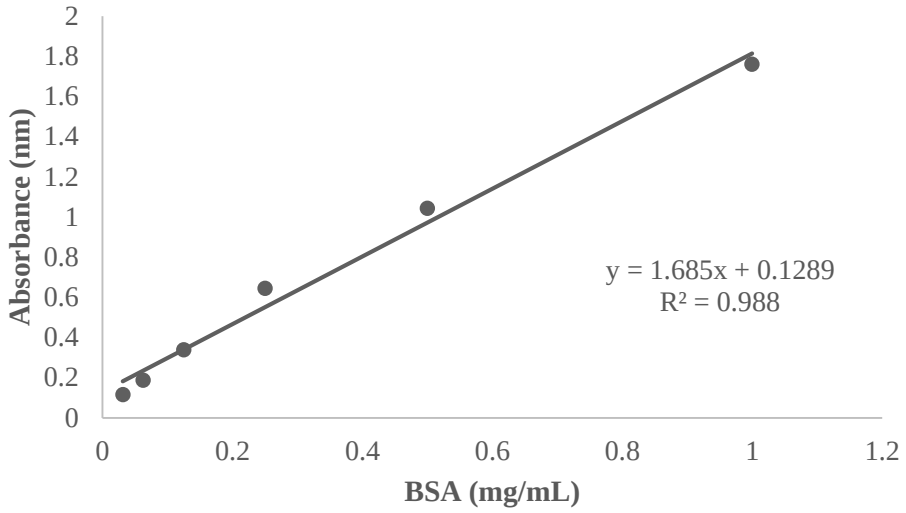

Figure A.2 Calibration curve for Lowry Method prepared by Bovine Serum Albumin (BSA) to determine total soluble protein contents in glycated soy protein

Absorbance (at 750 nm) = 1.685 * (mg BSA/ml) + 0.1289 where $R^2 = 0.988$

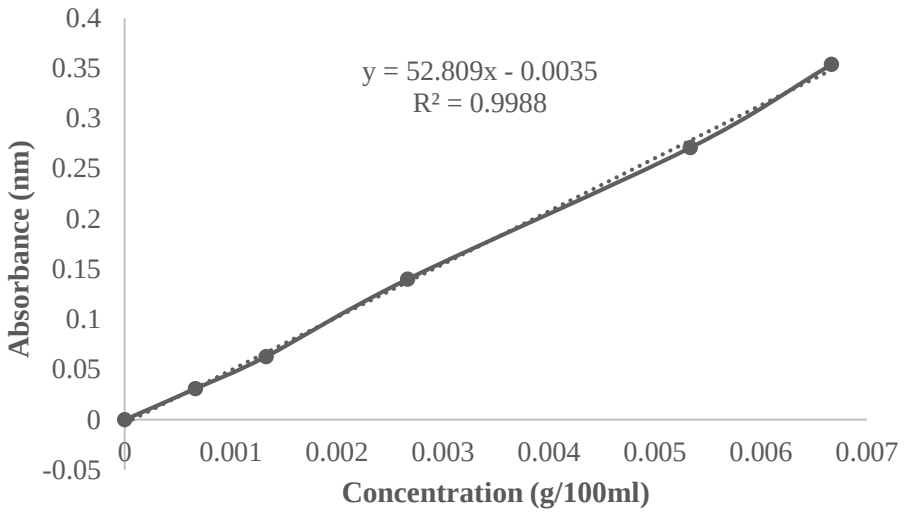

Figure A. 3 Calibration curve for OPA Method prepared by glycine to determine free amino groups (FAG) in glycated soy protein

Absorbance (at 340 nm) = 52.809 * (mg BSA/ml) –0.0035 where $R^2 = 0.9988$

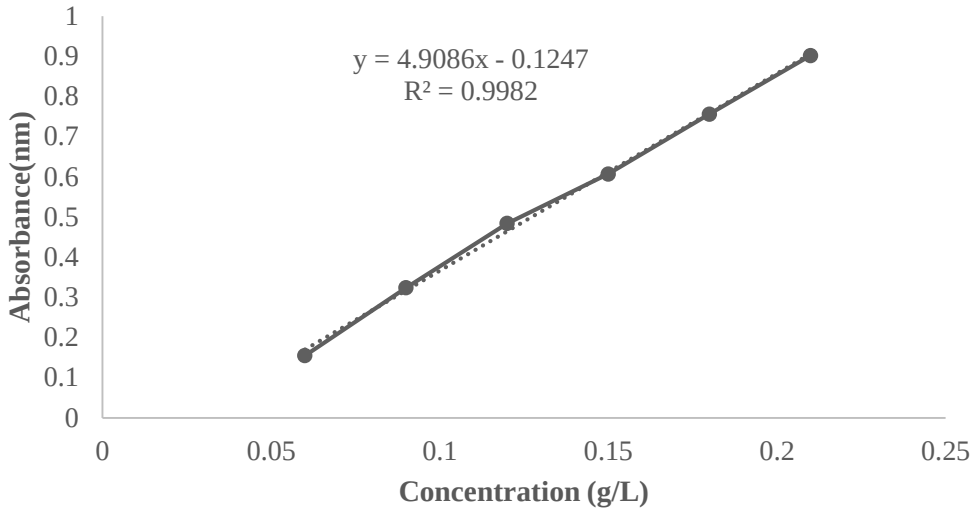

Figure A. 4 Calibration curve for DNS Method prepared by dextrose solution to determine reducing sugar amount in glycated soy protein

Absorbance (at 540 nm) = 4.9086 * (g/L) – 0.1247 where $R^2 = 0.9982$

B. COMPARATIVE FIGURES

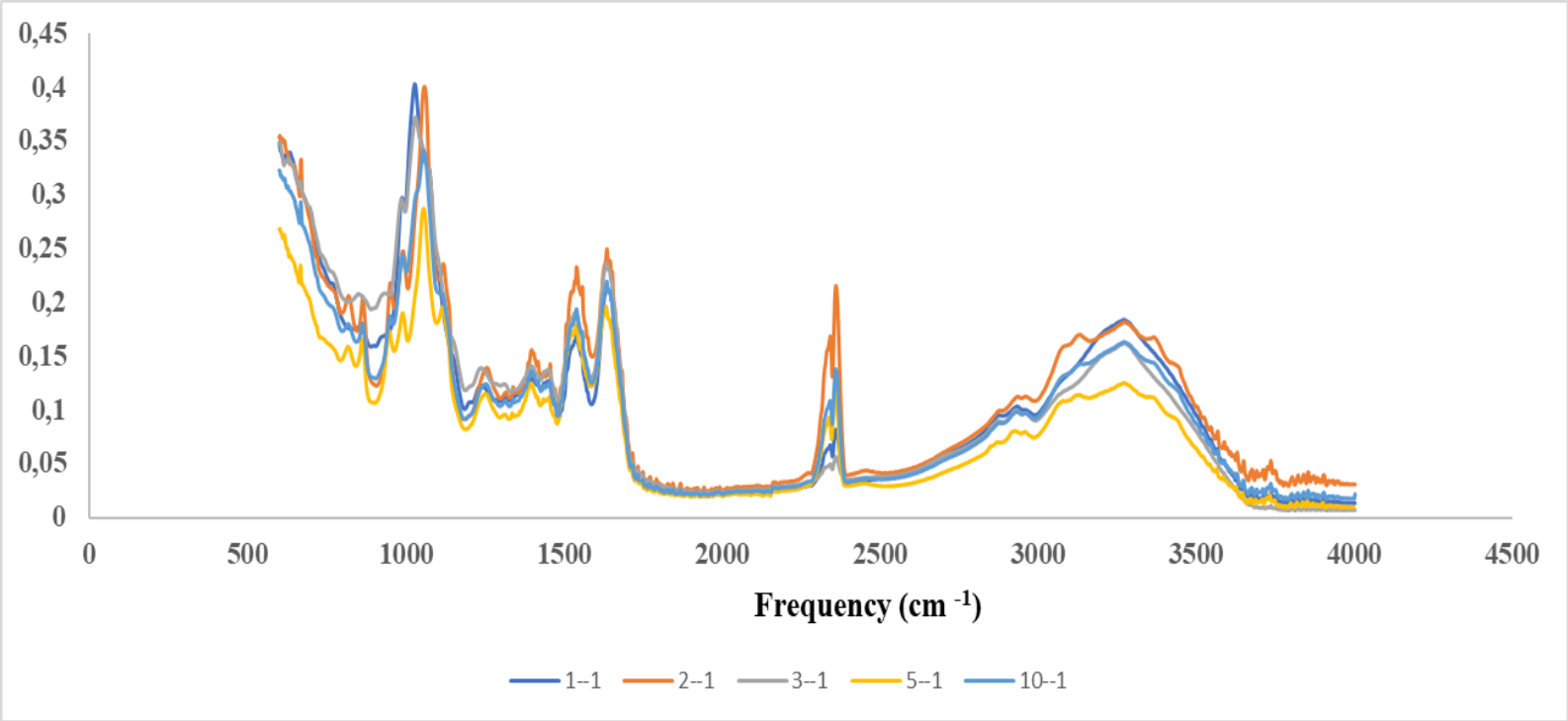

Figure B.1 Compounds of glycated soy protein with dextrose at pH 7 / FD conditions

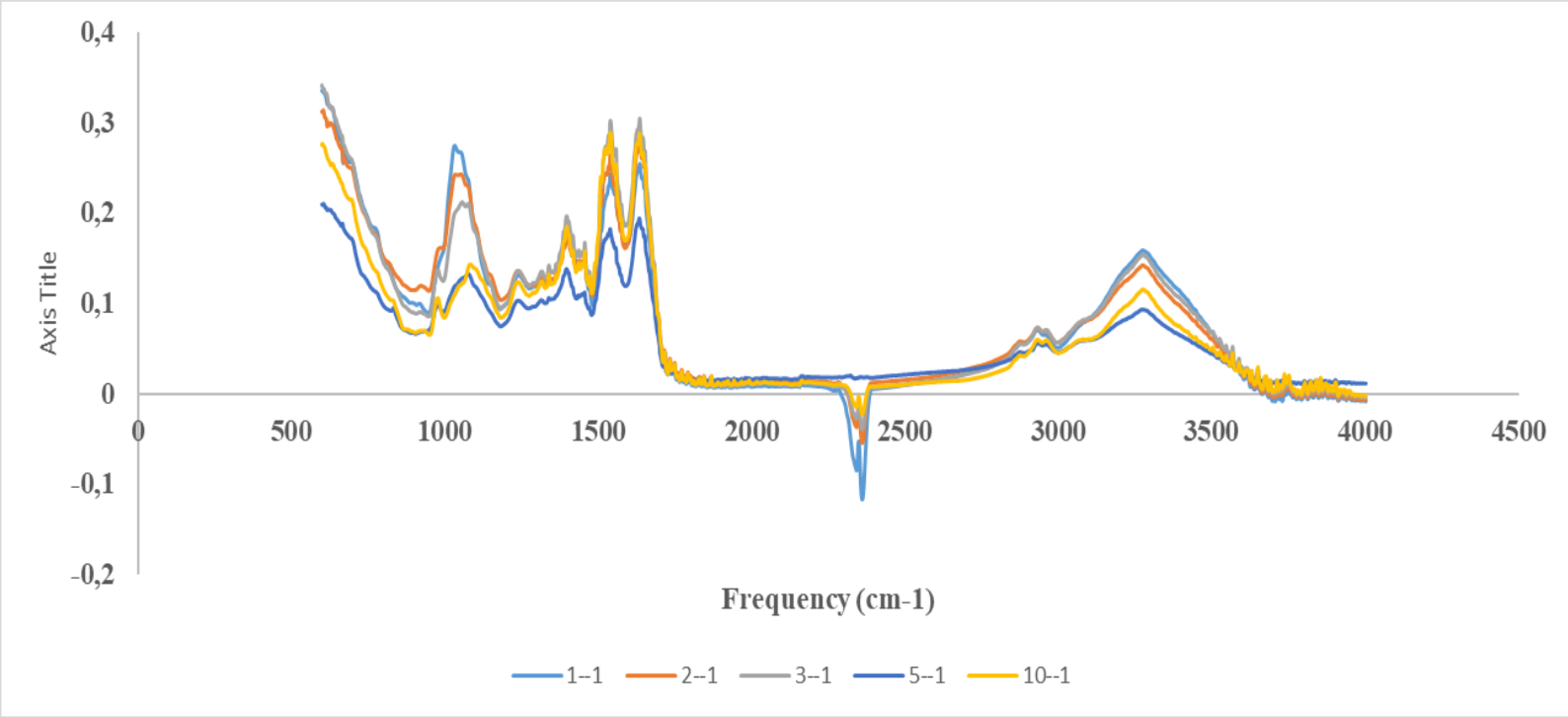

Figure B.2 Compounds of glycated soy protein with dextrose at pH 10 / FD conditions

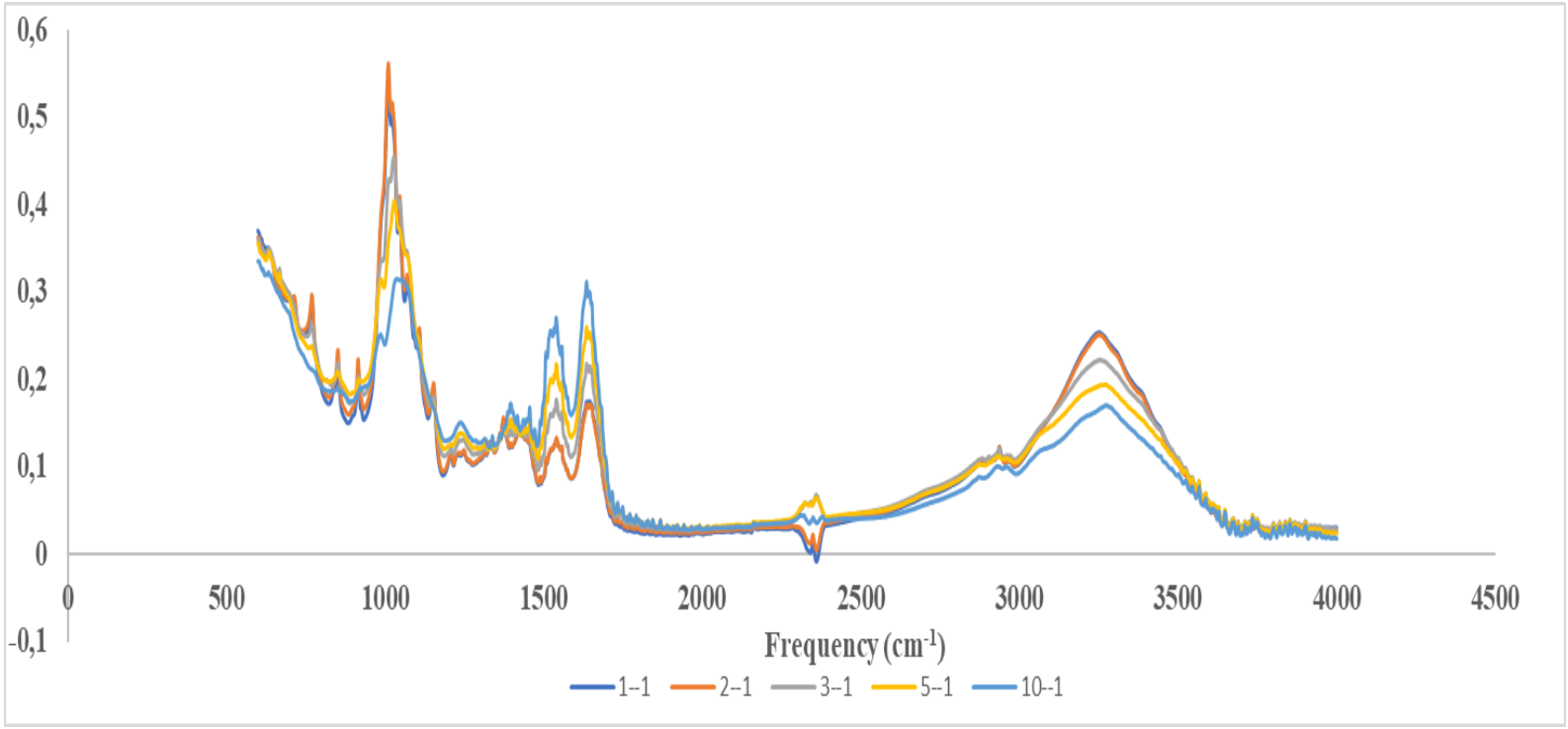

Figure B.3 Compounds of glycated soy protein with dextrose at pH 7 / SD conditions

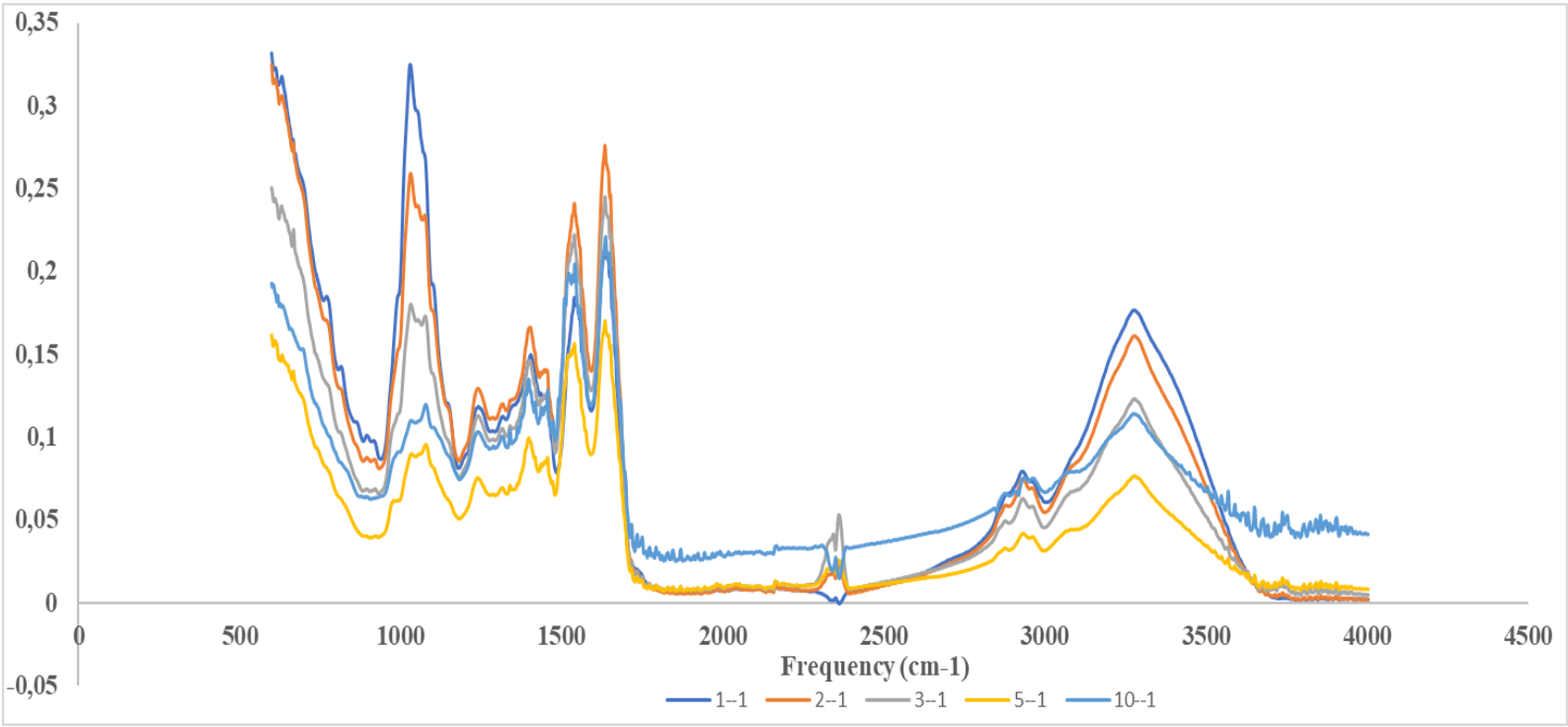

Figure B.4 Compounds of glycated soy protein with dextrose at pH 10 / SD conditions

