IN VITRO DIGESTIBILITY OF SOY-PROTEIN ISOLATE CONTAINING SOFT CANDIES FORMULATED WITH D-PSICOSE

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IN VITRO DIGESTIBILITY OF SOY-PROTEIN ISOLATE CONTAINING SOFT CANDIES FORMULATED WITH D-PSICOSE

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ABSTRACT

IN VITRO DIGESTIBILITY OF SOY-PROTEIN ISOLATE CONTAINING SOFT CANDIES FORMULATED WITH D-PSICOSE

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Commercial soft candy products are known to contain high amount of sugar and therefore they are considered as not healthy and high calorie sweets. Most of the candy present in the market are made by sucrose and as a gelling agent, gelatin and sometimes starch or pectin is being used. In this study, D-Allulose (rare sugar), soy protein isolate (SPI) and pectin were used to prepare different soft candy formulations. D-Allulose was used as a replacement of sucrose at different ratios (Sucrose/D-Allulose: 0/35, 10/25, 20/15, 35/0) so that high calorie concern will be out of the subject since D-Allulose's caloric value is approximately 0.4 Kcal/g. Furthermore, pectin is a plant based gelling agent and also known as dietary fiber. Therefore, it will be a good replacement compared to gelatin, that is an animal-based gelling agent, considering vegans, vegetarians and consumers who are trying to eat halal food. Finally, adding SPI to the formulation could definitely increase the perception of the product for the confectionary industry. Pectin was used at a concentration of 4% (w/w) whereas SPI was added at 2% (w/w). In addition to the conventional candy characterization experiments, digestion behavior of the candies in simulated gastric media were also examined and characterization experiments were also conducted during digestion. During gastric digestion, brix of the gels was recorded. Before

digestion, physical properties, such as hardness, moisture, water activity, pH, color were measured for the candies and morphologies of the candies were determined using scanning electron microscope (SEM) experiments. In addition to these physical measurements, Time-Domain NMR (TD-NMR) experiments were also conducted. T_2 relaxation times were measured to determine how the water distribution in the samples changed in the samples before and after digestion and to observe how the rare sugar D-Allulose changed this distribution. To observe how D-Allulose and SPI changed the molecular dynamics of the soft candies, Fast Field Cycling (FFC) NMR Relaxometry experiments (through T_1 relaxation times) were conducted as well. X-ray diffraction experiments were performed to measure the crystallization behavior of the confectioneries. D-Allulose was found to increase the crystallization ability of pectin-containing candies, whereas sucrose containing samples experienced no crystallization. Higher hardness values were obtained for the soy protein containing candies due to pectin-soy protein interaction. Also, higher moisture content was obtained soy protein containing candies. Moreover, mathematical modelling was performed by using Power law model (R^2>0.98) and the dissolution constant was calculated for the samples. Dissolution constant of the SPI containing candies showed no significant difference ($p>0.05$) as sugar type changed while for non-soy protein containing candies, it was significant ($p<0.05$). Sugar type and SPI addition was found to have impact on soft candy formulations.

Keywords: Time Domain NMR (TD-NMR), D-Allulose, Pectin-based soft candy, soy protein isolate (SPI), digestion

ÖZ

SOY PROTEİN İZOLATI VE D-PSİKOZ İÇEREN PEKTİN BAZLI ŞEKERLEMELERİN YAPAY MİDE ORTAMINDA SİNDİRİM DAVRANIŞININ İNCELENMESİ

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Ticari yumuşak şeker ürünlerinin yüksek miktarda şeker içerdiği bilinmektedir ve bu nedenle sağlıklı ve yüksek kalorili olmayan tatlılar olarak kabul edilir. Piyasada bulunan şekerin çoğu sakarozdan yapılır ve bir jelleştirici madde olarak jelatin ve bazen nişasta veya pektin kullanılır. Bu çalışmada, farklı yumuşak şeker formülasyonları hazırlamak için D-Allüloz (nadir şeker), soya proteini izolatı (SPI) ve pektin kullanılmıştır. D-Allulose sakarozun yerine kullanılmıştır (Sükroz / D-Allüloz: 0/35, 10/25, 20/15, 35/0), bu yüzden yüksek kalorili olması konu dışı kalacaktır, çünkü D-Alluloz'un kalori değeri yaklaşık 0.4 Kcal / g'dir. Ayrıca, pektin bitkisel bazlı bir jelleştirici maddedir. Bu nedenle veganlar, vejeteryanlar ve helal gıda tüketmeye özen gösterenler göz önüne alındığında hayvansal bazlı jel ajanı olan jelatine iyi bir alternative olarak görülmektedir. Son olarak, formülasyona soya proteini ilave edilmesi kesinlikle şekerleme endüstrisindeki değerini arttırmıştır. Pektin, %4 (w/w) konsantrasyonunda kullanılırken, SPI, %2 (w/w) oranında ilave edildi. Geleneksel şekerleme karakterizasyon deneylerine ek olarak, simule edilmiş mide ortamında şekerlerin sindirim davranışı da incelenmiş ve sindirim sırasında karakterizasyon deneyleri yapılmıştır. Simule edilmiş mide sindirimi sırasında jellerin briks değerleri kaydedildi. Sindirimden önce, şekerler için sertlik, nem, su aktivitesi, pH, renk gibi

fiziksel özellikler ölçülmüş ve şekerlerin morfolojileri taramalı elektron mikroskobu (SEM) deneyleri kullanılarak belirlenmiştir. Bu fiziksel ölçümlere ek olarak, Time-Domain NMR (TD-NMR) deneyleri de yapıldı. T_2 relaksasyon süreleri, numunelerdeki su dağılımının sindirimden önce ve sonra numunelerde nasıl değiştiğini belirlemek ve nadir D-Allüloz şekerin bu dağılımı nasıl değiştirdiğini gözlemlemek için ölçülmüştür. D-Alluloz ve SPI'nın yumuşak şekerlerin moleküler dinamiklerini nasıl değiştirdiğini görmek için, Hızlı Alan Döngülü (FFC) NMR Relaxometry deneyleri (T_1 relaksasyon süreleriyle) de yapıldı. Şekerlemelerin kristalleşme davranışını ölçmek için X ışını kırınımı deneyleri yapıldı. D-Allülozun, pektin içeren şekerlerin kristalleşme yeteneğini arttırdığı, sakaroz içeren numunelerin daha az kristalleşme yaşadığı bulunmuştur. Pektin-soya proteini etkileşimi nedeniyle soya proteini içeren şekerler için daha yüksek sertlik değerleri elde edilmiştir. Ayrıca, soya proteini içeren şekerlerde daha yüksek nem içeriği elde edilmiştir. Ayrıca, matematiksel modelleme Power kanun modeli ($R^2 > 0.98$) kullanılarak yapıldı ve örnekler için çözünme sabiti hesaplandı. SPI içeren şekerlerin çözülme sabiti, şeker tipi değiştikçe anlamlı bir fark göstermedi ($p > 0.05$), soya içermeyen şekerler için ise anlamlıydı ($p < 0.05$). Şeker tipi ve SPI ilavesinin yumuşak şeker formülasyonları üzerinde etkisi olduğu bulundu.

Anahtar Kelimeler: Time Domain NMR (TD-NMR), D-Alluloz, Pektin bazlı yumuşak şekerleme, Soya protein izolatı, sindirim

To my beloved family and my son...

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INTRODUCTION

1.1. D-Allulose

1.1.1. Properties of D-Allulose

Rare sugars are known as monosaccharides that are not commonly found in nature. Example of the rare sugars can be given as D-Tagatose, D-Allulose, D-Sorbose and D-Allose. Recently, D-Tagatose has been found as an important drug for type 2 diabetes treatment (Yan Tang, 2013). Also, it has been approved by Food and Drug Administration (FDA) as a low calorie sweetener (O'Brien-Nabors *et al.*, 2011). D-Allose, the C-3 epimer of D-Glucose, is also classified as a rare sugar. Unlike D-Tagatose and D-allulose, it is an aldohexose because it is a glucose epimer and can be produced from D-allulose with the enzyme L-rhamnose isomerase. D-Allulose which is also a rare sugar is the C-3 epimer of D-fructose. Although it is known as rare sugar, a way has been discovered to mass-produce it by the help of the enzyme D-tagatose 3-epimerase (DTE) which is obtained from *Pseudomonas cichorii or Rhodobacter sphaeroides* and the enzyme D-Allulose 3 epimerase fro*m Agrobacterium tumefaciens*. Ken Izumori a professor at Kagawa University, Japan introduced the *Izumoring* strategy, which is based on the hypothesis that all hexagons can be synthesized using this enzyme. The aforementioned enzymes can catalyse the isomerisation of D- fructose to D-Allulose (Ooshima and Sun, 2013). Therefore, D-Allulose can be obtained from the hydrolysis of sucrose followed by isomerisation of glucose to fructose (Chung *et al.*, 2012; Ochiai *et al.*, 2014). Using this method to produce D-Allulose is cost effective and provides an opportunity to use it in various research areas. In addition to these microorganisms and the Izumoring strategy, Ken

Izumori has discovered Zuina tree which produces D-Allulose naturally. İzumori has been trying to expand production of this plant (Ushijima, 2014; Hossain *et al.*, 2015).