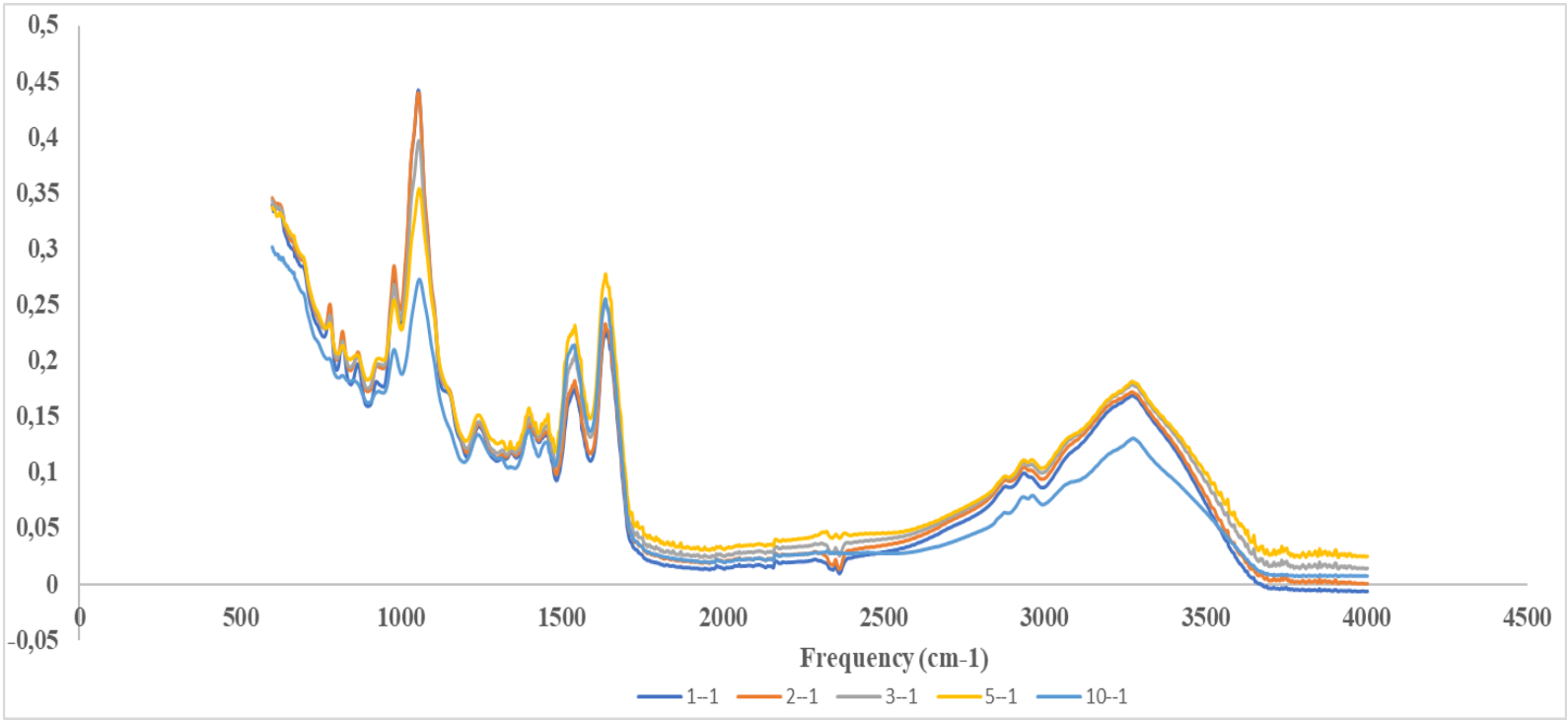

Figure B.5 Compounds of glycated soy protein with fructose at pH 7 / FD conditions

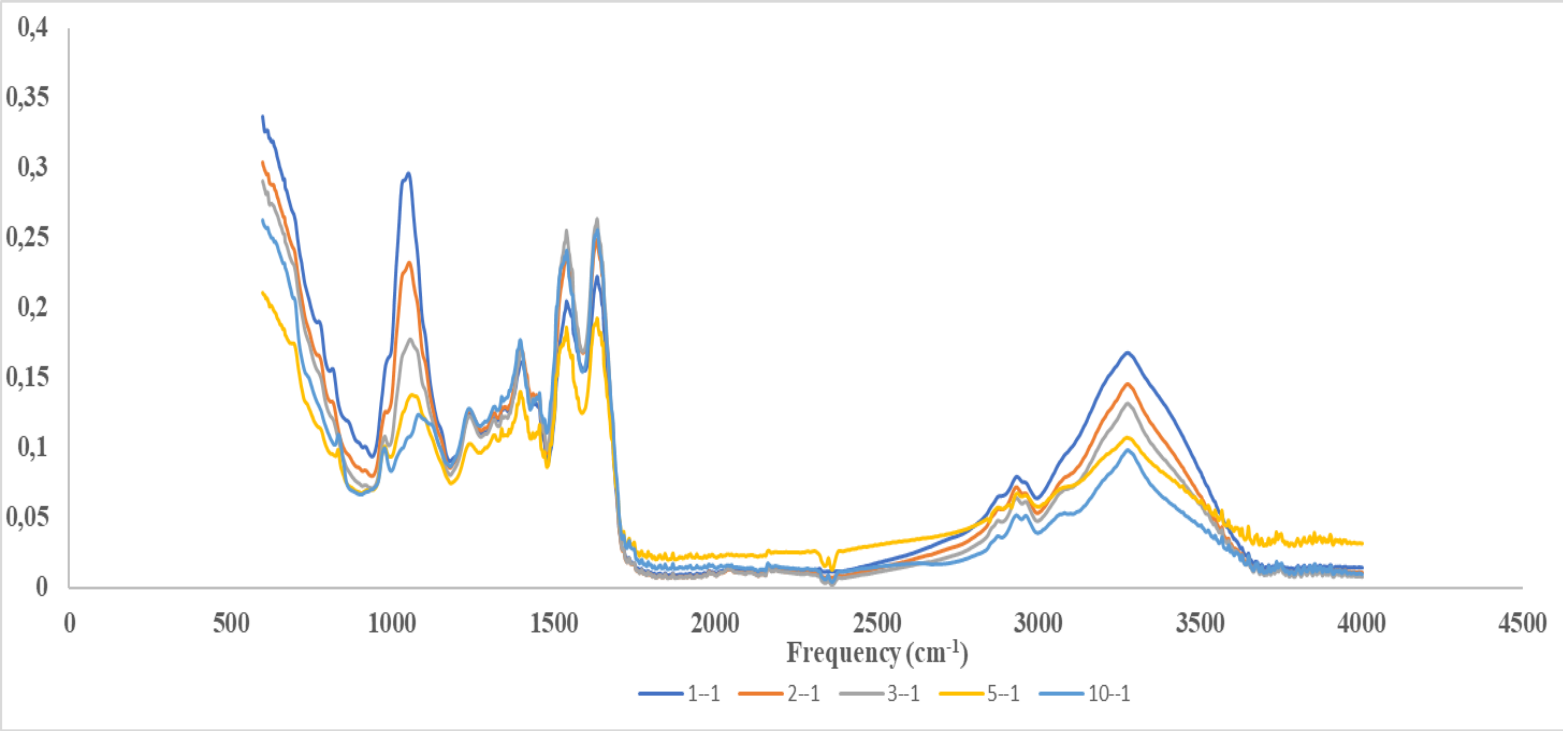

Figure B.6 Compounds of glycated soy protein with fructose at pH 10 / FD conditions

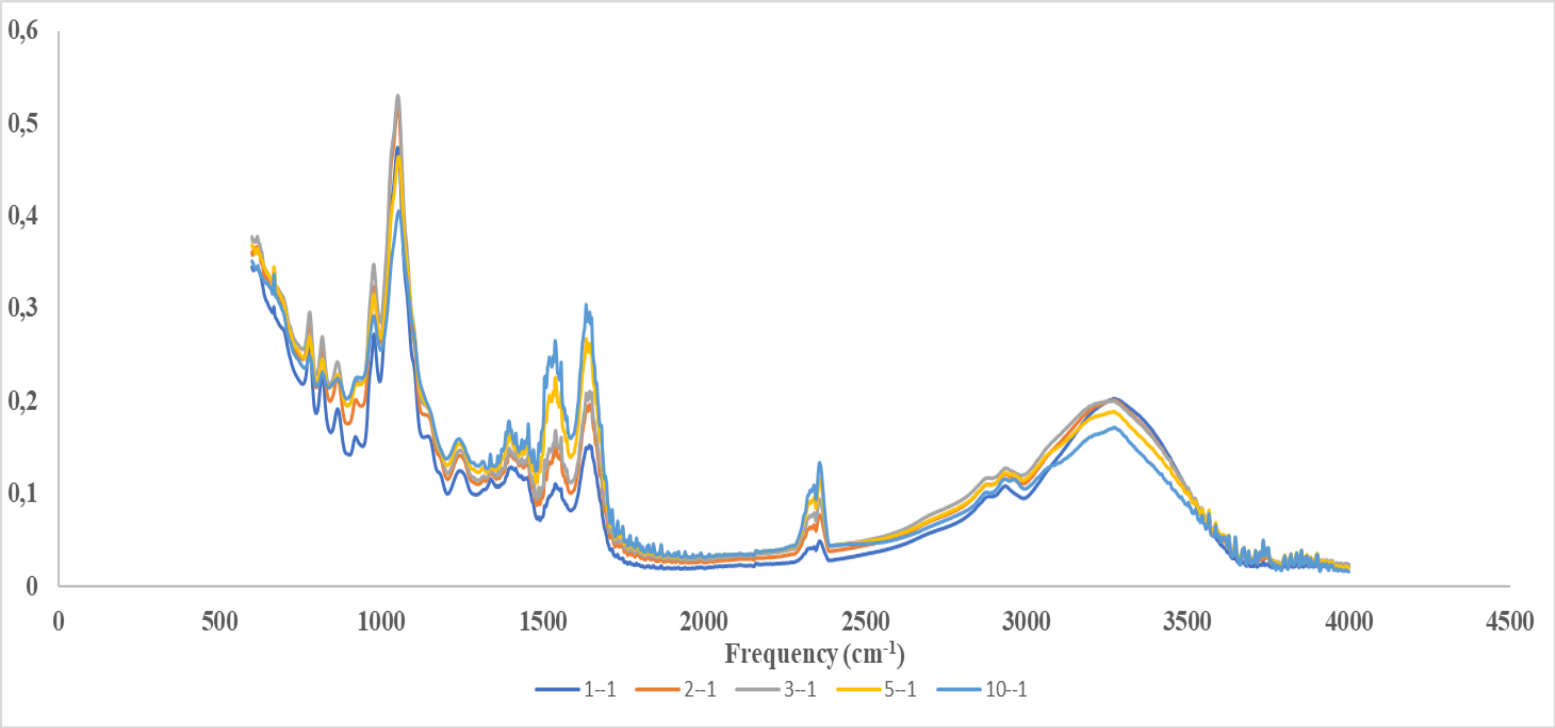

Figure B.7 Compounds of glycated soy protein with fructose at pH 7 / SD conditions

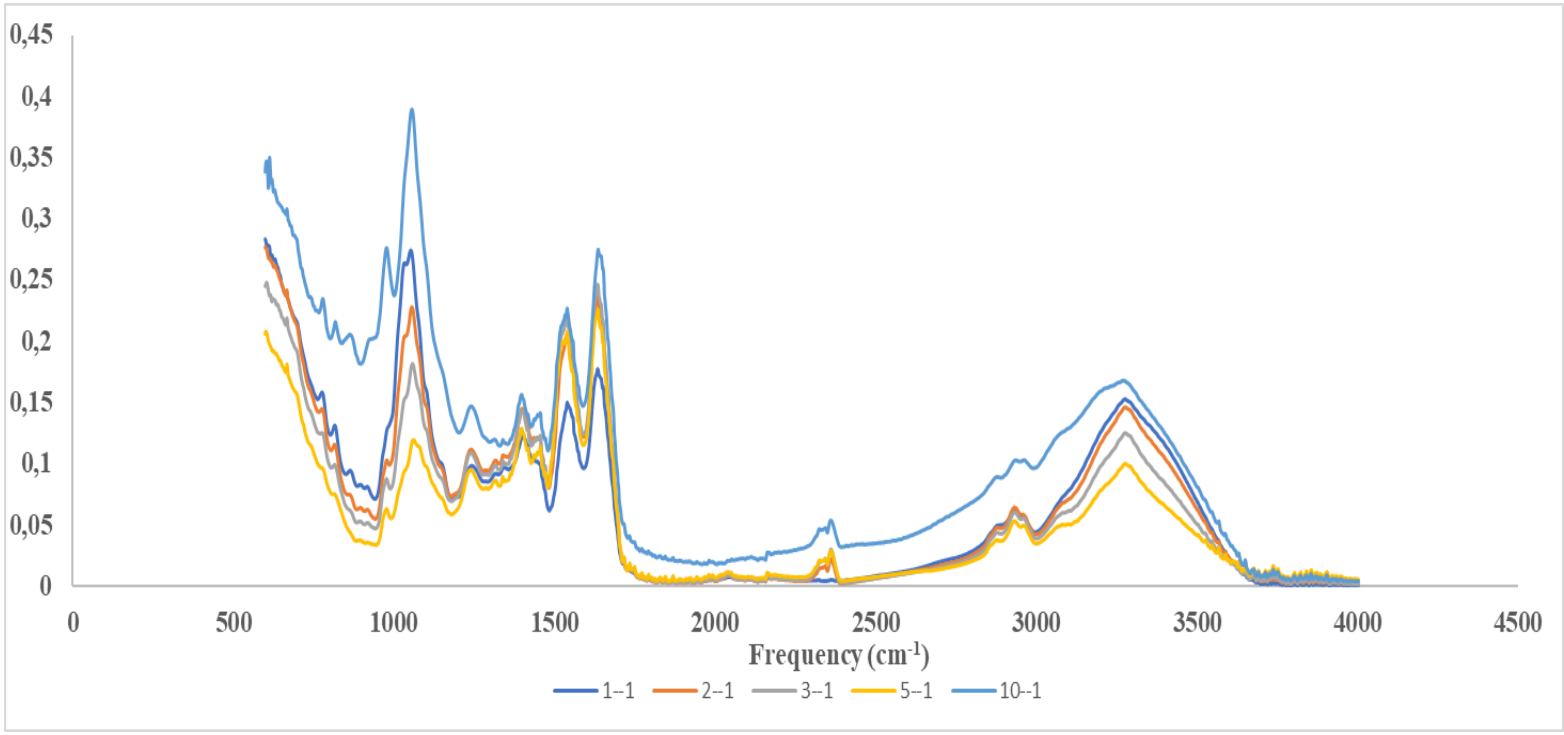

Figure B.8 Compounds of glycated soy protein with fructose at pH 10 / SD conditions

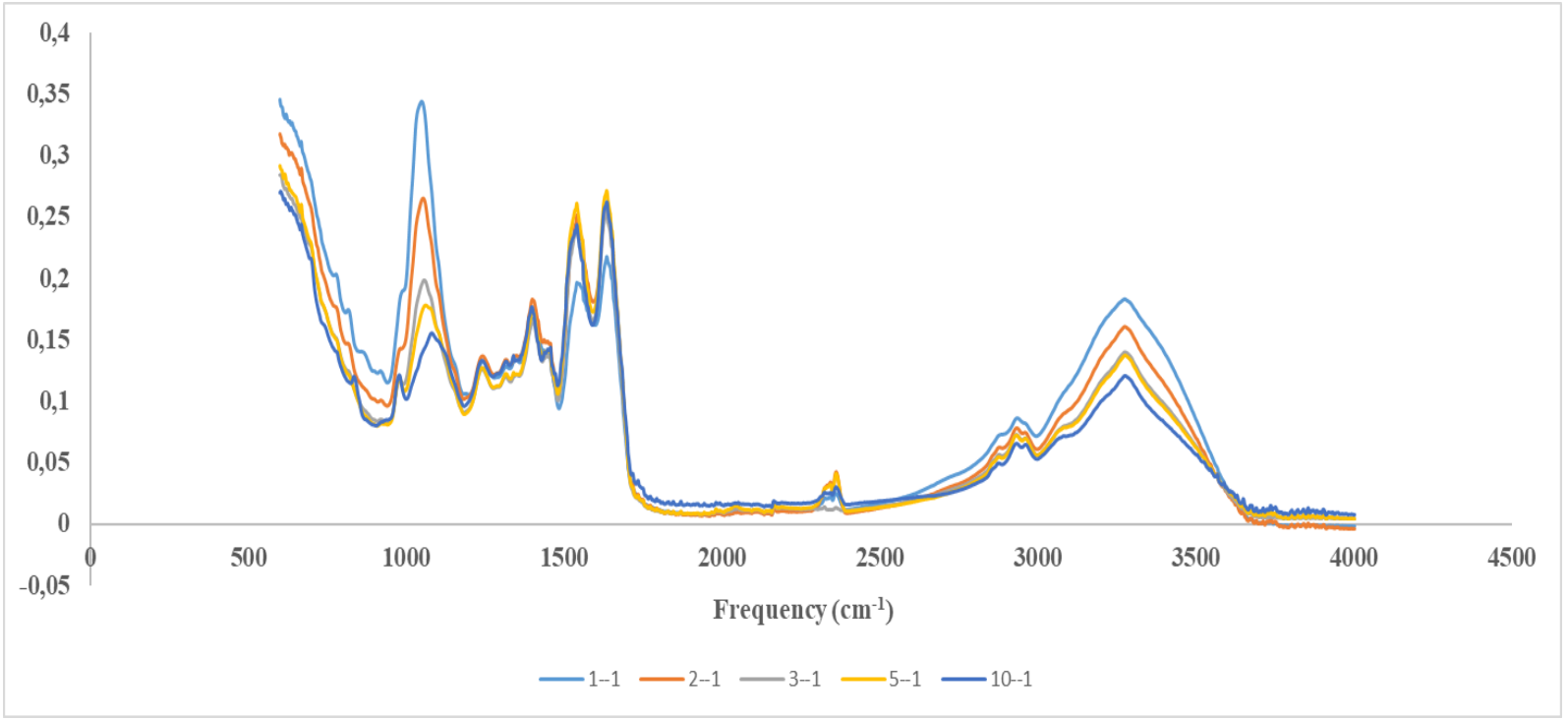

Figure B.9 Compounds of glycated soy protein with D-Psicose at pH 10 / FD conditions

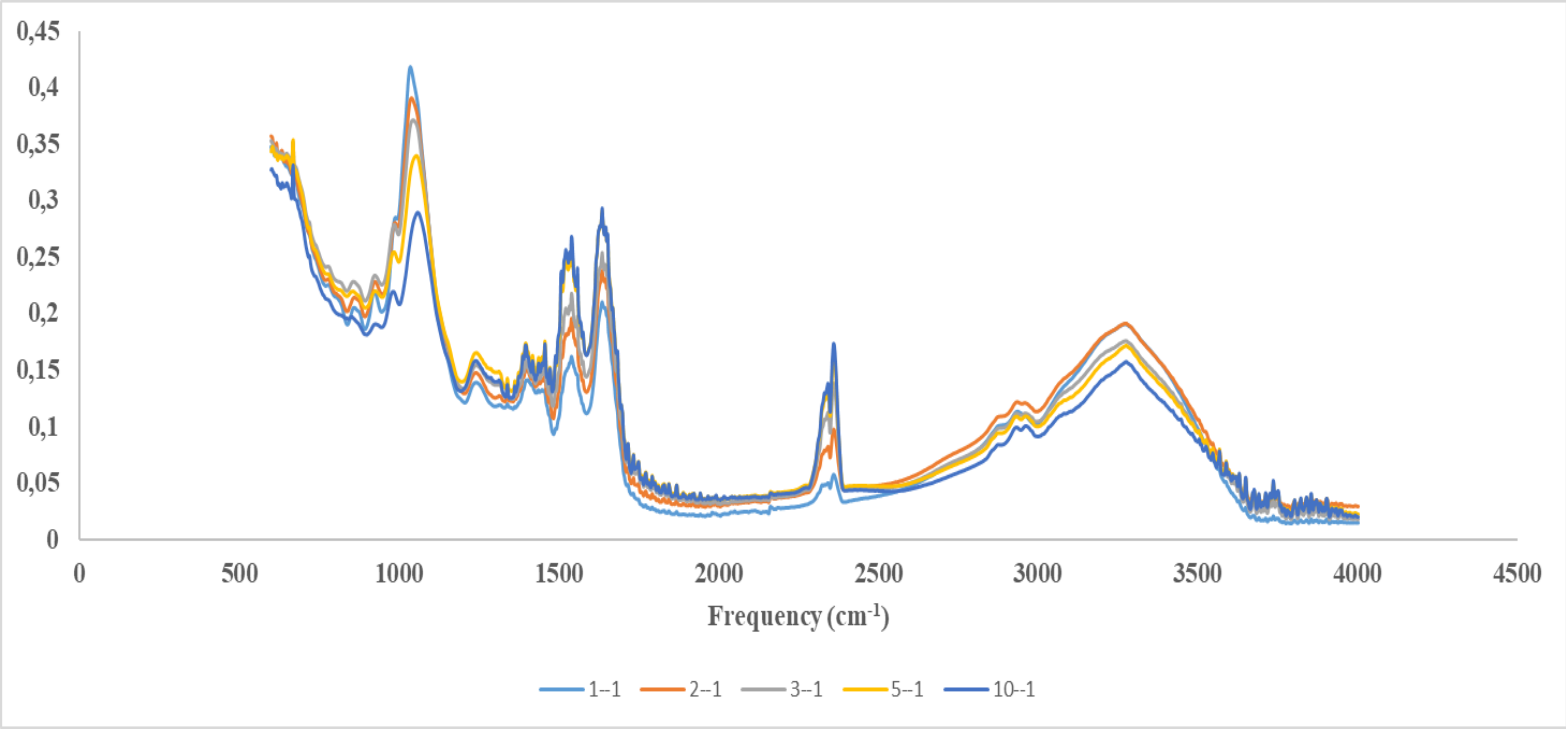

Figure B.10 Compounds of glycated soy protein with D-Psicose at pH 7 / SD conditions

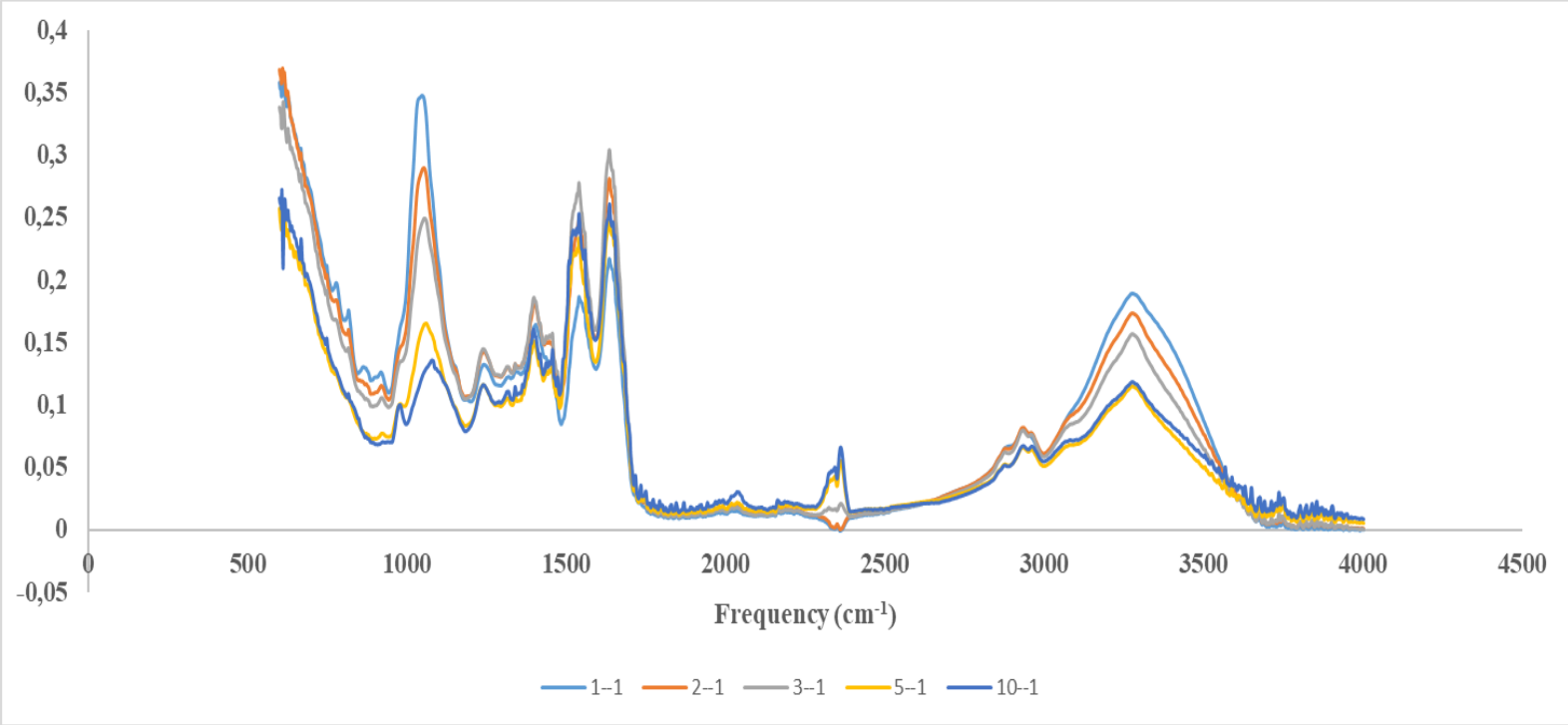

Figure B.11 Compounds of glycated soy protein with D-Psicose at pH 10 / SD conditions

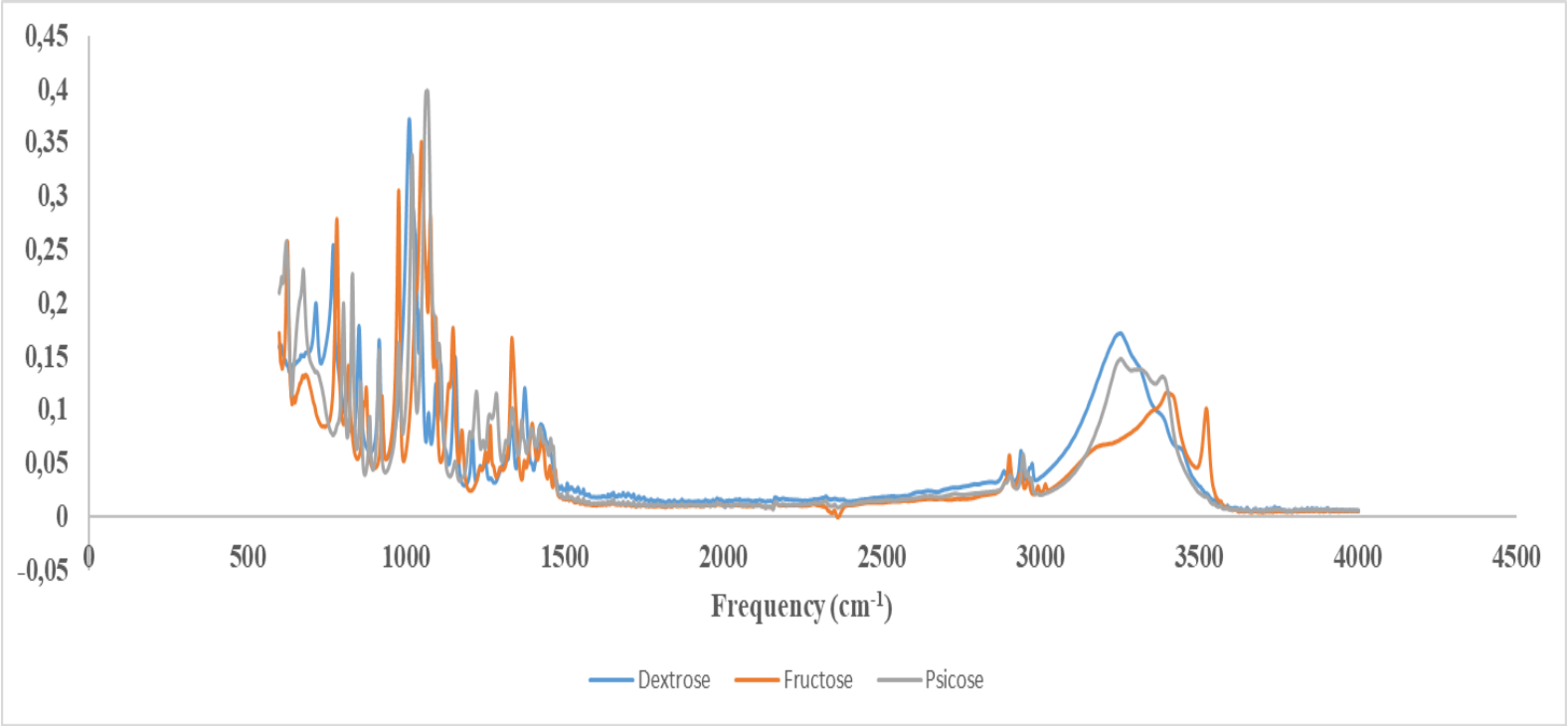

Figure B.12 Compounds of native dextrose, fructose and D-Psicose sugars

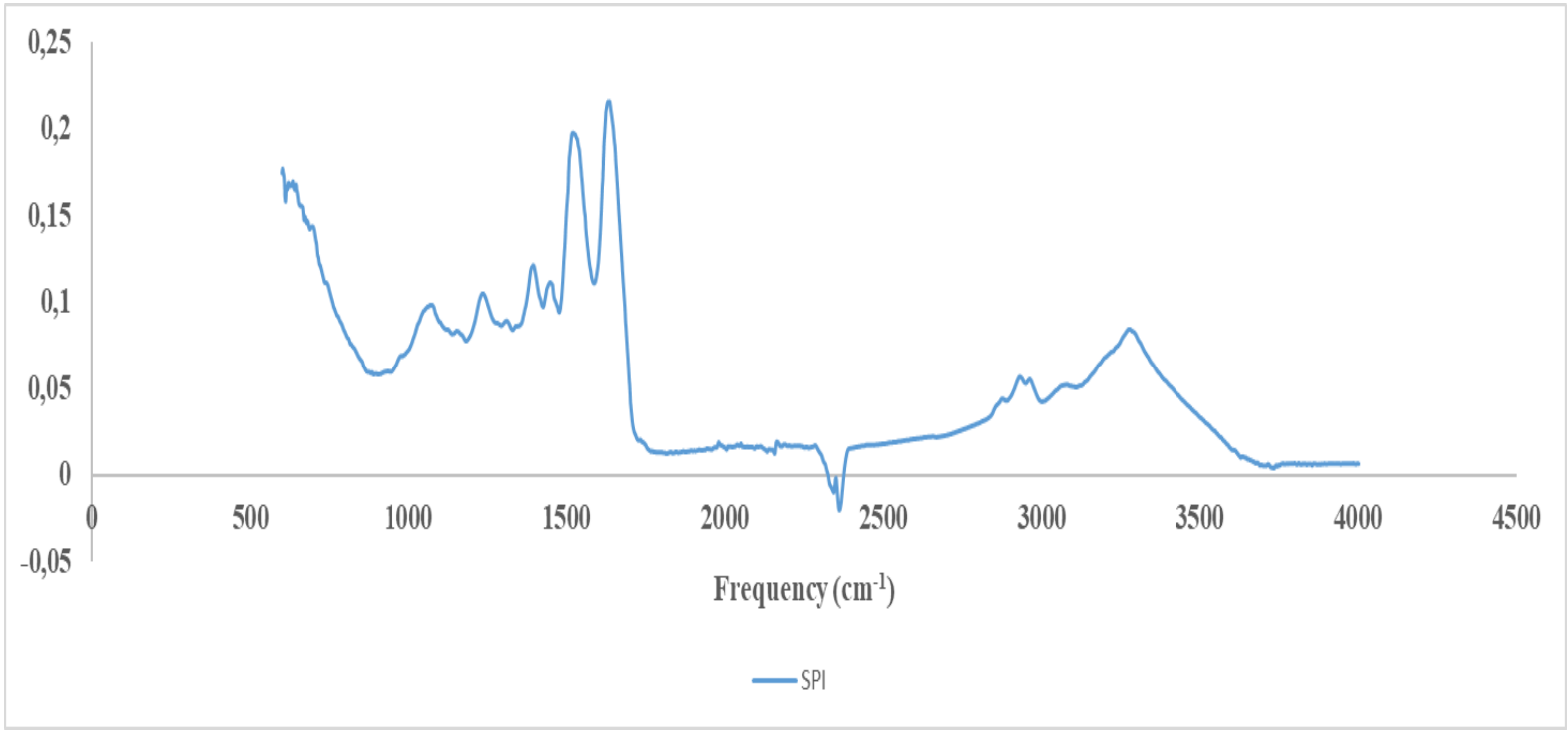

Figure B.13 Compounds of native soy protein isolate (SPI)

C. STATISTICAL ANALYSES

Table C. 1. ANOVA and Tukey's Comparison Test with 95% confidence level for determining antioxidant activity with DPPH Method.

General Linear Model: Results versus Sugar Type; SP Concentra; pH; FD/SD

```
Factor            Type   Levels  Values
Sugar Type        fixed       3  Dextrose; Fructose; Psicose
SP Concentration  fixed       5  50.00(1:1); 66.67(2:1); 75.00(3:1);
                                    83.33(5:1); 90.91(10:1)
pH                fixed       2  7; 10
FD/SD             fixed       2  1(FD); 2(SD)


Analysis of Variance for Results, using Adjusted SS for Tests

Source                                DF   Seq SS   Adj SS   Adj MS         F
Sugar Type                             2   636113   636113   318056   1668.13
SP Concentration                       4   153228   153228    38307    200.91
pH                                     1  4859665  4859665  4859665  25487.76
FD/SD                                  1   106970   106970   106970    561.03
Sugar Type*SP Concentration            8    54981    54981     6873     36.05
Sugar Type*pH                          2   153399   153399    76699    402.27
Sugar Type*FD/SD                       2    33004    33004    16502     86.55
SP Concentration*pH                    4    97794    97794    24449    128.23
SP Concentration*FD/SD                 4   375462   375462    93865    492.30
pH*FD/SD                               1     3009     3009     3009     15.78
Sugar Type*SP Concentration*pH         8   169581   169581    21198    111.18
Sugar Type*SP Concentration*FD/SD      8   138462   138462    17308     90.77
Sugar Type*pH*FD/SD                    2   102361   102361    51180    268.43
SP Concentration*pH*FD/SD              4   140007   140007    35002    183.58
Sugar Type*SP Concentration*pH*FD/SD   8   202214   202214    25277    132.57
Error                                120    22880    22880      191
Total                                179  7249129

Source                                   P
Sugar Type                           0.000
SP Concentration                     0.000
pH                                   0.000
FD/SD                                0.000
Sugar Type*SP Concentration          0.000
Sugar Type*pH                        0.000
Sugar Type*FD/SD                     0.000
SP Concentration*pH                  0.000
SP Concentration*FD/SD               0.000
pH*FD/SD                             0.000
Sugar Type*SP Concentration*pH       0.000
Sugar Type*SP Concentration*FD/SD    0.000
Sugar Type*pH*FD/SD                  0.000
SP Concentration*pH*FD/SD            0.000
```