D-Allulose is not just important as it is a rare sugar, but it is a potential sucrose substitute. The sweetness of D-Allulose is equivalent to 70% of the sucrose. Although sweetness is lower than sucrose, the caloric value of rare sugar is much lower approximately 0.39 kcal/g (O'Charoen *et al.*, 2014). In 2012, D-Allulose has also been accepted as GRAS (Generally Recognized as Safe) by FDA Moreover in April 2019, FDA announced that D-Allulose would not be included under 'sugars' category in food labels. In Europe, EFSA still did not approve the use of D-Allulose.

Now, Allulose is commercially available in the market. The producers are from Japan, South Korea and Unites States. In Japan, D-Allulose is now produced in the *International Institute of Rare Sugar Research and Education Center* at Kagawa University. Some other producers and sellers also exist in the market such as Bonumose (U.S.A), Astraea (Japan/U.S.A), , CJ Cheiljedang (South Korea), AllSweet Anderson Global Group (U.S.A), and Tate and Lyle (UK). The market price of the D-Allulose changes according to the seller and it is around ~$20/kg.

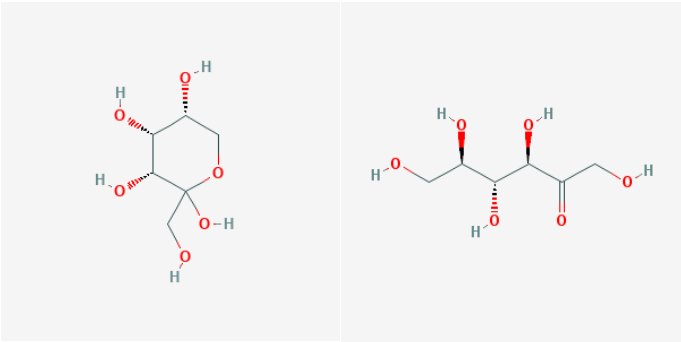

Figure 1.1. *Chemical structure D-Allulose*

1.1.2. Health Benefits and Advantages of D-Allulose

D-Allulose has now attracted attention the most over other rare sugars since it has many advantages. The first and most known advantage is that it has low caloric value and also it was found that that it had a strong effect on glycaemic index (Ochiai *et al.*, 2014). It was shown that it could prevent blood glucose level elevation. There are two mechanisms that can explain the prevention of blood glucose level. One of them is that D-Allulose inhibits α-glucosidase activity in the small intestine and therefore suppresses the absorption of glucose. The second mechanism is that D-Allulose enhances the conversion of glucose to glycogen in liver so the glycaemic response of the body decreases (Shintani *et al.*, 2017). Due to these properties against obesity and diabetes, it gained so much attention (Nagata *et al.*, 2018). Studies pointed out that in rats fed by D-Allulose, the sugar readily entered the bloodstream and approximately 70% of the D-Allulose was absorbed and left the body through urine within 24 hours completely (Hossain *et al.*, 2015). D-Allulose is absorbed in the small intestines but it is not significantly metabolized (Chung *et al.*, 2012; Ooshima and Sun, 2013) thus it does not contribute any calorie. In addition to its no-calorie property, it has been suggested that food intake is lowered when D-Allulose is used in the diet. In a study, it was reported that rats who were fed by D-Allulose containing diet had less food intake than rats who were fed by a cellulose or starch diet (Ochiai *et al.*, 2014). This property of D-Allulose was related to its anti-obesity feature and it was suggested that D-Allulose suppresses food intake (Ochiai *et al.*, 2014).

The products that contain D-Allulose has also found to show hypolipidemic activity. This means D-Allulose has a potential to decrease lipid concentration in blood. This can lead to lowered abdominal fat accumulation in the body and as a result lowering on the body weight gain. This mechanism can be explained by the effect of D-Allulose reducing and/or inhibiting the lipogenic enzyme activity such as *fatty acid synthase* (FAS) (Chung *et al.*, 2012). It was reported that fructose had an ability to enhance FAS activity more efficiently than glucose (Ochiai *et al.*, 2014). As a result, obesity and obesity related diseases was strongly linked to this enzyme activity. Moreover, it

was clinically proved that D-Allulose eliminated the fat accumulation in rats which were fed by 5% D-Allulose in a high sucrose diet (Ochiai *et al.*, 2014).

Another advantage of D-Allulose is that it has a high potential of antioxidant activity. Maillard reaction is the non-enzymatic reaction that takes place between reducing sugars and proteins. The end products of this reaction are called Maillard Reaction Products (MRP) and it is known that MRPs can have antioxidant activity (Amarowicz, 2009). It was proven that D-Allulose had more capacity to interact with proteins than other reducing hexose sugars. There has been a research conducted with egg white protein and D-Allulose and other six-carbon sugars. The MRPs that were formed by the reaction between D-Allulose and proteins showed better oxygen scavenging effect and antioxidant activity (Sun *et al.*, 2007). On the other hand, during Maillard reaction, D-Allulose showed stability against temperature and pH elevation. Therefore, higher pH and temperature resulted in intense brown colour, higher level of D-Allulose degradation. (Oshima *et al.*, 2014).

1.2. Pectin

Pectin is a water-soluble complex colloidal polysaccharide that is mainly found in plant cell walls. It consists of D-galacturonic acid units which are linked by α-1,4 glycosidic linkages. The primary role of pectin is to give strength to the plant. Chemical structure of pectin is shown in Figure 1.2. Despite the shown figure below, the exact chemical composition and structure is not clearly understood due to its very complicated structure and its changing nature during isolation or storage or processing. Generally, it is accepted that pectin is a heterogeneous molecule which consists of 2 main parts: *homogalacturonan* and *rhamnogalacturonan*. Homogalacturonan is the linear part of the pectin and composed of α-1,4 galacturonic acid units. These galacturonic acid groups contain carboxylic acid groups which may or may not methyl-esterified at carbon-6. Rhamnogalacturonan is responsible for the complex part of the pectin and composed of α-1,2 L-Rhamnosyl-α-1,L D-galactosylunoric acid units (Thakur *et al.*, 1997).

Generally, commercial pectin is obtained from apple pomace and citrus peels (Thakur *et al.,* 1997). Apple pomace has 15 to 20 % of pectin on dry basis, while citrus peel has 30 to 35% of pectin on dry basis (Lara-Espinoza *et al.,* 2018a). However, pectin obtained from these various sources do not have the same gelling property since they have different molecular weight and degree of esterification (Thakur *et al.,* 1997). When pectin is obtained, it is commercially used for its thickening, gelling and stabilizing properties. Due to its gelling property, it is mainly used in jam and jelly making processes.

There are two types of pectin which are classified by their degree of esterification. If degree of esterification of pectin is lower than 50, it is called low methoxyl pectin (LMP). Bivalent ions such as calcium chloride are needed to form LMP gels. If the degree of esterification is higher or equal to 50, then it is called high methoxyl pectin (HMP). Acidic conditions and sugar are needed to form HMP gels with the help of hydrogen bonding (Sessler *et al.,* 2013).

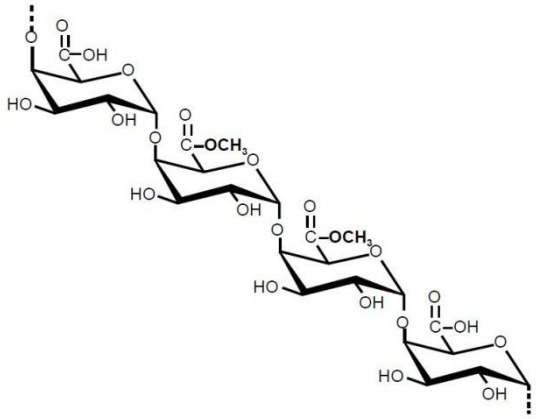

Figure 1.2. *Repeating parts of Pectin (A. Allwyn Sundar Raj*, S. Rubila, 2012)*

1.2.1. Gelation Mechanism of Pectin

High methoxyl pectin (HMP) gelation mechanism differs than low methoxyl pectin (LMP) gelation as mentioned above. For LMP gels, divalent ion such as calcium (Ca^{+2}) is required, no acid or sugar is needed to form a compact gel. For HMP gels, sugar and an acidic condition are essential so that pectin can form a strong gel structure. Sugar is added to obtain high methoxyl pectin gels because the environment must be dehydrated so that pectin molecules do not make H-bond with available water and they make bonds with each other to form the crosslinked structure (Buone, Donatella; Giacomazza, Daniela; Manno, Mauro; Martorana, Vincenzo; San Biagio, 2010; Sessler *et al.*, 2013). Pectin molecules form this network by both hydrogen bonding and hydrophobic interactions between each other (Thakur *et al.*, 1997). Figure 1.3. shows hydrogen bonds and hydrophobic interactions between methyl ester groups of pectin molecules at its junction zones. On the other hand, acid also plays important role since pectin molecules are negatively charged due to the carboxylic acid units present in the structure (Lam *et al.*, 2007). Charge of the pectin molecules is affected by H^+ ions and the carboxylic acid groups becomes protonated (-COOH) so that pectin molecules are forced to interact between each other since the repulsion due to negative charges has been removed (Thakur *et al.*, 1997). In fact, this protonation provides the hydrogen bonding and this the main gelling mechanism in HMP.