Table C. 1. Continued.

```
Total


S = 13.8082   R-Sq = 99.68%   R-Sq(adj) = 99.53%


Unusual Observations for Results

Obs  Results     Fit  SE Fit  Residual  St Resid
 35   881.40  907.40    7.97    -26.00     -2.31 R
 95   945.40  914.73    7.97     30.67      2.72 R
 97   897.40  920.07    7.97    -22.67     -2.01 R
 99   943.40  920.07    7.97     23.33      2.07 R
103   535.40  512.07    7.97     23.33      2.07 R
143   581.40  551.40    7.97     30.00      2.66 R
144   521.40  551.40    7.97    -30.00     -2.66 R
151   463.40  494.73    7.97    -31.33     -2.78 R


R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95.0% Confidence

Sugar Type   N   Mean  Grouping
Psicose     60  797.0  A
Fructose    60  711.6    B
Dextrose    60  652.2      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

SP
Concentration   N   Mean  Grouping
83.33          36  758.7  A
75.00          36  746.8    B
66.67          36  718.2      C
90.91          36  695.1        D
50.00          36  682.4          E

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

pH   N   Mean  Grouping
10  90  884.6  A
 7  90  556.0    B

Means that do not share a letter are significantly different.


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

Table C. 1. Continued.

FD/SD	N	Mean	Grouping
1	90	744.6	A
2	90	695.9	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Sugar Type	SP Concentration	N	Mean	Grouping
Psicose	83.33	12	826.6	A
Psicose	75.00	12	819.7	A
Psicose	90.91	12	816.1	A
Psicose	66.67	12	772.7	B
Fructose	83.33	12	765.2	B C
Psicose	50.00	12	750.1	C D
Fructose	75.00	12	733.7	D E
Fructose	66.67	12	728.7	E
Dextrose	75.00	12	687.1	F
Dextrose	83.33	12	684.4	F
Fructose	50.00	12	668.6	F G
Fructose	90.91	12	661.6	G
Dextrose	66.67	12	653.2	G
Dextrose	50.00	12	628.6	H
Dextrose	90.91	12	607.7	I

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Sugar Type	pH	N	Mean	Grouping
Psicose	10	30	959.5	A
Fructose	10	30	912.5	B
Dextrose	10	30	781.7	C
Psicose	7	30	634.6	D
Dextrose	7	30	522.7	E
Fructose	7	30	510.6	F

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Sugar Type	FD/SD	N	Mean	Grouping
Psicose	1	30	830.6	A
Psicose	2	30	763.5	B
Fructose	1	30	716.8	C
Fructose	2	30	706.3	D
Dextrose	1	30	686.5	E
Dextrose	2	30	617.9	F

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Table C. 1. Continued.

SP Concentration	pH	N	Mean	Grouping
83.33	10	18	962.2	A
75.00	10	18	916.6	B
66.67	10	18	882.4	C
50.00	10	18	833.6	D
90.91	10	18	828.1	D
75.00	7	18	577.1	E
90.91	7	18	562.2	F
83.33	7	18	555.3	F
66.67	7	18	554.1	F
50.00	7	18	531.2	G

Table C. 1. Continued.

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

SP Concentration	FD/SD	N	Mean	Grouping
83.33	1	18	793.7	A
75.00	1	18	792.7	A
66.67	1	18	770.5	B
90.91	2	18	761.1	B
50.00	1	18	737.1	C
83.33	2	18	723.7	C
75.00	2	18	701.0	D
66.67	2	18	666.0	E
90.91	1	18	629.2	F
50.00	2	18	627.7	F

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

pH	FD/SD	N	Mean	Grouping
10	1	45	904.9	A
10	2	45	864.3	B
7	1	45	584.4	C
7	2	45	527.5	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Sugar Type	SP Concentration	pH	N	Mean	Grouping
Psicose	90.91	10	6	1024.7	A
Psicose	83.33	10	6	1021.1	A
Fructose	83.33	10	6	1002.1	A
Fructose	75.00	10	6	969.7	B
Psicose	75.00	10	6	968.1	B

Table C. 1. Continued.

Fructose	66.67	10	6	959.7	B
Psicose	66.67	10	6	897.4	C
Psicose	50.00	10	6	886.1	C D
Dextrose	83.33	10	6	863.4	D
Fructose	50.00	10	6	860.1	D
Dextrose	75.00	10	6	812.1	E
Dextrose	66.67	10	6	790.1	E F
Fructose	90.91	10	6	771.1	F G
Dextrose	50.00	10	6	754.7	G
Dextrose	90.91	10	6	688.4	H
Psicose	75.00	7	6	671.4	H I
Psicose	66.67	7	6	648.1	I J
Psicose	83.33	7	6	632.1	J K
Psicose	50.00	7	6	614.1	K
Psicose	90.91	7	6	607.4	K
Dextrose	75.00	7	6	562.1	L
Fructose	90.91	7	6	552.1	L M
Fructose	83.33	7	6	528.4	M N
Dextrose	90.91	7	6	527.1	M N O
Dextrose	66.67	7	6	516.4	N O
Dextrose	83.33	7	6	505.4	N O P
Dextrose	50.00	7	6	502.4	N O P
Fructose	75.00	7	6	497.7	O P
Fructose	66.67	7	6	497.7	O P
Fructose	50.00	7	6	477.1	P

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Sugar Type	SP Concentration	FD/SD	N	Mean
Psicose	75.00	1	6	876.7
Psicose	83.33	1	6	847.7
Psicose	90.91	1	6	824.7
Psicose	90.91	2	6	807.4
Psicose	83.33	2	6	805.4
Psicose	50.00	1	6	804.4
Psicose	66.67	1	6	799.4
Fructose	66.67	1	6	782.4
Fructose	83.33	1	6	774.4
Fructose	75.00	1	6	765.7
Psicose	75.00	2	6	762.7
Dextrose	83.33	1	6	759.1
Fructose	83.33	2	6	756.1
Fructose	90.91	2	6	755.4
Psicose	66.67	2	6	746.1
Dextrose	75.00	1	6	735.7
Dextrose	66.67	1	6	729.7
Dextrose	90.91	2	6	720.4
Dextrose	50.00	1	6	713.1
Fructose	75.00	2	6	701.7
Psicose	50.00	2	6	695.7
Fructose	50.00	1	6	693.7

Table C. 1. Continued.

Fructose	66.67	2	6	675.1
Fructose	50.00	2	6	643.4
Dextrose	75.00	2	6	638.4
Dextrose	83.33	2	6	609.7
Dextrose	66.67	2	6	576.7
Fructose	90.91	1	6	567.7
Dextrose	50.00	2	6	544.1
Dextrose	90.91	1	6	495.1

Sugar Type	SP Concentration	FD/SD	Grouping
Psicose	75.00	1	A
Psicose	83.33	1	A B
Psicose	90.91	1	B C
Psicose	90.91	2	C D
Psicose	83.33	2	C D
Psicose	50.00	1	C D E
Psicose	66.67	1	C D E
Fructose	66.67	1	D E F
Fructose	83.33	1	E F G
Fructose	75.00	1	F G H
Psicose	75.00	2	F G H
Dextrose	83.33	1	F G H I
Fructose	83.33	2	F G H I
Fructose	90.91	2	F G H I
Psicose	66.67	2	G H I J
Dextrose	75.00	1	H I J K
Dextrose	66.67	1	I J K L
Dextrose	90.91	2	J K L M
Dextrose	50.00	1	K L M
Fructose	75.00	2	L M N
Psicose	50.00	2	M N
Fructose	50.00	1	M N
Fructose	66.67	2	N
Fructose	50.00	2	O
Dextrose	75.00	2	O P
Dextrose	83.33	2	P
Dextrose	66.67	2	Q
Fructose	90.91	1	Q R
Dextrose	50.00	2	R
Dextrose	90.91	1	S

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Sugar Type	pH	FD/SD	N	Mean	Grouping
Psicose	10	1	15	964.9	A
Psicose	10	2	15	954.1	A
Fructose	10	2	15	919.8	B
Fructose	10	1	15	905.3	B
Dextrose	10	1	15	844.5	C
Dextrose	10	2	15	719.0	D
Psicose	7	1	15	696.3	E
Psicose	7	2	15	572.9	F
Dextrose	7	1	15	528.6	G

Table C. 1. Continued.

Dextrose	7	2	15	516.7	G
Fructose	7	2	15	492.9	H

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

SP Concentration	pH	FD/SD	N	Mean	Grouping
83.33	10	1	9	1021.8	A
75.00	10	1	9	974.3	B
90.91	10	2	9	947.4	C
66.67	10	1	9	946.5	C
83.33	10	2	9	902.5	D
50.00	10	1	9	873.0	E
75.00	10	2	9	859.0	E
66.67	10	2	9	818.3	F
50.00	10	2	9	794.3	G
90.91	10	1	9	708.7	H
75.00	7	1	9	611.2	I
50.00	7	1	9	601.2	I
66.67	7	1	9	594.5	I J
90.91	7	2	9	574.7	J K
83.33	7	1	9	565.6	K L
90.91	7	1	9	549.6	L
83.33	7	2	9	545.0	L
75.00	7	2	9	543.0	L
66.67	7	2	9	513.6	M
50.00	7	2	9	461.2	N

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Sugar Type	SP Concentration	pH	FD/SD	N	Mean
Psicose	90.91	10	1	3	1045.4
Psicose	83.33	10	1	3	1038.7
Fructose	66.67	10	1	3	1037.4
Fructose	75.00	10	1	3	1014.1
Dextrose	83.33	10	1	3	1013.4
Fructose	83.33	10	1	3	1013.4
Psicose	90.91	10	2	3	1004.1
Psicose	83.33	10	2	3	1003.4
Fructose	83.33	10	2	3	990.7
Psicose	75.00	10	1	3	988.7
Fructose	90.91	10	2	3	973.4
Psicose	75.00	10	2	3	947.4
Fructose	75.00	10	2	3	925.4
Dextrose	75.00	10	1	3	920.1
Dextrose	66.67	10	1	3	914.7
Psicose	50.00	10	2	3	908.1
Psicose	66.67	10	2	3	907.4
Fructose	50.00	10	1	3	892.7

Table C. 1. Continued.

Fructose	66.67	10	2	3	882.1
Dextrose	90.91	10	2	3	864.7
Psicose	50.00	10	1	3	864.1
Dextrose	50.00	10	1	3	862.1
Fructose	50.00	10	2	3	827.4
Psicose	75.00	7	1	3	764.7
Psicose	50.00	7	1	3	744.7
Dextrose	83.33	10	2	3	713.4
Psicose	66.67	7	1	3	711.4
Dextrose	75.00	10	2	3	704.1
Dextrose	66.67	10	2	3	665.4
Psicose	83.33	7	1	3	656.7
Dextrose	50.00	10	2	3	647.4
Psicose	90.91	7	2	3	610.7
Psicose	83.33	7	2	3	607.4
Psicose	90.91	7	1	3	604.1
Psicose	66.67	7	2	3	584.7
Psicose	75.00	7	2	3	578.1
Dextrose	90.91	7	2	3	576.1
Dextrose	75.00	7	2	3	572.7
Fructose	90.91	10	1	3	568.7
Fructose	90.91	7	1	3	566.7
Dextrose	50.00	7	1	3	564.1
Dextrose	75.00	7	1	3	551.4
Dextrose	66.67	7	1	3	544.7
Fructose	90.91	7	2	3	537.4
Fructose	83.33	7	1	3	535.4
Fructose	66.67	7	1	3	527.4
Fructose	83.33	7	2	3	521.4
Fructose	75.00	7	1	3	517.4
Dextrose	90.91	10	1	3	512.1
Dextrose	83.33	7	2	3	506.1
Dextrose	83.33	7	1	3	504.7
Fructose	50.00	7	1	3	494.7
Dextrose	66.67	7	2	3	488.1
Psicose	50.00	7	2	3	483.4
Dextrose	90.91	7	1	3	478.1
Fructose	75.00	7	2	3	478.1
Fructose	66.67	7	2	3	468.1
Fructose	50.00	7	2	3	459.4
Dextrose	50.00	7	2	3	440.7

Sugar Type	SP Concentration	pH	FD/SD	Grouping
Psicose	90.91	10	1	A
Psicose	83.33	10	1	A
Fructose	66.67	10	1	A B
Fructose	75.00	10	1	A B C
Dextrose	83.33	10	1	A B C
Fructose	83.33	10	1	A B C
Psicose	90.91	10	2	A B C
Psicose	83.33	10	2	A B C
Fructose	83.33	10	2	B C D
Psicose	75.00	10	1	C D
Fructose	90.91	10	2	C D
Psicose	75.00	10	2	D E
Fructose	75.00	10	2	E F

Table C. 1. Continued.

Dextrose	75.00	10	1	E F
Dextrose	66.67	10	1	E F
Psicose	50.00	10	2	E F G
Psicose	66.67	10	2	E F G
Fructose	50.00	10	1	F G
Psicose	66.67	10	1	F G
Fructose	66.67	10	2	F G
Dextrose	90.91	10	2	G H
Psicose	50.00	10	1	G H
Dextrose	50.00	10	1	G H
Fructose	50.00	10	2	H
Psicose	75.00	7	1	I
Psicose	50.00	7	1	I J
Dextrose	83.33	10	2	J
Psicose	66.67	7	1	J K
Dextrose	75.00	10	2	J K
Dextrose	66.67	10	2	K L
Psicose	83.33	7	1	L M
Dextrose	50.00	10	2	L M N
Psicose	90.91	7	2	M N O
Psicose	83.33	7	2	N O
Psicose	90.91	7	1	N O
Psicose	66.67	7	2	O P
Psicose	75.00	7	2	O P Q
Dextrose	90.91	7	2	O P Q
Dextrose	75.00	7	2	O P Q R
Fructose	90.91	10	1	O P Q R
Fructose	90.91	7	1	O P Q R S
Dextrose	50.00	7	1	O P Q R S T
Dextrose	75.00	7	1	P Q R S T U
Dextrose	66.67	7	1	P Q R S T U
Fructose	90.91	7	2	Q R S T U V
Fructose	83.33	7	1	Q R S T U V
Fructose	66.67	7	1	R S T U V W
Fructose	83.33	7	2	S T U V W X
Fructose	75.00	7	1	T U V W X
Dextrose	90.91	10	1	U V W X Y
Dextrose	83.33	7	2	U V W X Y Z
Dextrose	83.33	7	1	U V W X Y Z
Fructose	50.00	7	1	V W X Y Z
Dextrose	66.67	7	2	W X Y Z
Psicose	50.00	7	2	W X Y Z AA

Table C. 1. Continued.

```
Dextrose    90.91          7  1                                          X
Y Z AA
Fructose    75.00          7  2                                          X
Y Z AA
Fructose    66.67          7  2                                           Y
Z AA
Fructose    50.00          7  2                                           Z
AA
Dextrose    50.00          7  2                                          AA

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

Table C. 2. ANOVA and Tukey's Comparison Test with 95% confidence level for determining degree of glycation

General Linear Model: Result x1000 versus Sugar Type; SP Concentration

```
Factor            Type   Levels  Values
Sugar Type        fixed       3  Dextrose; Fructose; Psicose
SP Concentration  fixed       5  50.00; 66.67; 75.00; 83.33; 90.91
FD/SD             fixed       2  0(SD); 1(FD)
PH                fixed       2  7; 10


Analysis of Variance for Result x1000, using Adjusted SS for Tests

Source                                  DF   Seq SS  Adj SS  Adj MS        F
Sugar Type                               2    20718   20718   10359   2550.76
SP Concentration                         4    29970   29970    7492   1844.92
FD/SD                                    1   171866  171866  171866  42319.90
PH                                       1    91576   91576   91576  22549.38
Sugar Type*SP Concentration              8    11181   11181    1398    344.14
Sugar Type*FD/SD                         2   451482  451482  225741  55586.03
Sugar Type*PH                            2   114994  114994   57497  14157.97
SP Concentration*FD/SD                   4    36573   36573    9143   2251.42
SP Concentration*PH                      4     1248    1247     312     76.80
FD/SD*PH                                 1    18605   18605   18605   4581.26
Sugar Type*SP Concentration*FD/SD        8    49680   49680    6210   1529.13
Sugar Type*SP Concentration*PH           8    13799   13799    1725    424.72
Sugar Type*FD/SD*PH                      2   573525  573525  286763  70611.89
SP Concentration*FD/SD*PH                4     1594    1594     399     98.14
Sugar Type*SP Concentration*FD/SD*PH     8    81557   81557   10195   2510.31
Error                                  120      487     487       4
Total                                  179  1668854
Source                                      P
Sugar Type                             0.000
SP Concentration                       0.000
FD/SD                                  0.000
PH                                     0.000
Sugar Type*SP Concentration            0.000
Sugar Type*FD/SD                       0.000
```

Table C. 2. Continued.

```
Sugar Type*PH                           0.000
SP Concentration*FD/SD                  0.000
SP Concentration*PH                     0.000
FD/SD*PH                                0.000
Sugar Type*SP Concentration*FD/SD       0.000
Sugar Type*SP Concentration*PH          0.000
Sugar Type*FD/SD*PH                     0.000
SP Concentration*FD/SD*PH               0.000
Sugar Type*SP Concentration*FD/SD*PH    0.000
Error
Total


S = 2.01522   R-Sq = 99.97%   R-Sq(adj) = 99.96%


Unusual Observations for Result x1000

      Result
Obs    x1000      Fit  SE Fit  Residual  St Resid
 48  242.000  238.667   1.163     3.333      2.03 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95.0% Confidence

Sugar Type   N   Mean  Grouping
Dextrose    60  125.6  A
Fructose    60  124.4    B
Psicose     60  102.3      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

SP
Concentration   N   Mean  Grouping
90.91          36  140.5  A
83.33          36  120.9    B
75.00          36  112.6      C
50.00          36  110.1        D
66.67          36  103.0          E

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

FD/SD   N   Mean  Grouping
0      90  148.3  A
1      90   86.5    B
Means that do not share a letter are significantly different.
```

Table C. 2. Continued.

Grouping Information Using Tukey Method and 95.0% Confidence

PH	N	Mean	Grouping
10	90	140.0	A
7	90	94.9	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Sugar Type	SP Concentration	N	Mean	Grouping
Fructose	90.91	12	160.4	A
Dextrose	90.91	12	143.8	B
Fructose	83.33	12	135.4	C
Dextrose	83.33	12	125.4	D
Fructose	75.00	12	122.8	D E
Dextrose	50.00	12	122.3	E
Dextrose	75.00	12	122.1	E
Psicose	90.91	12	117.3	F
Dextrose	66.67	12	114.4	G
Psicose	50.00	12	108.3	H
Fructose	66.67	12	103.8	I
Psicose	83.33	12	102.0	I J
Fructose	50.00	12	99.5	J
Psicose	75.00	12	93.0	K
Psicose	66.67	12	90.7	K

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Sugar Type	FD/SD	N	Mean	Grouping
Fructose	0	30	213.3	A
Dextrose	1	30	158.9	B
Psicose	0	30	139.4	C
Dextrose	0	30	92.3	D
Psicose	1	30	65.1	E
Fructose	1	30	35.5	F

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Sugar Type	PH	N	Mean	Grouping
Dextrose	10	30	171.1	A
Psicose	10	30	137.1	B
Fructose	7	30	137.0	B
Fructose	10	30	111.7	C
Dextrose	7	30	80.1	D
Psicose	7	30	67.5	E

Means that do not share a letter are significantly different.

Table C. 2. Continued.

Grouping Information Using Tukey Method and 95.0% Confidence

SP Concentration	FD/SD	N	Mean	Grouping
90.91	0	18	194.4	A
83.33	0	18	158.3	B
75.00	0	18	142.3	C
66.67	0	18	124.6	D
50.00	0	18	122.0	E
50.00	1	18	98.1	F
90.91	1	18	86.7	G
83.33	1	18	83.6	H
75.00	1	18	82.9	H I
66.67	1	18	81.3	I

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

SP Concentration	PH	N	Mean	Grouping
90.91	10	18	166.7	A
83.33	10	18	145.4	B
75.00	10	18	135.1	C
50.00	10	18	131.1	D
66.67	10	18	121.6	E
90.91	7	18	114.4	F
83.33	7	18	96.4	G
75.00	7	18	90.1	H
50.00	7	18	89.0	H
66.67	7	18	84.4	I

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

FD/SD	PH	N	Mean	Grouping
0	10	45	160.7	A
0	7	45	135.9	B
1	10	45	119.2	C
1	7	45	53.8	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Sugar Type	SP Concentration	FD/SD	N	Mean
Fructose	90.91	0	6	297.2
Fructose	83.33	0	6	241.8
Fructose	75.00	0	6	216.5
Fructose	66.67	0	6	175.2
Dextrose	90.91	1	6	173.0
Psicose	90.91	0	6	171.3

Table C. 2. Continued.

Dextrose	83.33	1	6	165.7	
Dextrose	75.00	1	6	164.0	
Dextrose	66.67	1	6	155.5	
Psicose	83.33	0	6	148.0	
Dextrose	50.00	1	6	136.5	
Fructose	50.00	0	6	135.7	
Psicose	75.00	0	6	130.2	
Psicose	66.67	0	6	125.3	
Psicose	50.00	0	6	122.2	
Dextrose	90.91	0	6	114.7	
Dextrose	50.00	0	6	108.2	
Psicose	50.00	1	6	94.5	
Dextrose	83.33	0	6	85.2	
Dextrose	75.00	0	6	80.2	
Dextrose	66.67	0	6	73.3	
Psicose	90.91	1	6	63.3	
Fructose	50.00	1	6	63.3	
Psicose	83.33	1	6	56.0	
Psicose	66.67	1	6	56.0	
Psicose	75.00	1	6	55.8	
Fructose	66.67	1	6	32.5	
Fructose	75.00	1	6	29.0	
Fructose	83.33	1	6	29.0	
Fructose	90.91	1	6	23.7	

Sugar Type	SP Concentration	FD/SD	Grouping
Fructose	90.91	0	A
Fructose	83.33	0	B
Fructose	75.00	0	C
Fructose	66.67	0	D
Dextrose	90.91	1	D
Psicose	90.91	0	D
Dextrose	83.33	1	E
Dextrose	75.00	1	E
Dextrose	66.67	1	F
Psicose	83.33	0	G
Dextrose	50.00	1	H
Fructose	50.00	0	H
Psicose	75.00	0	I
Psicose	66.67	0	J
Psicose	50.00	0	J
Dextrose	90.91	0	K
Dextrose	50.00	0	L
Psicose	50.00	1	M
Dextrose	83.33	0	N
Dextrose	75.00	0	O
Dextrose	66.67	0	P
Psicose	90.91	1	Q
Fructose	50.00	1	Q
Psicose	83.33	1	R
Psicose	66.67	1	R
Psicose	75.00	1	R
Fructose	66.67	1	S
Fructose	75.00	1	S
Fructose	83.33	1	S
Fructose	90.91	1	T

Table C. 2. Continued.

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Sugar Type	SP Concentration	PH	N	Mean	Grouping
Dextrose	50.00	10	6	182.8	A
Dextrose	90.91	10	6	173.2	B
Dextrose	83.33	10	6	169.0	B C
Dextrose	75.00	10	6	166.7	C D
Psicose	90.91	10	6	165.0	C D E
Dextrose	66.67	10	6	164.0	D E
Fructose	90.91	10	6	161.8	E F
Fructose	90.91	7	6	159.0	F
Fructose	83.33	7	6	147.0	G
Psicose	83.33	10	6	143.5	G
Psicose	50.00	10	6	134.0	H
Fructose	75.00	7	6	132.0	H
Psicose	75.00	10	6	125.2	I
Fructose	66.67	7	6	124.7	I
Fructose	83.33	10	6	123.8	I
Fructose	50.00	7	6	122.5	I
Psicose	66.67	10	6	117.7	J
Dextrose	90.91	7	6	114.5	J
Fructose	75.00	10	6	113.5	J
Fructose	66.67	10	6	83.0	K
Psicose	50.00	7	6	82.7	K
Dextrose	83.33	7	6	81.8	K L
Dextrose	75.00	7	6	77.5	L M
Fructose	50.00	10	6	76.5	M
Psicose	90.91	7	6	69.7	N
Dextrose	66.67	7	6	64.8	O
Psicose	66.67	7	6	63.7	O
Dextrose	50.00	7	6	61.8	O
Psicose	75.00	7	6	60.8	O
Psicose	83.33	7	6	60.5	O

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Sugar Type	FD/SD	PH	N	Mean	Grouping
Dextrose	1	10	15	288.1	A
Psicose	0	10	15	227.7	B
Fructose	0	7	15	226.3	B
Fructose	0	10	15	200.3	C
Dextrose	0	7	15	130.5	D
Psicose	1	7	15	83.9	E
Dextrose	0	10	15	54.1	F
Psicose	0	7	15	51.1	G
Fructose	1	7	15	47.8	H
Psicose	1	10	15	46.4	H
Dextrose	1	7	15	29.7	I
Fructose	1	10	15	23.2	J

Table C. 2. Continued.

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

SP Concentration	FD/SD	PH	N	Mean	Grouping
90.91	0	10	9	213.3	A
90.91	0	7	9	175.4	B
83.33	0	10	9	172.2	B
75.00	0	10	9	153.8	C
83.33	0	7	9	144.4	D
50.00	0	10	9	136.1	E
75.00	0	7	9	130.8	F
66.67	0	10	9	128.1	F G
50.00	1	10	9	126.1	G
66.67	0	7	9	121.1	H
90.91	1	10	9	120.0	H
83.33	1	10	9	118.7	H I
75.00	1	10	9	116.4	I J
66.67	1	10	9	115.0	J
50.00	0	7	9	107.9	K
50.00	1	7	9	70.1	L
90.91	1	7	9	53.3	M
75.00	1	7	9	49.4	N
83.33	1	7	9	48.4	N
66.67	1	7	9	47.7	N

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Sugar Type	SP Concentration	FD/SD	PH	N	Mean
Dextrose	90.91	1	10	3	321.0
Psicose	90.91	0	10	3	309.3
Dextrose	83.33	1	10	3	307.0
Fructose	90.91	0	10	3	305.3
Dextrose	75.00	1	10	3	295.0
Fructose	90.91	0	7	3	289.0
Dextrose	66.67	1	10	3	279.0
Psicose	83.33	0	10	3	258.0
Fructose	83.33	0	7	3	256.0
Dextrose	50.00	1	10	3	238.7
Fructose	75.00	0	7	3	228.0
Fructose	83.33	0	10	3	227.7
Psicose	75.00	0	10	3	218.0
Fructose	66.67	0	7	3	207.3
Fructose	75.00	0	10	3	205.0
Dextrose	90.91	0	7	3	204.0
Psicose	66.67	0	10	3	192.3
Psicose	50.00	0	10	3	161.0
Fructose	50.00	0	7	3	151.0
Fructose	66.67	0	10	3	143.0
Dextrose	83.33	0	7	3	139.3
Dextrose	50.00	0	10	3	127.0

Table C. 2. Continued.

Dextrose	75.00	0	7	3	122.0
Fructose	50.00	0	10	3	120.3
Psicose	50.00	1	10	3	107.0
Psicose	90.91	1	7	3	106.0
Dextrose	66.67	0	7	3	97.7
Fructose	50.00	1	7	3	94.0
Dextrose	50.00	0	7	3	89.3
Psicose	50.00	0	7	3	83.3
Psicose	83.33	1	7	3	83.0
Psicose	50.00	1	7	3	82.0
Psicose	75.00	1	7	3	79.3
Psicose	66.67	1	7	3	69.0
Psicose	66.67	0	7	3	58.3
Dextrose	66.67	0	10	3	49.0
Psicose	66.67	1	10	3	43.0
Psicose	75.00	0	7	3	42.3
Fructose	66.67	1	7	3	42.0
Dextrose	75.00	0	10	3	38.3
Psicose	83.33	0	7	3	38.0
Fructose	83.33	1	7	3	38.0
Fructose	75.00	1	7	3	36.0
Dextrose	50.00	1	7	3	34.3
Psicose	90.91	0	7	3	33.3
Dextrose	75.00	1	7	3	33.0
Fructose	50.00	1	10	3	32.7
Psicose	75.00	1	10	3	32.3
Dextrose	66.67	1	7	3	32.0
Dextrose	83.33	0	10	3	31.0
Psicose	83.33	1	10	3	29.0
Fructose	90.91	1	7	3	29.0
Dextrose	90.91	0	10	3	25.3
Dextrose	90.91	1	7	3	25.0
Dextrose	83.33	1	7	3	24.3
Fructose	66.67	1	10	3	23.0
Fructose	75.00	1	10	3	22.0
Psicose	90.91	1	10	3	20.7
Fructose	83.33	1	10	3	20.0
Fructose	90.91	1	10	3	18.3

Sugar Type	SP Concentration	FD/SD	PH	Grouping
Dextrose	90.91	1	10	A
Psicose	90.91	0	10	B
Dextrose	83.33	1	10	B
Fructose	90.91	0	10	B
Dextrose	75.00	1	10	C
Fructose	90.91	0	7	C
Dextrose	66.67	1	10	D
Psicose	83.33	0	10	E
Fructose	83.33	0	7	E
Dextrose	50.00	1	10	F
Fructose	75.00	0	7	G
Fructose	83.33	0	10	G
Psicose	75.00	0	10	H
Fructose	66.67	0	7	I
Fructose	75.00	0	10	I
Dextrose	90.91	0	7	I

Table C. 2. Continued.

Psicose	66.67	0	10	J
Psicose	50.00	0	10	K
Fructose	50.00	0	7	L
Fructose	66.67	0	10	M
Dextrose	83.33	0	7	M
Dextrose	50.00	0	10	N
Dextrose	75.00	0	7	N
Fructose	50.00	0	10	N
Psicose	50.00	1	10	O
Psicose	90.91	1	7	O
Dextrose	66.67	0	7	P
Fructose	50.00	1	7	P Q
Dextrose	50.00	0	7	Q R
Psicose	50.00	0	7	R S
Psicose	83.33	1	7	R S
Psicose	50.00	1	7	S
Psicose	75.00	1	7	S
Psicose	66.67	1	7	T
Psicose	66.67	0	7	U
Dextrose	66.67	0	10	V
Psicose	66.67	1	10	V W
Psicose	75.00	0	7	V W X
Fructose	66.67	1	7	W X
Dextrose	75.00	0	10	W X Y
Psicose	83.33	0	7	W X Y
Fructose	83.33	1	7	W X Y
Fructose	75.00	1	7	X Y Z
Dextrose	50.00	1	7	Y Z AA
Psicose	90.91	0	7	Y Z AA
Dextrose	75.00	1	7	Y Z AA
Fructose	50.00	1	10	Y Z AA
Psicose	75.00	1	10	Y Z AA
Dextrose	66.67	1	7	Y Z AA AB
Dextrose	83.33	0	10	Z AA AB AC
Psicose	83.33	1	10	AA AB AC AD
Fructose	90.91	1	7	AA AB AC AD
Dextrose	90.91	0	10	AB AC AD AE
Dextrose	90.91		1	7 AC AD AE AF

Table C. 2. Continued.

Dextrose AC AD AE AF	83.33	1	7
Fructose AD AE AF	66.67	1	10
Fructose AE AF	75.00	1	10
Psicose AE AF	90.91	1	10
Fructose AE AF	83.33	1	10
Fructose AF	90.91	1	10

Means that do not share a letter are significantly different.

Table C. 3. ANOVA and Tukey's Comparison Test with 95% confidence level for determining emulsification activity

General Linear Model: Percentage E versus Sugar Type; SP Concentration

Factor	Type	Levels	Values
Sugar Type	fixed	3	Dextrose; Fructose; Psicose
SP Concentration	fixed	5	50.00; 66.67; 75.00; 83.33; 90.91
FD/SD	fixed	2	1(FD); 2(SD)
PH	fixed	2	7; 10

Analysis of Variance for Percentage Emulsion Activity, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F
Sugar Type	2	161.49	161.49	80.75	18.87
SP Concentration	4	84.19	84.19	21.05	4.92
FD/SD	1	1386.11	1386.11	1386.11	323.96
PH	1	761.76	761.76	761.76	178.04
Sugar Type*SP Concentration	8	93.28	93.28	11.66	2.73
Sugar Type*FD/SD	2	601.19	601.19	300.59	70.25
Sugar Type*PH	2	1193.96	1193.96	596.98	139.53
SP Concentration*FD/SD	4	58.83	58.83	14.71	3.44
SP Concentration*PH	4	208.46	208.46	52.12	12.18
FD/SD*PH	1	1088.76	1088.76	1088.76	254.47
Sugar Type*SP Concentration*FD/SD	8	233.36	233.36	29.17	6.82
Sugar Type*SP Concentration*PH	8	131.26	131.26	16.41	3.83
Sugar Type*FD/SD*PH	2	814.66	814.66	407.33	95.20
SP Concentration*FD/SD*PH	4	27.55	27.55	6.89	1.61
Sugar Type*SP Concentration*FD/SD*PH	8	181.79	181.79	22.72	5.31
Error	120	513.43	513.43	4.28	
Total	179	7540.08			

Source	P
Sugar Type	0.000
SP Concentration	0.001

Table C. 3. Continued.

```
FD/SD                                   0.000
PH                                      0.000
Sugar Type*SP Concentration             0.009
Sugar Type*FD/SD                        0.000
Sugar Type*PH                           0.000
SP Concentration*FD/SD                  0.011
SP Concentration*PH                     0.000
FD/SD*PH                                0.000
Sugar Type*SP Concentration*FD/SD       0.000
Sugar Type*SP Concentration*PH          0.000
Sugar Type*FD/SD*PH                     0.000
SP Concentration*FD/SD*PH               0.176
Sugar Type*SP Concentration*FD/SD*PH    0.000
Error
Total


S = 2.06848   R-Sq = 93.19%   R-Sq(adj) = 89.84%


Unusual Observations for Percentage Emulsion Activity

     Percentage
       Emulsion
Obs    Activity      Fit  SE Fit  Residual  St Resid
 47     89.2857  84.3034  1.1942    4.9824      2.95 R
 49     82.6923  86.2061  1.1942   -3.5138     -2.08 R
 61     77.2727  80.9181  1.1942   -3.6453     -2.16 R
 65     85.1852  89.8310  1.1942   -4.6458     -2.75 R
 70     92.3077  87.3694  1.1942    4.9383      2.92 R
103     88.8889  85.1852  1.1942    3.7037      2.19 R
104     81.4815  85.1852  1.1942   -3.7037     -2.19 R
155     74.0741  79.0123  1.1942   -4.9383     -2.92 R
163     88.4615  83.8082  1.1942    4.6534      2.76 R
166     87.5000  83.9286  1.1942    3.5714      2.11 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95.0% Confidence

Sugar Type   N  Mean  Grouping
Fructose    60  84.8  A
Psicose     60  83.2    B
Dextrose    60  82.6    B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

SP
Concentration   N  Mean  Grouping
50.00          36  84.2  A
75.00          36  84.2  A
83.33          36  84.0  A
90.91          36  82.9  A B
```

Table C. 3. Continued.

```
66.67          36  82.6    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

FD/SD   N  Mean  Grouping
2      90  86.3  A
1      90  80.8    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

PH   N  Mean  Grouping
10  90  85.6  A
 7  90  81.5    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

            SP
Sugar Type  Concentration   N  Mean  Grouping
Fructose    83.33          12  86.5  A
Fructose    75.00          12  85.4  A B
Psicose     50.00          12  84.6  A B C
Fructose    50.00          12  84.4  A B C
Fructose    66.67          12  84.3  A B C
Psicose     75.00          12  83.6  A B C
Dextrose    50.00          12  83.6  A B C
Fructose    90.91          12  83.6  A B C
Dextrose    75.00          12  83.4    B C
Psicose     66.67          12  82.9    B C D
Dextrose    90.91          12  82.9    B C D
Psicose     83.33          12  82.8    B C D
Dextrose    83.33          12  82.6    B C D
Psicose     90.91          12  82.3      C D
Dextrose    66.67          12  80.4        D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Sugar Type  FD/SD   N  Mean  Grouping
Dextrose    2      30  87.1  A
Psicose     2      30  86.8  A
Fructose    2      30  85.1    B
Fructose    1      30  84.6    B
Psicose     1      30  79.7      C
Dextrose    1      30  78.0        D

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

Table C. 3. Continued.

Grouping Information Using Tukey Method and 95.0% Confidence

Sugar Type	PH	N	Mean	Grouping
Dextrose	10	30	88.3	A
Fructose	7	30	85.0	B
Fructose	10	30	84.7	B
Psicose	10	30	83.9	B C
Psicose	7	30	82.6	C
Dextrose	7	30	76.9	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

SP Concentration	FD/SD	N	Mean	Grouping
50.00	2	18	87.6	A
83.33	2	18	87.2	A
75.00	2	18	86.2	A B
90.91	2	18	85.9	A B
66.67	2	18	84.7	B
75.00	1	18	82.1	C
83.33	1	18	80.7	C D
50.00	1	18	80.7	C D
66.67	1	18	80.4	C D
90.91	1	18	79.9	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

SP Concentration	PH	N	Mean	Grouping
83.33	10	18	87.4	A
75.00	10	18	86.4	A B
90.91	10	18	85.3	A B C
66.67	10	18	84.6	B C
50.00	10	18	84.3	B C
50.00	7	18	84.1	C D
75.00	7	18	81.9	D E
83.33	7	18	80.5	E
90.91	7	18	80.5	E
66.67	7	18	80.5	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

FD/SD	PH	N	Mean	Grouping
2	7	45	86.7	A
2	10	45	85.9	A B
1	10	45	85.3	B
1	7	45	76.3	C

Table C. 3. Continued.

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Sugar Type	SP Concentration	FD/SD	N	Mean	Grouping
Dextrose	50.00	2	6	89.5	A
Psicose	83.33	2	6	88.1	A B
Psicose	66.67	2	6	87.9	A B
Dextrose	75.00	2	6	87.9	A B
Dextrose	83.33	2	6	87.1	A B
Dextrose	90.91	2	6	87.0	A B
Psicose	50.00	2	6	87.0	A B
Fructose	83.33	1	6	86.6	A B C
Fructose	66.67	1	6	86.6	A B C
Fructose	50.00	2	6	86.4	A B C D
Fructose	83.33	2	6	86.3	A B C D
Psicose	90.91	2	6	85.5	A B C D
Fructose	75.00	2	6	85.5	A B C D
Fructose	75.00	1	6	85.4	A B C D
Psicose	75.00	2	6	85.2	A B C D
Fructose	90.91	2	6	85.2	A B C D
Dextrose	66.67	2	6	84.2	B C D
Fructose	50.00	1	6	82.3	C D E
Psicose	50.00	1	6	82.1	C D E F
Fructose	66.67	2	6	82.1	C D E F
Psicose	75.00	1	6	82.0	D E F G
Fructose	90.91	1	6	81.9	D E F G
Psicose	90.91	1	6	79.0	E F G H
Dextrose	75.00	1	6	79.0	E F G H
Dextrose	90.91	1	6	78.7	E F G H
Dextrose	83.33	1	6	78.1	E F G H
Psicose	66.67	1	6	78.0	E F G H
Dextrose	50.00	1	6	77.7	F G H
Psicose	83.33	1	6	77.5	G H
Dextrose	66.67	1	6	76.6	H

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Sugar Type	SP Concentration	PH	N	Mean	Grouping
Dextrose	90.91	10	6	90.4	A
Dextrose	75.00	10	6	90.3	A
Dextrose	83.33	10	6	89.2	A B
Fructose	83.33	10	6	87.4	A B C
Dextrose	50.00	10	6	86.0	A B C D
Psicose	50.00	7	6	85.9	A B C D
Fructose	75.00	7	6	85.7	B C D E
Psicose	83.33	10	6	85.6	B C D E
Fructose	83.33	7	6	85.5	B C D E
Dextrose	66.67	10	6	85.3	B C D E
Fructose	75.00	10	6	85.2	B C D E
Fructose	50.00	7	6	85.1	B C D E

Table C. 3. Continued.

Fructose	66.67	10	6	84.4	C D E F
Fructose	90.91	7	6	84.3	C D E F
Fructose	66.67	7	6	84.3	C D E F
Psicose	66.67	10	6	84.2	C D E F
Psicose	75.00	10	6	83.7	C D E F
Fructose	50.00	10	6	83.7	C D E F
Psicose	75.00	7	6	83.5	C D E F
Psicose	50.00	10	6	83.3	C D E F
Fructose	90.91	10	6	82.8	C D E F
Psicose	90.91	10	6	82.7	D E F
Psicose	90.91	7	6	81.9	D E F
Psicose	66.67	7	6	81.7	D E F
Dextrose	50.00	7	6	81.2	E F
Psicose	83.33	7	6	80.0	F G
Dextrose	75.00	7	6	76.5	G H
Dextrose	83.33	7	6	76.0	G H
Dextrose	66.67	7	6	75.5	G H
Dextrose	90.91	7	6	75.3	H

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Sugar Type	FD/SD	PH	N	Mean	Grouping
Dextrose	1	10	15	89.2	A
Dextrose	2	10	15	87.3	A B
Psicose	2	7	15	87.1	A B
Dextrose	2	7	15	86.9	A B
Psicose	2	10	15	86.5	B C
Fructose	2	7	15	86.2	B C D
Fructose	1	10	15	85.4	B C D
Fructose	2	10	15	84.0	C D
Fructose	1	7	15	83.8	D E
Psicose	1	10	15	81.3	E
Psicose	1	7	15	78.1	F
Dextrose	1	7	15	66.9	G

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

SP Concentration	FD/SD	PH	N	Mean	Grouping
50.00	2	7	9	89.3	A
83.33	2	10	9	88.2	A B
75.00	1	10	9	87.0	A B C
75.00	2	7	9	86.6	A B C
83.33	1	10	9	86.6	A B C
83.33	2	7	9	86.1	A B C D
90.91	2	7	9	86.1	A B C D
50.00	2	10	9	86.0	A B C D
75.00	2	10	9	85.8	A B C D
90.91	2	10	9	85.8	A B C D
66.67	2	7	9	85.6	B C D
66.67	1	10	9	85.4	B C D

Table C. 3. Continued.

90.91	1	10	9	84.9			B	C	D													
66.67	2	10	9	83.9				C	D													
50.00	1	10	9	82.6					D													
50.00	1	7	9	78.8						E												
75.00	1	7	9	77.3						E	F											
66.67	1	7	9	75.4						E	F											
90.91	1	7	9	74.9							F											
83.33	1	7	9	74.9							F											

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Sugar Type	SP Concentration	FD/SD	PH	N	Mean	Grouping									
Dextrose	83.33	1	10	3	92.1	A									
Dextrose	90.91	1	10	3	92.0	A									
Dextrose	75.00	1	10	3	91.4	A	B								
Dextrose	50.00	2	7	3	91.3	A	B								
Psicose	83.33	2	10	3	90.9	A	B	C							
Psicose	50.00	2	7	3	90.1	A	B	C	D						
Fructose	66.67	1	10	3	89.8	A	B	C	D						
Dextrose	75.00	2	10	3	89.3	A	B	C	D	E					
Dextrose	90.91	2	10	3	88.9	A	B	C	D	E	F				
Psicose	66.67	2	10	3	88.2	A	B	C	D	E	F	G			
Dextrose	83.33	2	7	3	87.8	A	B	C	D	E	F	G	H		
Psicose	66.67	2	7	3	87.7	A	B	C	D	E	F	G	H		
Dextrose	50.00	2	10	3	87.7	A	B	C	D	E	F	G	H		
Fructose	75.00	2	7	3	87.7	A	B	C	D	E	F	G	H		
Fructose	83.33	1	10	3	87.4	A	B	C	D	E	F	G	H		
Fructose	83.33	2	10	3	87.4	A	B	C	D	E	F	G	H		
Fructose	75.00	1	10	3	87.0	A	B	C	D	E	F	G	H	I	
Fructose	90.91	2	7	3	86.6	A	B	C	D	E	F	G	H	I	
Psicose	90.91	2	7	3	86.4	A	B	C	D	E	F	G	H	I	
Fructose	50.00	2	10	3	86.4	A	B	C	D	E	F	G	H	I	
Dextrose	83.33	2	10	3	86.4	A	B	C	D	E	F	G	H	I	
Dextrose	75.00	2	7	3	86.4	A	B	C	D	E	F	G	H	I	
Fructose	50.00	2	7	3	86.4	A	B	C	D	E	F	G	H	I	
Dextrose	66.67	1	10	3	86.2	A	B	C	D	E	F	G	H	I	
Fructose	83.33	1	7	3	85.9	A	B	C	D	E	F	G	H	I	J
Psicose	75.00	2	7	3	85.7	A	B	C	D	E	F	G	H	I	J
Psicose	83.33	2	7	3	85.4	A	B	C	D	E	F	G	H	I	J
Dextrose	90.91	2	7	3	85.2	A	B	C	D	E	F	G	H	I	J
Fructose	83.33	2	7	3	85.2	A	B	C	D	E	F	G	H	I	J
Fructose	66.67	2	7	3	85.2	A	B	C	D	E	F	G	H	I	J
Psicose	75.00	2	10	3	84.8		B	C	D	E	F	G	H	I	J
Psicose	90.91	2	10	3	84.6		B	C	D	E	F	G	H	I	J
Dextrose	66.67	2	10	3	84.4		B	C	D	E	F	G	H	I	J
Dextrose	50.00	1	10	3	84.3		B	C	D	E	F	G	H	I	J K
Dextrose	66.67	2	7	3	84.0			C	D	E	F	G	H	I	J K
Psicose	50.00	2	10	3	83.9			C	D	E	F	G	H	I	J K
Fructose	90.91	2	10	3	83.8			C	D	E	F	G	H	I	J K
Fructose	75.00	1	7	3	83.8			C	D	E	F	G	H	I	J K
Fructose	50.00	1	7	3	83.8				D	E	F	G	H	I	J K
Fructose	66.67	1	7	3	83.4				D	E	F	G	H	I	J K
Fructose	75.00	2	10	3	83.3				D	E	F	G	H	I	J K

Table C. 3. Continued.

Psicose	75.00	1	10	3	82.7			E	F	G	H	I	J	K	L	
Psicose	50.00	1	10	3	82.6			E	F	G	H	I	J	K	L	
Fructose	90.91	1	7	3	82.1				F	G	H	I	J	K	L	
Fructose	90.91	1	10	3	81.8				F	G	H	I	J	K	L	
Psicose	50.00	1	7	3	81.6					G	H	I	J	K	L	M
Psicose	75.00	1	7	3	81.3					G	H	I	J	K	L	M
Fructose	50.00	1	10	3	80.9						H	I	J	K	L	M
Psicose	90.91	1	10	3	80.8						H	I	J	K	L	M
Psicose	83.33	1	10	3	80.3							I	J	K	L	M
Psicose	66.67	1	10	3	80.2							I	J	K	L	M
Fructose	66.67	2	10	3	79.0								J	K	L	M
Psicose	90.91	1	7	3	77.3									K	L	M	N
Psicose	66.67	1	7	3	75.7										L	M	N
Psicose	83.33	1	7	3	74.6											M	N
Dextrose	50.00	1	7	3	71.2												N	O
Dextrose	66.67	1	7	3	67.1													O
Dextrose	75.00	1	7	3	66.7													O
Dextrose	90.91	1	7	3	65.4													O
Dextrose	83.33	1	7	3	64.2													O

Means that do not share a letter are significantly different.

Table C. 4. ANOVA and Tukey's Comparison Test with 95% confidence level for determining solubility of the glycated soy protein

General Linear Model: Results versus Sugar Type; SP Concentra; pH; FD/SD

Factor	Type	Levels	Values
Sugar Type	fixed	3	Dextrose; Fructose; Psicose
SP Concentration	fixed	5	50,00; 66,67; 75,00; 83,33; 90,91
pH	fixed	2	7; 10
FD/SD	fixed	2	FD; SD

Analysis of Variance for Results, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS
Sugar Type	2	117,977	117,977	58,989
SP Concentration	4	1620,978	1620,978	405,244
pH	1	1283,258	1283,258	1283,258
FD/SD	1	12,142	12,142	12,142
Sugar Type*SP Concentration	8	244,244	244,244	30,530
Sugar Type*pH	2	296,604	296,604	148,302
Sugar Type*FD/SD	2	417,964	417,964	208,982
SP Concentration*pH	4	59,385	59,385	14,846
SP Concentration*FD/SD	4	307,336	307,336	76,834
pH*FD/SD	1	32,900	32,900	32,900
Sugar Type*SP Concentration*pH	8	298,640	298,640	37,330
Sugar Type*SP Concentration*FD/SD	8	82,392	82,392	10,299

Table C. 4. Continued.