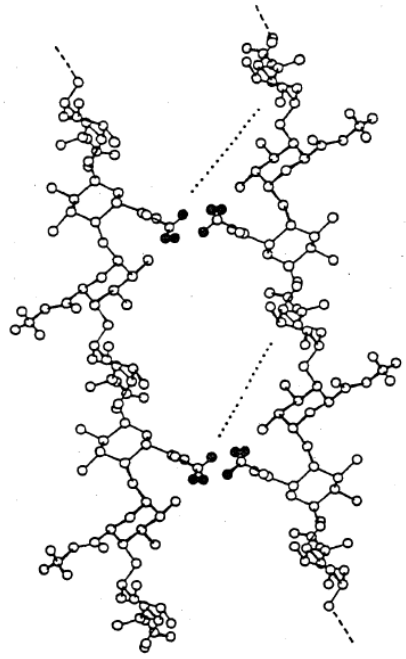

Figure 1.3. *Hydrogen bond (dotted lines) and hydrophobic interactions (filled circles) between pectin molecules at junction zones*

On the other hand, low methoxyl pectin (LMP) gel mechanism is completely different from HMP. Here, no sugar or acidic condition is needed. LMP gels can easily be formed without these ingredients. The only thing needed is divalent cations such as Ca^{+2}. By adding divalent cation, at the smooth regions of homogalacturonic acid units, intermolecular junction zones are formed between carboxylic acid groups. These zones are attributed to egg box model as shown in Figure 1.4. Electrostatic interaction and the ionic bonding of carboxylic acid groups are the main associations to form LMP gels (Lara-Espinoza *et al.*, 2018b).

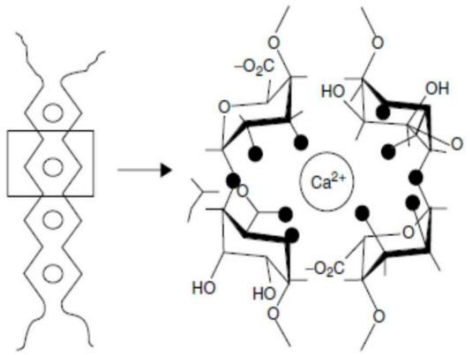

Figure 1.4. *Egg box model for the gelation of Low Methoxyl Pectin gels*

1.3. Soy Protein

Soybean has been widely used in Asia over 300 years and now lately it is getting attention all over the world due to its high protein content. Soybean approximately consists of 38% of protein, 18% of oil, 14% of water, 15% of soluble carbohydrate and 15% of insoluble carbohydrate.

Soybean protein is considered as complete source of protein since it contains all the essential amino acids human body needs (Michelfelder, 2009) hence it fulfils a good amino acid balance. As being a complete source of protein, it is one of the most popular vegetarian sources for protein in the human diet (Michelfelder, 2009). There are different versions of soybean proteins in the market. These are generally classified as soy protein isolates (SPIs), soy protein concentrates (SPCs) and texturized soy protein. Amino acid content of soybean proteins per 16 g of Nitrogen is given in Table 1.1. (Wolf, 1970; Singh *et al.*, 2008).

In addition to its amino acid content, soy protein also adds value to the product. It enhances the quality through improving texture, water binding ability, oil binding capacity, foam ability, gelation, emulsification, viscosity, etc. Soy proteins show excellent gelling and emulsifying properties which can strengthen the food structure. Also, they show higher water holding capacity and oil holding capacity due to their polar structure (Nishinari *et al.*, 2014).

Soy proteins mainly consist of globulins which are insoluble at their isoelectric point that is between pH 4-5 (Wolf, 1970; Nishinari *et al.*, 2014; Luisa *et al.*, 2017). They become soluble when pH is below or above the isoelectric point.

Aside from these, soy proteins have numerous health benefits to human. Soy protein contains essential components which are proved to reduce cholesterol and the risk of cardiovascular diseases (Food and Drug Administration, 1999). They are known to reduce total cholesterol, low-density lipoprotein (LDL) (also known as bad cholesterol) and triglyceride levels in the blood stream. Also, High-density lipoprotein (HDL) known as good cholesterol was found to increase with soy protein consumption. Moreover, for women, soy protein is effective on adverse of the menopausal hot flashes. In postmenopausal period, bone density reduction is very common. Soy protein is proved to be effective on the fracture of the bones and is known to improve bone mineral density (Wolf, 1970; Michelfelder, 2009).

Table 1.1. *Amino acid composition of forms of Soybean Protein* (Wolf, 1970)

	(Gram amino acid per 16 grams of Nitrogen)		
Amino Acid	**Soy protein isolates**	**Concentrates**	**Meals**
Cysteine	1.00	1.60	1.60
Isoleucine	5.00	4.90	5.10
Leucine	7.90	8.00	7.70
Lysine	5.70	6.60	6.90
Methionine	1.30	1.30	1.60
Phenylalanine	5.90	5.30	5.00
Threonine	3.80	4.30	4.30
Tryptophan	1.00	1.40	1.30
Valine	5.20	5.00	5.40

Table 1.2. *Energy values of different forms of soy protein* (Wolf, 1970)

Soy protein product	Energy (kcal/100g)
Soybean concentrate	328
Soybean Isolate	334
Texturized Soy Protein	340