```
Sugar Type*pH*FD/SD                      2    69,739    69,739    34,869
SP Concentration*pH*FD/SD                4    36,800    36,800     9,200
Sugar Type*SP Concentration*pH*FD/SD     8   197,690   197,690    24,711
Error                                  120    36,286    36,286     0,302
Total                                  179  5114,333

Source                                        F      P
Sugar Type                             195,08  0,000
SP Concentration                      1340,18  0,000
pH                                    4243,85  0,000
FD/SD                                   40,16  0,000
Sugar Type*SP Concentration            100,97  0,000
Sugar Type*pH                          490,45  0,000
Sugar Type*FD/SD                       691,12  0,000
SP Concentration*pH                     49,10  0,000
SP Concentration*FD/SD                 254,10  0,000
pH*FD/SD                               108,80  0,000
Sugar Type*SP Concentration*pH         123,45  0,000
Sugar Type*SP Concentration*FD/SD       34,06  0,000
Sugar Type*pH*FD/SD                    115,32  0,000
SP Concentration*pH*FD/SD               30,43  0,000
Sugar Type*SP Concentration*pH*FD/SD    81,72  0,000
Error
Total


S = 0,549891   R-Sq = 99,29%   R-Sq(adj) = 98,94%


Unusual Observations for Results

Obs  Results      Fit  SE Fit  Residual  St Resid
  1  12,2772  11,3437  0,3175    0,9335      2,08 R
 61  10,8532  11,9133  0,3175   -1,0601     -2,36 R
 73  13,9462  12,8061  0,3175    1,1401      2,54 R
152  27,2529  25,3780  0,3175    1,8749      4,18 R
153  23,2420  25,3780  0,3175   -2,1360     -4,76 R
156  17,3516  18,2530  0,3175   -0,9014     -2,01 R
163  12,8235  14,1420  0,3175   -1,3185     -2,94 R
167  27,4190  29,4205  0,3175   -2,0015     -4,46 R
168  31,0027  29,4205  0,3175    1,5822      3,52 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Sugar Type   N  Mean  Grouping
Fructose    60  11,9  A
Psicose     60  11,4     B
Dextrose    60  10,0        C

Means that do not share a letter are significantly different.


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

Table C. 4. Continued.

```
SP
Concentration   N  Mean  Grouping
50,00          36  17,0  A
66,67          36  10,5    B
75,00          36  10,2    B
90,91          36   9,4      C
83,33          36   8,6        D

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

pH   N  Mean  Grouping
 7  90  13,8  A
10  90   8,4    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

FD/SD   N  Mean  Grouping
FD     90  11,4  A
SD     90  10,9    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

            SP
Sugar Type  Concentration   N  Mean  Grouping
Psicose     50,00          12  19,3  A
Fructose    50,00          12  17,0    B
Dextrose    50,00          12  14,6      C
Fructose    66,67          12  12,4        D
Fructose    75,00          12  11,7        D E
Psicose     90,91          12  10,9          E F
Dextrose    75,00          12  10,7            F
Psicose     66,67          12  10,3            F
Fructose    83,33          12   9,5              G
Fructose    90,91          12   9,0              G H
Dextrose    66,67          12   8,7                H I
Psicose     83,33          12   8,5                H I J
Dextrose    90,91          12   8,3                H I J
Psicose     75,00          12   8,1                  I J
Dextrose    83,33          12   7,8                    J

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Sugar Type  pH   N  Mean  Grouping
Psicose      7  30  15,3  A
Fructose     7  30  15,1  A
```

Table C. 4. Continued.

```
Dextrose     7  30  10,9    B
Dextrose    10  30   9,1      C
Fructose    10  30   8,7        D
Psicose     10  30   7,6          E

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Sugar Type  FD/SD   N  Mean  Grouping
Fructose    FD     30  14,1  A
Psicose     FD     30  11,5    B
Dextrose    SD     30  11,5    B
Psicose     SD     30  11,4    B
Fructose    SD     30   9,7      C
Dextrose    FD     30   8,5        D

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

SP
Concentration  pH   N  Mean  Grouping
50,00           7  18  20,5  A
50,00          10  18  13,5    B
75,00           7  18  13,1    B
66,67           7  18  12,3      C
90,91           7  18  12,2      C
83,33           7  18  10,8        D
66,67          10  18   8,6          E
75,00          10  18   7,2            F
90,91          10  18   6,6              G
83,33          10  18   6,4              G

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

SP
Concentration  FD/SD   N  Mean  Grouping
50,00          FD     18  18,5  A
50,00          SD     18  15,4    B
66,67          FD     18  11,6      C
90,91          SD     18  11,4      C
75,00          FD     18  11,1      C
66,67          SD     18   9,3        D
75,00          SD     18   9,2        D
83,33          SD     18   9,0        D
83,33          FD     18   8,2          E
90,91          FD     18   7,4            F

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

Table C. 4. Continued.

Grouping Information Using Tukey Method and 95,0% Confidence

pH	FD/SD	N	Mean	Grouping
7	SD	45	14,0	A
7	FD	45	13,6	B
10	FD	45	9,1	C
10	SD	45	7,8	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Sugar Type	SP Concentration	pH	N	Mean	Grouping
Psicose	50,00	7	6	26,8	A
Fructose	50,00	7	6	18,6	B
Dextrose	50,00	7	6	16,0	C
Fructose	75,00	7	6	15,8	C
Fructose	50,00	10	6	15,4	C
Fructose	66,67	7	6	15,1	C
Psicose	90,91	7	6	15,0	C
Fructose	90,91	7	6	13,5	D
Dextrose	75,00	7	6	13,4	D
Dextrose	50,00	10	6	13,2	D
Psicose	66,67	7	6	12,9	D E
Fructose	83,33	7	6	12,7	D E
Psicose	83,33	7	6	11,8	E
Psicose	50,00	10	6	11,8	E
Psicose	75,00	7	6	10,1	F
Fructose	66,67	10	6	9,8	F
Dextrose	66,67	7	6	9,0	F G
Dextrose	90,91	10	6	8,4	G H
Dextrose	66,67	10	6	8,4	G H
Dextrose	90,91	7	6	8,1	G H I
Dextrose	75,00	10	6	8,0	G H I
Dextrose	83,33	7	6	8,0	G H I
Psicose	66,67	10	6	7,7	H I
Dextrose	83,33	10	6	7,6	H I
Fructose	75,00	10	6	7,5	H I J
Psicose	90,91	10	6	6,9	I J K
Fructose	83,33	10	6	6,3	J K L
Psicose	75,00	10	6	6,2	K L
Psicose	83,33	10	6	5,2	L M
Fructose	90,91	10	6	4,4	M

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Sugar Type	SP Concentration	FD/SD	N	Mean	Grouping
Fructose	50,00	FD	6	21,3	A
Psicose	50,00	FD	6	21,1	A
Psicose	50,00	SD	6	17,5	B
Dextrose	50,00	SD	6	16,0	C

Table C. 4. Continued.

Fructose	66,67	FD	6	15,2	C D
Psicose	90,91	SD	6	14,5	D E
Fructose	75,00	FD	6	13,6	E F
Dextrose	50,00	FD	6	13,2	F
Fructose	50,00	SD	6	12,7	F G
Psicose	66,67	FD	6	11,6	G H
Dextrose	90,91	SD	6	11,3	H
Dextrose	75,00	SD	6	10,9	H I
Fructose	83,33	FD	6	10,8	H I J
Dextrose	75,00	FD	6	10,5	H I J K
Dextrose	83,33	SD	6	9,9	I J K L
Fructose	75,00	SD	6	9,7	J K L
Fructose	90,91	FD	6	9,6	J K L
Fructose	66,67	SD	6	9,6	J K L
Psicose	75,00	FD	6	9,3	K L M
Dextrose	66,67	SD	6	9,3	K L M
Psicose	66,67	SD	6	9,0	L M N
Psicose	83,33	SD	6	8,9	L M N
Fructose	90,91	SD	6	8,3	M N O
Fructose	83,33	SD	6	8,2	M N O
Psicose	83,33	FD	6	8,1	M N O P
Dextrose	66,67	FD	6	8,1	N O P
Psicose	90,91	FD	6	7,4	O P
Psicose	75,00	SD	6	6,9	P
Dextrose	83,33	FD	6	5,6	Q
Dextrose	90,91	FD	6	5,2	Q

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Sugar Type	pH	FD/SD	N	Mean	Grouping
Fructose	7	FD	15	17,8	A
Psicose	7	SD	15	16,1	B
Psicose	7	FD	15	14,6	C
Dextrose	7	SD	15	13,3	D
Fructose	7	SD	15	12,5	E
Fructose	10	FD	15	10,4	F
Dextrose	10	SD	15	9,7	G
Dextrose	10	FD	15	8,5	H
Dextrose	7	FD	15	8,5	H
Psicose	10	FD	15	8,5	H
Fructose	10	SD	15	6,9	I
Psicose	10	SD	15	6,7	I

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

SP Concentration	pH	FD/SD	N	Mean	Grouping
50,00	7	FD	9	22,0	A
50,00	7	SD	9	18,9	B
50,00	10	FD	9	15,1	C
90,91	7	SD	9	15,1	C

Table C. 4. Continued.

75,00	7	FD	9	14,3	C
66,67	7	FD	9	13,1	D
83,33	7	SD	9	12,2	D E
75,00	7	SD	9	12,0	E
50,00	10	SD	9	11,9	E
66,67	7	SD	9	11,6	E
66,67	10	FD	9	10,2	F
83,33	7	FD	9	9,4	F
90,91	7	FD	9	9,3	F
75,00	10	FD	9	8,0	G
90,91	10	SD	9	7,7	G H
66,67	10	SD	9	7,1	H I
83,33	10	FD	9	6,9	H I
75,00	10	SD	9	6,4	I J
83,33	10	SD	9	5,8	J
90,91	10	FD	9	5,5	J

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Sugar Type	SP Concentration	pH	FD/SD	N	Mean
Psicose	50,00	7	FD	3	29,4
Fructose	50,00	7	FD	3	25,4
Psicose	50,00	7	SD	3	24,2
Dextrose	50,00	7	SD	3	20,7
Psicose	90,91	7	SD	3	20,5
Fructose	66,67	7	FD	3	18,3
Fructose	75,00	7	FD	3	18,1
Fructose	50,00	10	FD	3	17,2
Dextrose	50,00	10	FD	3	15,1
Fructose	90,91	7	FD	3	14,1
Dextrose	75,00	7	FD	3	14,0
Psicose	83,33	7	SD	3	13,9
Psicose	66,67	7	FD	3	13,6
Fructose	75,00	7	SD	3	13,5
Fructose	50,00	10	SD	3	13,5
Fructose	83,33	7	FD	3	13,1
Psicose	50,00	10	FD	3	12,9
Dextrose	75,00	7	SD	3	12,8
Fructose	90,91	7	SD	3	12,8
Fructose	66,67	10	FD	3	12,2
Fructose	83,33	7	SD	3	12,2
Psicose	66,67	7	SD	3	12,1
Fructose	66,67	7	SD	3	12,0
Fructose	50,00	7	SD	3	11,9
Dextrose	90,91	7	SD	3	11,9
Dextrose	50,00	10	SD	3	11,3
Dextrose	50,00	7	FD	3	11,2
Dextrose	90,91	10	SD	3	10,8
Psicose	50,00	10	SD	3	10,8
Psicose	75,00	7	FD	3	10,6
Dextrose	83,33	7	SD	3	10,6
Dextrose	66,67	7	SD	3	10,6
Psicose	83,33	7	FD	3	9,7

Table C. 4. Continued.

Psicose	66,67	10	FD	3	9,6
Psicose	75,00	7	SD	3	9,6
Psicose	90,91	7	FD	3	9,5
Dextrose	83,33	10	SD	3	9,3
Fructose	75,00	10	FD	3	9,1
Dextrose	75,00	10	SD	3	9,1
Dextrose	66,67	10	FD	3	8,7
Psicose	90,91	10	SD	3	8,5
Fructose	83,33	10	FD	3	8,4
Dextrose	66,67	10	SD	3	8,1
Psicose	75,00	10	FD	3	8,1
Dextrose	66,67	7	FD	3	7,5
Fructose	66,67	10	SD	3	7,3
Dextrose	75,00	10	FD	3	6,9
Psicose	83,33	10	FD	3	6,5
Dextrose	90,91	10	FD	3	6,0
Dextrose	83,33	10	FD	3	5,9
Psicose	66,67	10	SD	3	5,8
Fructose	75,00	10	SD	3	5,8
Dextrose	83,33	7	FD	3	5,3
Psicose	90,91	10	FD	3	5,3
Fructose	90,91	10	FD	3	5,1
Dextrose	90,91	7	FD	3	4,4
Psicose	75,00	10	SD	3	4,3
Fructose	83,33	10	SD	3	4,2
Psicose	83,33	10	SD	3	3,9
Fructose	90,91	10	SD	3	3,7

Sugar Type	SP Concentration	pH	FD/SD	Grouping
Psicose	50,00	7	FD	A
Fructose	50,00	7	FD	B
Psicose	50,00	7	SD	B
Dextrose	50,00	7	SD	C
Psicose	90,91	7	SD	C
Fructose	66,67	7	FD	D
Fructose	75,00	7	FD	D
Fructose	50,00	10	FD	D
Dextrose	50,00	10	FD	E
Fructose	90,91	7	FD	E F
Dextrose	75,00	7	FD	E F G
Psicose	83,33	7	SD	E F G H
Psicose	66,67	7	FD	E F G H I
Fructose	75,00	7	SD	E F G H I
Fructose	50,00	10	SD	E F G H I
Fructose	83,33	7	FD	F G H I J
Psicose	50,00	10	FD	F G H I J K
Dextrose	75,00	7	SD	F G H I J K
Fructose	90,91	7	SD	F G H I J K
Fructose	66,67	10	FD	G H I J K L
Fructose	83,33	7	SD	G H I J K L
Psicose	66,67	7	SD	H I J K L
Fructose	66,67	7	SD	I J K L
Fructose	50,00	7	SD	I J K L
Dextrose	90,91	7	SD	I J K L
Dextrose	50,00	10	SD	J K L M
Dextrose	50,00	7	FD	K L M

Table C. 4. Continued.

Dextrose	90,91	10	SD	L M N
Psicose	50,00	10	SD	L M N
Psicose	75,00	7	FD	L M N
Dextrose	83,33	7	SD	L M N
Dextrose	66,67	7	SD	L M N
Psicose	83,33	7	FD	M N O
Psicose	66,67	10	FD	M N O
Psicose	75,00	7	SD	M N O
Psicose	90,91	7	FD	M N O
Dextrose	83,33	10	SD	N O P
Fructose	75,00	10	FD	N O P
Dextrose	75,00	10	SD	N O P Q
Dextrose	66,67	10	FD	O P Q R
Psicose	90,91	10	SD	O P Q R
Fructose	83,33	10	FD	O P Q R
Dextrose	66,67	10	SD	O P Q R S
Psicose	75,00	10	FD	O P Q R S
Dextrose	66,67	7	FD	P Q R S T
Fructose	66,67	10	SD	Q R S T
Dextrose	75,00	10	FD	R S T U
Psicose	83,33	10	FD	S T U
Dextrose	90,91	10	FD	T U V
Dextrose	83,33	10	FD	T U V
Psicose	66,67	10	SD	T U V
Fructose	75,00	10	SD	T U V
Dextrose	83,33	7	FD	U V W
Psicose	90,91	10	FD	U V W
Fructose	90,91	10	FD	U V W
Dextrose	90,91	7	FD	V W
Psicose	75,00	10	SD	V W
Fructose	83,33	10	SD	V W
Psicose	83,33	10	SD	W
Fructose	90,91	10	SD	W

Means that do not share a letter are significantly different.

Table C. 5. ANOVA and Tukey's Comparison Test with 95% confidence level for determining free amino groups of the glycated soy protein

General Linear Model: Results x 10 versus Sugar Type; SP Concentra; ...

```
Factor            Type   Levels  Values
Sugar Type        fixed       3  Dextrose; Fructose; Psicose
SP Concentration  fixed       5  50,00; 66,67; 75,00; 83,33; 90,91
pH                fixed       2  7; 10
FD/SD             fixed       2  FD; SD


Analysis of Variance for Results x 10000, using Adjusted SS for Tests

Source                                DF   Seq SS   Adj SS   Adj MS         F
Sugar Type                             2   131375   131375    65687    7325,90
SP Concentration                       4   226028   226028    56507    6302,05
pH                                     1    45264    45264    45264    5048,14
FD/SD                                  1  1086690  1086690  1086690  121195,14
Sugar Type*SP Concentration            8    40377    40377     5047     562,88
Sugar Type*pH                          2    15102    15102     7551     842,15
Sugar Type*FD/SD                       2   142133   142133    71066    7925,82
SP Concentration*pH                    4    76134    76134    19034    2122,75
SP Concentration*FD/SD                 4   218305   218305    54576    6086,73
pH*FD/SD                               1    48126    48126    48126    5367,31
Sugar Type*SP Concentration*pH         8    23864    23864     2983     332,68
Sugar Type*SP Concentration*FD/SD      8    41047    41047     5131     572,22
Sugar Type*pH*FD/SD                    2    18942    18942     9471    1056,29
SP Concentration*pH*FD/SD              4    78214    78214    19554    2180,74
Sugar Type*SP Concentration*pH*FD/SD   8    23338    23338     2917     325,36
Error                                120     1076     1076        9
Total                                179  2216014

Source                                    P
Sugar Type                            0,000
SP Concentration                      0,000
pH                                    0,000
FD/SD                                 0,000
Sugar Type*SP Concentration           0,000
Sugar Type*pH                         0,000
Sugar Type*FD/SD                      0,000
SP Concentration*pH                   0,000
SP Concentration*FD/SD                0,000
pH*FD/SD                              0,000
Sugar Type*SP Concentration*pH        0,000
Sugar Type*SP Concentration*FD/SD     0,000
Sugar Type*pH*FD/SD                   0,000
SP Concentration*pH*FD/SD             0,000
Sugar Type*SP Concentration*pH*FD/SD  0,000
Error
Total


S = 2,99440   R-Sq = 99,95%   R-Sq(adj) = 99,93%

Unusual Observations for Results x 10000
```

Table C. 5. Continued.

Obs	Results x 10000	Fit	SE Fit	Residual	St Resid	
16	63,189	69,288	1,729	-6,099	-2,49	R
18	75,387	69,288	1,729	6,099	2,49	R
32	110,480	103,015	1,729	7,465	3,05	R
33	96,172	103,015	1,729	-6,843	-2,80	R
41	165,740	158,720	1,729	7,019	2,87	R
49	178,279	186,242	1,729	-7,963	-3,26	R
50	193,366	186,242	1,729	7,124	2,91	R
66	60,640	66,329	1,729	-5,689	-2,33	R
68	95,716	88,493	1,729	7,223	2,95	R
69	83,333	88,493	1,729	-5,159	-2,11	R
71	100,423	93,292	1,729	7,131	2,92	R
72	87,209	93,292	1,729	-6,082	-2,49	R
79	83,668	77,086	1,729	6,582	2,69	R
82	82,923	76,622	1,729	6,301	2,58	R
83	70,320	76,622	1,729	-6,301	-2,58	R
87	119,820	126,023	1,729	-6,203	-2,54	R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

Sugar Type	N	Mean	Grouping
Dextrose	60	124,731	A
Psicose	60	74,459	B
Fructose	60	62,327	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

SP Concentration	N	Mean	Grouping
90,91	36	150,274	A
83,33	36	96,149	B
75,00	36	78,989	C
66,67	36	62,572	D
50,00	36	47,878	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

pH	N	Mean	Grouping
10	90	103,030	A
7	90	71,315	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

FD/SD	N	Mean	Grouping

Table C. 5. Continued.

```
SD      90  164,871  A
FD      90    9,473    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

             SP
Sugar Type   Concentration   N     Mean  Grouping
Dextrose     90,91          12  217,342  A
Psicose      90,91          12  143,529    B
Dextrose     83,33          12  138,722      C
Dextrose     75,00          12  116,048        D
Fructose     90,91          12   89,950          E
Dextrose     66,67          12   82,672            F
Psicose      83,33          12   75,596              G
Fructose     83,33          12   74,128              G
Dextrose     50,00          12   68,871                H
Fructose     75,00          12   64,225                  I
Psicose      75,00          12   56,692                    J
Psicose      66,67          12   54,116                    J K
Fructose     66,67          12   50,927                      K
Psicose      50,00          12   42,360                        L
Fructose     50,00          12   32,404                          M

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Sugar Type  pH   N     Mean  Grouping
Dextrose    10   30  129,387  A
Dextrose     7   30  120,075    B
Psicose     10   30  101,551      C
Fructose    10   30   78,152        D
Psicose      7   30   47,366          E
Fructose     7   30   46,502          E

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Sugar Type  FD/SD   N     Mean  Grouping
Dextrose    SD     30  241,354  A
Psicose     SD     30  139,633    B
Fructose    SD     30  113,627      C
Fructose    FD     30   11,027        D
Psicose     FD     30    9,284        D E
Dextrose    FD     30    8,108          E

Means that do not share a letter are significantly different.


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

Table C. 5. Continued.

SP Concentration	pH	N	Mean	Grouping
90,91	10	18	206,408	A
83,33	10	18	109,981	B
90,91	7	18	94,140	C
75,00	10	18	82,767	D
83,33	7	18	82,316	D
75,00	7	18	75,210	E
66,67	10	18	65,652	F
66,67	7	18	59,491	G
50,00	10	18	50,342	H
50,00	7	18	45,415	I

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

SP Concentration	FD/SD	N	Mean	Grouping
90,91	SD	18	289,920	A
83,33	SD	18	182,644	B
75,00	SD	18	148,770	C
66,67	SD	18	116,376	D
50,00	SD	18	86,647	E
90,91	FD	18	10,628	F
83,33	FD	18	9,654	F
75,00	FD	18	9,207	F
50,00	FD	18	9,110	F
66,67	FD	18	8,767	F

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

pH	FD/SD	N	Mean	Grouping
10	SD	45	197,080	A
7	SD	45	132,662	B
7	FD	45	9,967	C
10	FD	45	8,979	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Sugar Type	SP Concentration	pH	N	Mean	Grouping
Dextrose	90,91	10	6	269,141	A
Psicose	90,91	10	6	231,672	B
Dextrose	90,91	7	6	165,544	C
Dextrose	83,33	10	6	152,035	D
Dextrose	75,00	7	6	131,392	E
Dextrose	83,33	7	6	125,408	E
Fructose	90,91	10	6	118,410	F
Dextrose	75,00	10	6	100,705	G

Table C. 5. Continued.

Dextrose	66,67	7	6	95,839	G
Fructose	83,33	10	6	94,520	G
Psicose	83,33	10	6	83,388	H
Dextrose	50,00	7	6	82,193	H
Fructose	75,00	10	6	76,873	H I
Psicose	75,00	10	6	70,724	I J
Dextrose	66,67	10	6	69,505	J
Psicose	83,33	7	6	67,805	J K
Psicose	66,67	10	6	64,866	J K
Fructose	66,67	10	6	62,587	K L
Fructose	90,91	7	6	61,490	K L M
Psicose	50,00	10	6	57,107	L M N
Dextrose	50,00	10	6	55,548	M N
Psicose	90,91	7	6	55,386	M N
Fructose	83,33	7	6	53,736	N
Fructose	75,00	7	6	51,578	N
Psicose	66,67	7	6	43,367	O
Psicose	75,00	7	6	42,661	O
Fructose	66,67	7	6	39,267	O
Fructose	50,00	10	6	38,370	O
Psicose	50,00	7	6	27,613	P
Fructose	50,00	7	6	26,439	P

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Sugar Type	SP Concentration	FD/SD	N	Mean	Grouping
Dextrose	90,91	SD	6	425,924	A
Psicose	90,91	SD	6	277,018	B
Dextrose	83,33	SD	6	268,421	C
Dextrose	75,00	SD	6	224,082	D
Fructose	90,91	SD	6	166,818	E
Dextrose	66,67	SD	6	157,671	F
Psicose	83,33	SD	6	142,372	G
Fructose	83,33	SD	6	137,139	G H
Dextrose	50,00	SD	6	130,674	H
Fructose	75,00	SD	6	117,182	I
Psicose	75,00	SD	6	105,047	J
Psicose	66,67	SD	6	99,542	J
Fructose	66,67	SD	6	91,915	K
Psicose	50,00	SD	6	74,186	L
Fructose	50,00	SD	6	55,081	M
Fructose	90,91	FD	6	13,082	N
Fructose	75,00	FD	6	11,269	N
Fructose	83,33	FD	6	11,118	N
Psicose	50,00	FD	6	10,534	N
Psicose	90,91	FD	6	10,040	N
Fructose	66,67	FD	6	9,939	N
Fructose	50,00	FD	6	9,728	N
Dextrose	83,33	FD	6	9,022	N
Psicose	83,33	FD	6	8,821	N
Dextrose	90,91	FD	6	8,760	N
Psicose	66,67	FD	6	8,690	N
Psicose	75,00	FD	6	8,337	N

Table C. 5. Continued.

```
Dextrose    75,00          FD     6    8,015                              N
Dextrose    66,67          FD     6    7,672                              N
Dextrose    50,00          FD     6    7,068                              N

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Sugar Type  pH  FD/SD   N     Mean  Grouping
Dextrose    10  SD     15  248,776  A
Dextrose     7  SD     15  233,933    B
Psicose     10  SD     15  194,277      C
Fructose    10  SD     15  148,188        D
Psicose      7  SD     15   84,989          E
Fructose     7  SD     15   79,066            F
Fructose     7  FD     15   13,939              G
Dextrose    10  FD     15    9,998                H
Psicose      7  FD     15    9,744                H I
Psicose     10  FD     15    8,825                H I
Fructose    10  FD     15    8,116                H I
Dextrose     7  FD     15    6,218                  I

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

SP
Concentration  pH  FD/SD  N     Mean  Grouping
90,91          10  SD     9  403,202  A
83,33          10  SD     9  210,812    B
90,91           7  SD     9  176,639      C
75,00          10  SD     9  157,176        D
83,33           7  SD     9  154,475        D
75,00           7  SD     9  140,364          E
66,67          10  SD     9  122,867            F
66,67           7  SD     9  109,886              G
50,00          10  SD     9   91,345                H
50,00           7  SD     9   81,949                  I
90,91           7  FD     9   11,642                    J
83,33           7  FD     9   10,157                    J
75,00           7  FD     9   10,057                    J
90,91          10  FD     9    9,613                    J
50,00          10  FD     9    9,338                    J
83,33          10  FD     9    9,150                    J
66,67           7  FD     9    9,096                    J
50,00           7  FD     9    8,881                    J
66,67          10  FD     9    8,438                    J
75,00          10  FD     9    8,358                    J

Means that do not share a letter are significantly different.

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

Table C. 5. Continued.

Sugar Type	Concentration	pH	FD/SD	N	Mean
Dextrose	90,91	10	SD	3	528,131
Psicose	90,91	10	SD	3	454,180
Dextrose	90,91	7	SD	3	323,718
Dextrose	83,33	10	SD	3	292,731
Dextrose	75,00	7	SD	3	255,978
Dextrose	83,33	7	SD	3	244,110
Fructose	90,91	10	SD	3	227,294
Dextrose	75,00	10	SD	3	192,185
Dextrose	66,67	7	SD	3	186,242
Fructose	83,33	10	SD	3	180,985
Dextrose	50,00	7	SD	3	159,616
Psicose	83,33	10	SD	3	158,720
Fructose	75,00	10	SD	3	145,871
Psicose	75,00	10	SD	3	133,473
Dextrose	66,67	10	SD	3	129,100
Psicose	83,33	7	SD	3	126,023
Psicose	66,67	10	SD	3	121,998
Fructose	66,67	10	SD	3	117,502
Fructose	90,91	7	SD	3	106,341
Psicose	50,00	10	SD	3	103,015
Dextrose	50,00	10	SD	3	101,732
Psicose	90,91	7	SD	3	99,856
Fructose	83,33	7	SD	3	93,292
Fructose	75,00	7	SD	3	88,493
Psicose	66,67	7	SD	3	77,086
Psicose	75,00	7	SD	3	76,622
Fructose	50,00	10	SD	3	69,288
Fructose	66,67	7	SD	3	66,329
Psicose	50,00	7	SD	3	45,357
Fructose	50,00	7	SD	3	40,874
Fructose	90,91	7	FD	3	16,638
Fructose	75,00	7	FD	3	14,664
Fructose	83,33	7	FD	3	14,180
Fructose	66,67	7	FD	3	12,206
Fructose	50,00	7	FD	3	12,004
Dextrose	83,33	10	FD	3	11,339
Psicose	50,00	10	FD	3	11,198
Psicose	90,91	7	FD	3	10,916
Dextrose	90,91	10	FD	3	10,151
Dextrose	66,67	10	FD	3	9,909
Psicose	50,00	7	FD	3	9,869
Psicose	66,67	7	FD	3	9,647
Psicose	83,33	7	FD	3	9,587
Fructose	90,91	10	FD	3	9,526
Dextrose	50,00	10	FD	3	9,365
Dextrose	75,00	10	FD	3	9,224
Psicose	90,91	10	FD	3	9,163
Psicose	75,00	7	FD	3	8,700
Psicose	83,33	10	FD	3	8,055
Fructose	83,33	10	FD	3	8,055
Psicose	75,00	10	FD	3	7,975
Fructose	75,00	10	FD	3	7,874
Psicose	66,67	10	FD	3	7,733
Fructose	66,67	10	FD	3	7,672
Fructose	50,00	10	FD	3	7,451
Dextrose	90,91	7	FD	3	7,370

Table C. 5. Continued.

Dextrose	75,00	7	FD	3	6,806
Dextrose	83,33	7	FD	3	6,705
Dextrose	66,67	7	FD	3	5,436
Dextrose	50,00	7	FD	3	4,771

Sugar Type	SP Concentration	pH	FD/SD	Grouping
Dextrose	90,91	10	SD	A
Psicose	90,91	10	SD	B
Dextrose	90,91	7	SD	C
Dextrose	83,33	10	SD	D
Dextrose	75,00	7	SD	E
Dextrose	83,33	7	SD	F
Fructose	90,91	10	SD	G
Dextrose	75,00	10	SD	H
Dextrose	66,67	7	SD	H I
Fructose	83,33	10	SD	I
Dextrose	50,00	7	SD	J
Psicose	83,33	10	SD	J
Fructose	75,00	10	SD	K
Psicose	75,00	10	SD	L
Dextrose	66,67	10	SD	L M
Psicose	83,33	7	SD	L M N
Psicose	66,67	10	SD	M N
Fructose	66,67	10	SD	N
Fructose	90,91	7	SD	O
Psicose	50,00	10	SD	O P
Dextrose	50,00	10	SD	O P
Psicose	90,91	7	SD	O P
Fructose	83,33	7	SD	P Q
Fructose	75,00	7	SD	Q
Psicose	66,67	7	SD	R
Psicose	75,00	7	SD	R
Fructose	50,00	10	SD	R S
Fructose	66,67	7	SD	S
Psicose	50,00	7	SD	T
Fructose	50,00	7	SD	T
Fructose	90,91	7	FD	U
Fructose	75,00	7	FD	U
V				
Fructose	83,33	7	FD	U
V				
Fructose	66,67	7	FD	U
V				
Fructose	50,00	7	FD	U
V				
Dextrose	83,33	10	FD	U
V				
Psicose	50,00	10	FD	U
V				
Psicose	90,91	7	FD	U
V				
Dextrose	90,91	10	FD	U
V				
Dextrose	66,67	10	FD	U
V				

Table C. 5. Continued.

Psicose	50,00	7	FD	U
V				
Psicose	66,67	7	FD	U
V				
Psicose	83,33	7	FD	U
V				
Fructose	90,91	10	FD	U
V				
Dextrose	50,00	10	FD	U
V				
Dextrose	75,00	10	FD	U
V				
Psicose	90,91	10	FD	U
V				
Psicose	75,00	7	FD	U
V				
Psicose	83,33	10	FD	U
V				
Fructose	83,33	10	FD	U
V				
Psicose	75,00	10	FD	U
V				
Fructose	75,00	10	FD	U
V				
Psicose	66,67	10	FD	U
V				
Fructose	66,67	10	FD	U
V				
Fructose	50,00	10	FD	U
V				
Dextrose	90,91	7	FD	U
V				
Dextrose	75,00	7	FD	U
V				
Dextrose	83,33	7	FD	U
V				
Dextrose	66,67	7	FD	V
Dextrose	50,00	7	FD	V

Means that do not share a letter are significantly different.

Table C. 6. ANOVA and Tukey's Comparison Test with 95% confidence level for determining hydration behavior of the glycated soy protein with NMR Relaxometry.

General Linear Model: T2 Results versus Sugar Type; SP Concentra; ...

Factor	Type	Levels	Values
Sugar Type	fixed	3	Dextrose; Fructose; Psicose
SP Concentration	fixed	5	50.00; 66.67; 75.00; 83.33; 90.91
FD/NFD/SD	fixed	3	0(NFD); 1(FD); 2(SD)
pH	fixed	2	7; 10

Table C. 6. Continued.

Analysis of Variance for T2 Results, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Sugar Type	2	149210	149210	74605	991.25	0.000
SP Concentration	4	635920	635920	158980	2112.30	0.000
FD/NFD/SD	2	772860	772860	386430	5134.32	0.000
pH	1	17285	17285	17285	229.66	0.000
Sugar Type*SP Concentration	8	40372	40372	5047	67.05	0.000
Sugar Type*FD/NFD/SD	4	155380	155380	38845	516.12	0.000
Sugar Type*pH	2	16794	16794	8397	111.57	0.000
SP Concentration*FD/NFD/SD	8	97648	97648	12206	162.18	0.000
SP Concentration*pH	4	15417	15417	3854	51.21	0.000
FD/NFD/SD*pH	2	117731	117731	58866	782.12	0.000
Sugar Type*SP Concentration* FD/NFD/SD	16	82529	82529	5158	68.53	0.000
Sugar Type*SP Concentration*pH	8	19851	19851	2481	32.97	0.000
Sugar Type*FD/NFD/SD*pH	4	23893	23893	5973	79.36	0.000
SP Concentration*FD/NFD/SD*pH	8	46349	46349	5794	76.98	0.000
Sugar Type*SP Concentration* FD/NFD/SD*pH	16	35048	35048	2190	29.10	0.000
Error	180	13548	13548	75		
Total	269	2239835				

S = 8.67549 R-Sq = 99.40% R-Sq(adj) = 99.10%

Unusual Observations for T2 Results

Obs	T2 Results	Fit	SE Fit	Residual	St Resid	
16	147.726	162.865	5.009	-15.139	-2.14	R
62	443.727	418.092	5.009	25.635	3.62	R
63	380.572	418.092	5.009	-37.521	-5.30	R
64	396.592	435.267	5.009	-38.675	-5.46	R
65	454.709	435.267	5.009	19.442	2.74	R
66	454.501	435.267	5.009	19.233	2.72	R
68	253.709	271.481	5.009	-17.772	-2.51	R
92	194.379	209.572	5.009	-15.193	-2.14	R
106	147.726	162.865	5.009	-15.139	-2.14	R
122	401.803	386.743	5.009	15.060	2.13	R
130	158.671	174.935	5.009	-16.263	-2.30	R
131	158.795	174.935	5.009	-16.140	-2.28	R
132	207.337	174.935	5.009	32.403	4.57	R
151	336.490	351.128	5.009	-14.638	-2.07	R
256	408.680	428.329	5.009	-19.650	-2.77	R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95.0% Confidence

Sugar Type	N	Mean	Grouping
Psicose	90	177.2	A
Fructose	90	162.7	B
Dextrose	90	121.7	C

Means that do not share a letter are significantly different.

Table C. 6. Continued.

Grouping Information Using Tukey Method and 95.0% Confidence

SP Concentration	N	Mean	Grouping
50.00	54	241.1	A
66.67	54	165.0	B
75.00	54	138.9	C
83.33	54	124.9	D
90.91	54	99.3	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

FD/NFD/SD	N	Mean	Grouping
1 (FD)	90	226.6	A
2 (SD)	90	135.6	B
0 (NFD)	90	99.4	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

pH	N	Mean	Grouping
7	135	161.8	A
10	135	145.8	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Sugar Type	SP Concentration	N	Mean	Grouping
Psicose	50.00	18	280.5	A
Fructose	50.00	18	260.5	B
Psicose	66.67	18	202.2	C
Dextrose	50.00	18	182.3	D
Fructose	66.67	18	167.9	E
Psicose	75.00	18	153.1	F
Psicose	83.33	18	143.6	F G
Fructose	75.00	18	143.3	F G
Fructose	83.33	18	134.3	G H
Dextrose	66.67	18	124.9	H I
Dextrose	75.00	18	120.4	I
Fructose	90.91	18	107.4	J
Psicose	90.91	18	106.4	J K
Dextrose	83.33	18	96.8	K
Dextrose	90.91	18	84.0	L

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Table C. 6. Continued.

Sugar Type	FD/NFD/SD	N	Mean	Grouping
Psicose	1	30	274.9	A
Fructose	1	30	253.8	B
Psicose	2	30	163.0	C
Dextrose	1	30	151.0	D
Fructose	2	30	131.4	E
Dextrose	2	30	112.4	F
Fructose	0	30	102.9	G
Dextrose	0	30	101.6	G
Psicose	0	30	93.6	H

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Sugar Type	pH	N	Mean	Grouping
Psicose	7	45	179.9	A
Psicose	10	45	174.4	B
Fructose	7	45	164.8	C
Fructose	10	45	160.6	C
Dextrose	7	45	140.8	D
Dextrose	10	45	102.5	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

SP Concentration	FD/NFD/SD	N	Mean	Grouping
50.00	1	18	333.8	A
66.67	1	18	247.7	B
50.00	2	18	243.8	B
75.00	1	18	214.5	C
83.33	1	18	202.3	D
50.00	0	18	145.7	E
66.67	2	18	140.5	E F
90.91	1	18	134.5	F
75.00	2	18	110.7	G
66.67	0	18	106.8	G
83.33	2	18	95.1	H
75.00	0	18	91.5	H
90.91	2	18	87.7	H
83.33	0	18	77.3	I
90.91	0	18	75.7	I

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

SP Concentration	pH	N	Mean	Grouping
50.00	7	27	260.7	A
50.00	10	27	221.4	B
66.67	7	27	167.9	C

Table C. 6. Continued.