1.3.1. Soy protein isolate (SPI)

Soybean contains approximately 40% protein and almost 20% of oil and that oil is removed so that soy protein isolate is obtained at a high purity over 90%. Among this 90% of the protein, β-conglycinin and glycinin are the dominant proteins (Nishinari *et al.*, 2014). Due to this high protein content, soy protein isolate can be classified as the most functional product of soy protein. Among other products, SPI has highest protein content and has the highest water binding capacity (35g/100g) (Were *et al.*, 2006). Soy protein isolate can be used in a variety of food applications. Due to the differences on the procedure of producing soy protein isolates, their purposes differ. Some isolates can be used for their ability to emulsify fat and bind water while some isolates can be used for its enhancive effect on texture , controlling viscosity by making them creamy or more appropriate for mouthfeel (Wolf, 1970). For gel structure, soy protein isolate makes firmer, harder and more resilient gels than other soy protein products (Jideani, 2012).

1.3.2. Soy protein-pectin interaction

Soy protein and pectin interaction has been widely studied. Confections, hydrogels, biopolymers and emulsion-filled gels are some of the studies to understand the complexity of pectin-soy protein interaction (Lam *et al.*, 2007; Jaramillo *et al.*, 2011; Sessler *et al.*, 2013; Luisa *et al.*, 2017; Feng *et al.*, 2019). The common point obtained from all these studies is that at its isoelectric point, soy protein was almost insoluble. However, adding polysaccharide to the network increases solubility of the soy protein by preventing protein aggregation due to electrostatic interaction.

During pectin gelation, soy protein increased the textural attributes of gel network. Soy protein isolate is added to the system since it is desired to dehydrate the environment by its excellent water binding ability and so that pectin gel network is strengthened. Another expectation from soy protein in gelation is that since pectin is negatively charged and soy protein is positively charged in acidic conditions (pH below 4.6) (Jaramillo *et al.*, 2011), there might be a electrostatic interaction between them so that gel network is strengthened. As a result, mechanical strength, overall physical attributes, appearance and dissolution behaviour of the pectin gel structure differs when soy protein is added to the system (Sessler *et al.*, 2013).

1.4. Confectionary Products

Confectionary products have been in human's life for over 3,000 years (Mansvelt *et al.*, 2012). Confectionary products consist of hard candy, soft candy, jellies, gummies, chewing gum, fondants, marshmallow, chocolate and chocolate based products (Chaven, 2014).

When confectionary word comes up, the first word comes to mind is sugar. 3,000 years ago, the main ingredient was honey to give sweetness. Nowadays, the main ingredient has changed to sugar and its derivatives due to lower cost and easy access to main ingredient. Sugar can be extracted from sugar beets and sugar canes. Moreover, instead of sugar, invert sugar, glucose syrup, High Fructose Corn Syrup (HFCS), molasses and golden syrup are also used as main ingredients. Corn syrup is generally used for these products since it gives elasticity and also the cost of the candy can be reduced significantly. Other minor ingredients on the formulations can be listed as condensed milk, milk powder, butter, emulsifier, fats and flavours. However since sweetness of corn syrup is not enough it is still needed to be used together with sucrose.

Confections can be categorized by their physical state whether it is amorphous/non-crystalline, or it is crystalline. Non-crystalline ones can be categorized as caramels,

hard candy, jellies and gummies while crystalline ones are fondants, creams, chocolates, and fudges (Mansvelt *et al.*, 2012).

1.4.1. Jellies and Gummies

These products are often known with their chewy like structure. To give chewy like structure, pectin, starch, gelatin or other agents may be used. While pectin and starch are irreversible gelling agents, gelatin is a thermoreversible gelling agent (Edwards, 2009). Pectin requires sugar and acid to form a gel (Lara-Espinoza *et al.*, 2018b) while starch needs boiling water to gelatinize (Siegwein *et al.*, 2011). For starch based candies, the sugar concentration is should not be above 50% since higher sweetener concentration can block starch gelatinization (Mansvelt *et al.*, 2012). Gelatin needs to be soaked in water before mixing with other ingredients and cooking (Pocan *et al.*, 2019). Moisture content of these products generally changes between 8 to 22% while equilibrium relative humidity changes between 50 to 75 % (Ergun *et al.*, 2010).

1.4.2. Hard Candy

Hard candy products as name implies are hard and have strict shape such as lollipop or candy canes. These are in glassy state and not in crystalline form. To obtain hard candy, the ratio of sugar to corn syrup is generally 70 to 30; however, this may vary by the product up to 50 to 50 if it is centre-filled hard candies. The average moisture content of these products are between 1-3% and equilibrium relative humidity changes between 26 to 32% (Ergun *et al.*, 2010; Mansvelt *et al.*, 2012). Generally, ingredients are sugar and corn syrup. These are mixed and boiled until all the moisture is evaporated where moisture level is approximately 2%. After evaporation, flavour, colour and organic acid such as citric acid are added. These are added after evaporation since the loss of these compounds is quite easy. Addition of acidulant is usually done for enhancing the fruit flavour (Smidova *et al.*, 2003). Typical hard candy flow chart is given in Figure 1.5.

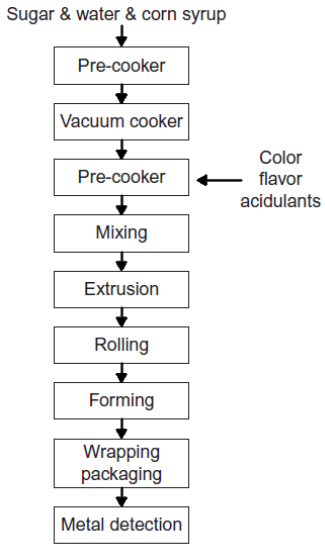

Figure 1.5. *Flow Chart of Hard Candy Processing* (Chaven, 2014)

1.5. Nuclear Magnetic Resonance

1.5.1. Time Domain (TD) Nuclear Magnetic Resonance (NMR)

Time domain NMR also mostly known as low field NMR due to magnetic fields used for these experiments being lower compared to higher field spectroscopy systems, is becoming more popular for food characterisation. TD-NMR is a non-invasive and non-destructive technique used for obtaining information about the food samples both chemical, structural and at molecular level.

NMR was not a method for food samples when it was first discovered due to unaffordable cost, lack of NMR equipment specifically designed for food samples and the lack of NMR knowledge among food scientist. However, recently, TD-NMR devices have started to be used for food samples since now portable, low-cost, and bench top types are in use at frequencies around 10-25 MHz.

In TD-NMR experiments, relaxation times are usually obtained and interpreted. NMR Relaxometry is a technique based on the measurement of relaxation times T_1 and T_2. T_1 is defined as the rate of recovery of longitudinal magnetization of the spins and T_2

is characterized by the exponential decay of transverse magnetization when short time radio frequency pulse (RF) is applied. T_1 is also known as the longitudinal relaxation time which can be defined as the energy release to the environment by the nuclei which is in excited state whereas T_2 is known as the transverse relaxation time and it can be defined as the energy release between adjacent nuclei (Hashemi *et al.*, 2012; Kirtil and Oztop, 2016; Parlak and Güzeler, 2016).

T_1 and T_2 relaxation yield exponential signal curves. Foods as being complex samples could have more than one relaxation time. In such cases, relaxation signal should be fitted to a multiexponential model. Fitting multiexponential decay and finding the components requires the use of Inverse Laplace transform which could be an ill posed problem in certain cases. Different algorithms have been developed for that purpose. Once the multiexponential fitting is achieved a relaxation spectrum is obtained. A relaxation spectrum is the output of the NMR signals which is obtained by applying Inverse Laplace Transform method to the signal curve. This relaxation spectra gives information about the proton pools that food samples contain (Pocan *et al.*, 2019)

In the literature, NMR relaxometry experiments has been conducted for different types of foods. In meat and poultry products it was used to examine the origin of the product as well as changes occurred after slaughtering (Graham *et al.*, 2010). In another study it was used to understand the water holding capacity and mobility of the water in cheese (Castell-Palou *et al.*, 2011). NMR was also used in fruits and vegetables to determine the chemical composition. For instance, to determine the formic acid content of apple juice, NMR relaxometry was a great tool (Berregi *et al.*, 2007). Swelling capacity of protein hydrogels were also studied in by this technique (Ozel *et al.*, 2017c). Although soft candies could be considered as a hydrogel, TD-NMR relaxometry studies on confectionary products are seemed to be less studied in the literature.

1.5.2. Fast Field Cycling (FFC) NMR Relaxometry

Fast Field cycling is also an NMR technique that can operate from low field to high field and it is getting more attention as new applications are discovered. FFC has become more famous lately, since latest developments made it possible to use it on bench top. FFC NMR works in a wide range of magnetic field strength with a single equipment to measure spin-lattice relaxation time (T_1) (Steele *et al.*, 2016) or longitudinal spin relaxation rate which is $1/T_1$ (R_1). R_1 of the sample can show differences when there is a change in the molecular dynamics of the sample such as change in viscosity, change of physical state of material, change in concentration etc. Once T_1 data is collected a Nuclear Magnetic Resonance Dispersion (NMRD) profile is obtained. NMR dispersion curve is the plot of R_1 or T_1 vs Larmor Frequency of the magnetic field. So, more information could be obtained about the molecular dynamics of samples. It can operate between very low frequency values like a few kHz to very high frequency values like 100 MHz (Steele, 2018).