```
66.67          10  27  162.1      C
75.00           7  27  152.4        D
83.33          10  27  126.2          E
75.00          10  27  125.5          E
83.33           7  27  123.6          E
90.91           7  27  104.6            F
90.91          10  27   94.0              G

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

FD/NFD/SD  pH   N   Mean  Grouping
1          10  45  239.2  A
1           7  45  213.9    B
2           7  45  172.2      C
0           7  45   99.4        D
0          10  45   99.4        D
2          10  45   99.0        D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

             SP
Sugar Type   Concentration  FD/NFD/SD  N   Mean
Fructose     50.00          1          6  412.9
Psicose      50.00          1          6  384.6
Psicose      66.67          1          6  340.1
Psicose      50.00          2          6  320.6
Psicose      75.00          1          6  259.1
Fructose     66.67          1          6  257.7
Psicose      83.33          1          6  250.0
Fructose     50.00          2          6  230.6
Fructose     83.33          1          6  225.5
Fructose     75.00          1          6  219.1
Dextrose     50.00          1          6  203.8
Dextrose     50.00          2          6  180.2
Psicose      66.67          2          6  167.4
Dextrose     75.00          1          6  165.4
Dextrose     50.00          0          6  162.9
Fructose     90.91          1          6  153.6
Dextrose     66.67          1          6  145.3
Psicose      90.91          1          6  140.9
Fructose     50.00          0          6  137.9
Psicose      50.00          0          6  136.3
Fructose     66.67          2          6  133.2
Dextrose     83.33          1          6  131.3
Dextrose     66.67          2          6  120.9
Psicose      75.00          2          6  115.5
Fructose     66.67          0          6  112.8
Dextrose     75.00          2          6  109.1
Dextrose     90.91          1          6  109.0
Dextrose     66.67          0          6  108.4
Fructose     75.00          2          6  107.6
```

Table C. 6. Continued.

Psicose	90.91	2	6	105.8
Psicose	83.33	2	6	105.5
Fructose	75.00	0	6	103.1
Psicose	66.67	0	6	99.1
Fructose	83.33	2	6	97.9
Fructose	90.91	2	6	87.5
Dextrose	75.00	0	6	86.7
Psicose	75.00	0	6	84.7
Dextrose	83.33	2	6	82.0
Fructose	90.91	0	6	81.2
Fructose	83.33	0	6	79.6
Dextrose	83.33	0	6	77.0
Psicose	83.33	0	6	75.2
Dextrose	90.91	0	6	73.1
Psicose	90.91	0	6	72.6
Dextrose	90.91	2	6	69.7

Sugar Type	SP Concentration	FD/NFD/SD	Grouping
Fructose	50.00	1	A
Psicose	50.00	1	B
Psicose	66.67	1	C
Psicose	50.00	2	C
Psicose	75.00	1	D
Fructose	66.67	1	D
Psicose	83.33	1	D E
Fructose	50.00	2	E F
Fructose	83.33	1	F
Fructose	75.00	1	F G
Dextrose	50.00	1	G
Dextrose	50.00	2	H
Psicose	66.67	2	H I
Dextrose	75.00	1	H I J
Dextrose	50.00	0	H I J
Fructose	90.91	1	I J K
Dextrose	66.67	1	J K L
Psicose	90.91	1	K L M
Fructose	50.00	0	K L M
Psicose	50.00	0	K L M
Fructose	66.67	2	L M N
Dextrose	83.33	1	L M N O
Dextrose	66.67	2	M N O P
Psicose	75.00	2	N O P Q
Fructose	66.67	0	O P Q
Dextrose	75.00	2	P Q
Dextrose	90.91	1	P Q
Dextrose	66.67	0	P Q
Fructose	75.00	2	P Q
Psicose	90.91	2	P Q R
Psicose	83.33	2	P Q R
Fructose	75.00	0	P Q R S
Psicose	66.67	0	Q R S
Fructose	83.33	2	Q R S T
Fructose	90.91	2	R S T U

Table C. 6. Continued.

```
Dextrose    75.00          0                                                R S
T U
Psicose     75.00          0                                                  S
T U
Dextrose    83.33          2                                                  T
U
Fructose    90.91          0                                                  T
U
Fructose    83.33          0                                                  T
U
Dextrose    83.33          0                                                  U
Psicose     83.33          0                                                  U
Dextrose    90.91          0                                                  U
Psicose     90.91          0                                                  U
Dextrose    90.91          2                                                  U

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

            SP
Sugar Type  Concentration  pH  N   Mean  Grouping
Psicose     50.00           7  9  305.2  A
Fructose    50.00           7  9  263.1    B
Fructose    50.00          10  9  257.9    B
Psicose     50.00          10  9  255.7    B
Psicose     66.67          10  9  220.1      C
Dextrose    50.00           7  9  213.9      C
Psicose     66.67           7  9  184.2        D
Fructose    66.67           7  9  172.1        D E
Fructose    66.67          10  9  163.7          E F
Psicose     75.00           7  9  155.0            F G
Dextrose    75.00           7  9  151.8            F G H
Psicose     75.00          10  9  151.2            F G H
Dextrose    50.00          10  9  150.7            F G H
Fructose    75.00           7  9  150.3            F G H
Fructose    83.33          10  9  147.6              G H
Dextrose    66.67           7  9  147.4              G H
Psicose     83.33           7  9  146.1              G H
Psicose     83.33          10  9  141.1              G H
Fructose    75.00          10  9  136.3                H I
Fructose    83.33           7  9  121.1                I J
Fructose    90.91           7  9  117.5                  J K
Psicose     90.91           7  9  108.9                  J K L
Psicose     90.91          10  9  104.0                    K L M
Dextrose    83.33           7  9  103.5                    K L M
Dextrose    66.67          10  9  102.3                    K L M N
Fructose    90.91          10  9   97.4                      L M N
Dextrose    83.33          10  9   90.0                        M N O
Dextrose    75.00          10  9   89.0                        M N O
Dextrose    90.91           7  9   87.4                          N O
Dextrose    90.91          10  9   80.5                            O

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

Table C. 6. Continued.

Grouping Information Using Tukey Method and 95.0% Confidence

Sugar Type	FD/NFD/SD	pH	N	Mean	Grouping
Psicose	1	10	15	307.9	A
Fructose	1	10	15	265.4	B
Fructose	1	7	15	242.1	C
Psicose	1	7	15	241.9	C
Psicose	2	7	15	204.1	D
Dextrose	2	7	15	163.0	E
Dextrose	1	7	15	157.8	E F
Fructose	2	7	15	149.4	F G
Dextrose	1	10	15	144.1	G
Psicose	2	10	15	121.8	H
Fructose	2	10	15	113.3	H I
Fructose	0	7	15	102.9	I J
Fructose	0	10	15	102.9	I J
Dextrose	0	7	15	101.6	J
Dextrose	0	10	15	101.6	J
Psicose	0	7	15	93.6	J
Psicose	0	10	15	93.6	J
Dextrose	2	10	15	61.8	K

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

SP Concentration	FD/NFD/SD	pH	N	Mean	Grouping
50.00	1	10	9	351.7	A
50.00	2	7	9	320.7	B
50.00	1	7	9	315.8	B
66.67	1	10	9	278.2	C
75.00	1	7	9	227.2	D
83.33	1	10	9	224.6	D
66.67	1	7	9	217.2	D E
75.00	1	10	9	201.9	E
83.33	1	7	9	179.9	F
66.67	2	7	9	179.8	F
50.00	2	10	9	166.9	F
50.00	0	7	9	145.7	G
50.00	0	10	9	145.7	G
90.91	1	10	9	139.4	G H
75.00	2	7	9	138.4	G H
90.91	1	7	9	129.6	H
83.33	2	7	9	113.5	I
90.91	2	7	9	108.5	I
66.67	0	7	9	106.8	I J
66.67	0	10	9	106.8	I J
66.67	2	10	9	101.2	I J
75.00	0	10	9	91.5	J K
75.00	0	7	9	91.5	J K
75.00	2	10	9	83.0	K L
83.33	0	7	9	77.3	K L M
83.33	0	10	9	77.3	K L M
83.33	2	10	9	76.8	K L M
90.91	0	10	9	75.7	L M

Table C. 6. Continued.

```
90.91          0           7  9   75.7                          L M
90.91          2          10  9   66.8                            M

Means that do not share a letter are significantly different.

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

Sugar Type	SP Concentration	FD/NFD/SD	pH	N	Mean
Fructose	50.00	1	10	3	439.1
Psicose	66.67	1	10	3	435.3
Psicose	50.00	2	7	3	428.3
Psicose	50.00	1	10	3	418.1
Fructose	50.00	1	7	3	386.7
Psicose	50.00	1	7	3	351.1
Fructose	83.33	1	10	3	276.0
Psicose	75.00	1	10	3	271.5
Dextrose	50.00	2	7	3	269.2
Fructose	66.67	1	10	3	265.0
Fructose	50.00	2	7	3	264.6
Psicose	83.33	1	10	3	255.8
Fructose	66.67	1	7	3	250.5
Psicose	75.00	1	7	3	246.8
Psicose	66.67	1	7	3	244.9
Psicose	83.33	1	7	3	244.3
Fructose	75.00	1	7	3	224.6
Fructose	75.00	1	10	3	213.6
Psicose	50.00	2	10	3	212.8
Dextrose	75.00	1	7	3	210.1
Dextrose	50.00	1	7	3	209.6
Psicose	66.67	2	7	3	208.7
Dextrose	50.00	1	10	3	198.1
Fructose	50.00	2	10	3	196.6
Dextrose	66.67	2	7	3	177.5
Fructose	83.33	1	7	3	174.9
Fructose	90.91	1	7	3	173.7
Dextrose	50.00	0	7	3	162.9
Dextrose	50.00	0	10	3	162.9
Psicose	90.91	1	10	3	159.1
Dextrose	75.00	2	7	3	158.6
Dextrose	66.67	1	7	3	156.3
Fructose	66.67	2	7	3	153.1
Dextrose	83.33	1	10	3	142.0
Fructose	50.00	0	7	3	137.9
Fructose	50.00	0	10	3	137.9
Psicose	50.00	0	7	3	136.3
Psicose	50.00	0	10	3	136.3
Dextrose	66.67	1	10	3	134.2
Psicose	75.00	2	7	3	133.6
Fructose	90.91	1	10	3	133.4
Psicose	90.91	2	7	3	131.3
Psicose	66.67	2	10	3	126.0
Dextrose	90.91	1	10	3	125.7
Fructose	75.00	2	7	3	123.1
Psicose	90.91	1	7	3	122.6
Dextrose	75.00	1	10	3	120.7

Table C. 6. Continued.

Dextrose	83.33	1	7	3	120.5
Psicose	83.33	2	7	3	118.7
Fructose	66.67	2	10	3	113.3
Dextrose	83.33	2	7	3	113.0
Fructose	66.67	0	7	3	112.8
Fructose	66.67	0	10	3	112.8
Fructose	83.33	2	7	3	108.7
Dextrose	66.67	0	7	3	108.4
Dextrose	66.67	0	10	3	108.4
Fructose	75.00	0	10	3	103.1
Fructose	75.00	0	7	3	103.1
Psicose	66.67	0	10	3	99.1
Psicose	66.67	0	7	3	99.1
Fructose	90.91	2	7	3	97.5
Psicose	75.00	2	10	3	97.5
Dextrose	90.91	2	7	3	96.7
Dextrose	90.91	1	7	3	92.3
Psicose	83.33	2	10	3	92.3
Fructose	75.00	2	10	3	92.0
Dextrose	50.00	2	10	3	91.2
Fructose	83.33	2	10	3	87.2
Dextrose	75.00	0	10	3	86.7
Dextrose	75.00	0	7	3	86.7
Psicose	75.00	0	10	3	84.7
Psicose	75.00	0	7	3	84.7
Fructose	90.91	0	10	3	81.2
Fructose	90.91	0	7	3	81.2
Psicose	90.91	2	10	3	80.2
Fructose	83.33	0	7	3	79.6
Fructose	83.33	0	10	3	79.6
Fructose	90.91	2	10	3	77.4
Dextrose	83.33	0	7	3	77.0
Dextrose	83.33	0	10	3	77.0
Psicose	83.33	0	7	3	75.2
Psicose	83.33	0	10	3	75.2
Dextrose	90.91	0	10	3	73.1
Dextrose	90.91	0	7	3	73.1
Psicose	90.91	0	10	3	72.6
Psicose	90.91	0	7	3	72.6
Dextrose	66.67	2	10	3	64.3
Dextrose	75.00	2	10	3	59.5
Dextrose	83.33	2	10	3	51.0
Dextrose	90.91	2	10	3	42.7

Sugar Type	SP Concentration	FD/NFD/SD	pH	Grouping
Fructose	50.00	1	10	A
Psicose	66.67	1	10	A
Psicose	50.00	2	7	A
Psicose	50.00	1	10	A
Fructose	50.00	1	7	B
Psicose	50.00	1	7	C
Fructose	83.33	1	10	D
Psicose	75.00	1	10	D E
Dextrose	50.00	2	7	D E
Fructose	66.67	1	10	D E
Fructose	50.00	2	7	D E

Table C. 6. Continued.

Psicose	83.33	1	10	D E
Fructose	66.67	1	7	D E F
Psicose	75.00	1	7	D E F
Psicose	66.67	1	7	E F
Psicose	83.33	1	7	E F G
Fructose	75.00	1	7	F G H
Fructose	75.00	1	10	G H
Psicose	50.00	2	10	H
Dextrose	75.00	1	7	H
Dextrose	50.00	1	7	H
Psicose	66.67	2	7	H
Dextrose	50.00	1	10	H I
Fructose	50.00	2	10	H I
Dextrose	66.67	2	7	I J
Fructose	83.33	1	7	I J
Fructose	90.91	1	7	I J
Dextrose	50.00	0	7	J K
Dextrose	50.00	0	10	J K
Psicose	90.91	1	10	J K L
Dextrose	75.00	2	7	J K L
Dextrose	66.67	1	7	J K L M
Fructose	66.67	2	7	J K L M N
Dextrose	83.33	1	10	K L M N O
Fructose	50.00	0	7	K L M N O P
Fructose	50.00	0	10	K L M N O P
Psicose	50.00	0	7	K L M N O P
Psicose	50.00	0	10	K L M N O P
Dextrose	66.67	1	10	K L M N O P
Psicose	75.00	2	7	K L M N O P Q
Fructose	90.91	1	10	K L M N O P Q
Psicose	90.91	2	7	L M N O P Q
Psicose	66.67	2	10	M N O P Q R
Dextrose	90.91	1	10	M N O P Q
Fructose	75.00	2	7	N O P Q R
Psicose	90.91	1	7	N O P Q R S
Dextrose	75.00	1	10	O P Q R S T
Dextrose	83.33	1	7	O P Q R S T
Psicose	83.33	2	7	O P Q R S T
Fructose	66.67	2	10	O P Q R S T U
Dextrose	83.33	2	7	O P Q R S T U
Fructose	66.67	0	7	O P Q R S T U
Fructose	66.67	0	10	O P Q R S T U
Fructose	83.33	2	7	P Q R S T U V
Dextrose	66.67	0	7	P Q R S T U V

Table C. 6. Continued.

Dextrose	66.67	0	10	P Q R S T U V
Fructose	75.00	0	10	Q R S T U V W
Fructose	75.00	0	7	Q R S T U V W
Psicose	66.67	0	10	R S T U V W
Psicose	66.67	0	7	R S T U V W
Fructose	90.91	2	7	R S T U V W
Psicose	75.00	2	10	R S T U V W
Dextrose	90.91	2	7	R S T U V W
Dextrose	90.91	1	7	S T U V W X
Psicose	83.33	2	10	S T U V W X
Fructose	75.00	2	10	S T U V W X
Dextrose	50.00	2	10	T U V W X
Fructose	83.33	2	10	U V W X Y
Dextrose	75.00	0	10	U V W X Y
Dextrose	75.00	0	7	U V W X Y
Psicose	75.00	0	10	U V W X Y
Psicose	75.00	0	7	U V W X Y
Fructose	90.91	0	10	V W X Y Z
Fructose	90.91	0	7	V W X Y Z
Psicose	90.91	2	10	V W X Y Z
Fructose	83.33	0	7	V W X Y Z
Fructose	83.33	0	10	V W X Y Z
Fructose	90.91	2	10	W X Y Z
Dextrose	83.33	0	7	W X Y Z
Dextrose	83.33	0	10	W X Y Z
Psicose	83.33	0	7	W X Y Z
Psicose	83.33	0	10	W X Y Z
Dextrose	90.91	0	10	W X Y Z AA

Table C. 6. Continued.

Dextrose	90.91	0	7	W X Y Z AA
Psicose	90.91	0	10	W X Y Z AA
Psicose	90.91	0	7	W X Y Z AA
Dextrose	66.67	2	10	X Y Z AA
Dextrose	75.00	2	10	Y Z AA
Dextrose	83.33	2	10	Z AA
Dextrose	90.91	2	10	

Means that do not share a letter are significantly different.