From the obtained T_1 curves, a lot of information can be collected. Characterization of porous systems (Godefroy and Callaghan, 2003), determination of activation energies (Ladd-Parada *et al.*, 2019), differentiation of amorphous and crystalline participation in a polymeric medium, calculation of diffusion coefficient (Kimmich and Anoardo, 2004) can be determined by the help of FFC relaxation.

There are also some food applications of FFC NMR which are not that much. It was used in cheese and blueberry for characterization purposes and also it was used in vinegar to examine adulteration (Godefroy *et al.*, 2003; Baroni *et al.*, 2009; Capitani *et al.*, 2014). To characterize frankfurter from different meat sources, FFC was also used (Uguz *et al.*, 2019). Although it has been used in different food samples for different purposes, FFC has not been used in confectionary products neither characterization nor differentiation purposes.

1.6. *In Vitro* Digestion

In vitro digestion models are commonly used to asses digestibility, physical and chemical changes and release behaviour of food samples. Generally *in vitro* digestion model consists of three parts: mouth, stomach and gastrointestinal tract (GI). Mouth and stomach are considered as where food samples are disintegrated, and GI tract is considered as where nutrients are absorbed (Kong and Singh, 2008a). Disintegration of the samples is important for digestion since it affects absorption.

Digestion starts in the mouth. Mouth digestion allows particles to become smaller in size by the help of chewing action. In mouth digestion, α-amylase is the responsible enzyme for digestion (Hepher, 2010). In addition to α-amylase, the saliva consists of salt, mucous and water. Also, by the help of saliva, mouth digestion helps the food sample to become lubricated and hydrated so that swallowing action gets easier. Moreover, with these slippery and smaller particles, food samples digestion in the stomach becomes easier as surface area of the sample gets bigger. The size of the sample after chewing changes with the food texture, moisture content, fat content etc. As an example, vegetables have higher size than nuts after mastication (Peyron *et al.*, 2004). Mouth digestion is faster than other steps and approximate digestion time is 280 ms for almost all kind of food samples (Bornhorst and Singh, 2012).

Stomach consists of different compartment as it can be seen in Figure 1.6. As the food is eaten, the layers are formed. Large and solid food particles are layered at the bottom as low-density food particles are layered at the top (Bornhorst and Singh, 2012). Gastric digestion has been less understood since there are various of factors that affect digestion. For example, mechanism of peristaltic movement is not fully comprehended or the effect of enzymes in gastric environment differs, pH of the stomach varies with the food swallowed etc. That's why every digestion modelling system has its own properties. Generally, average pH of the stomach is between 1.8-2.2 as it is at the fast state; however, after eating a meal, pH of the stomach is increased for a long time and it takes approximately 5 hours to get back to original pH value (Kong and Singh,

2008a; Mennah-Govela *et al.*, 2015a). Gastric juice mainly consists of water, pepsinogen (inactive state of pepsin), concentrated HCl and mucous. Gastric digestion has 3 main functions which are storage, mixing and emptying. Emptying step is very crucial for digestion since it is closely related to some diseases such as obesity and diabetes. If rapid emptying takes place, this may trigger overconsumption of calories. On the other hand, if slow emptying takes place, this could lead to reflux (Kong and Singh, 2008a).

- For *in vitro* digestion systems, the temperature is generally set to 37^{o}C to simulate the body temperature (Hur *et al.*, 2011a; Mennah-Govela and Bornhorst, 2016a, 2017).
- The general digestion time is 2 hours for *in vitro* gastric digestion (Hur *et al.*, 2011b).
- Pepsin, α-amylase, lipase, bile salts and pancreatin are the most common enzymes used in *in vitro* digestion systems (Hur *et al.*, 2011a).

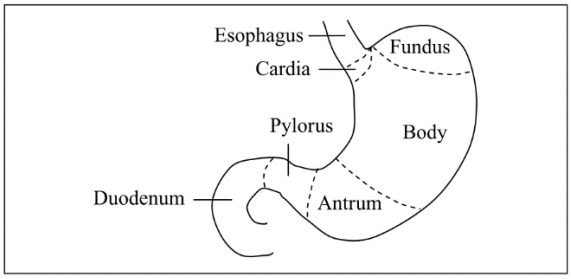

Figure 1.6. *Compartments of human stomach* (Kong and Singh, 2008a)

1.7. Objective of the Study

The objective of this study to obtain and characterize soy protein containing pectin based soft candies formulated with D-Allulose before and after *in vitro* gastric digestion. Use of different NMR Relaxometry techniques and interpreting these data with the physical properties of the candies can be defined as the specific objectives of the study.

Moreover, in the literature, there are studies that explored the gastric digestion of food and carbohydrates (Kong and Singh, 2008b; Bornhorst and Singh, 2012; Kaur *et al.*, 2014; Mennah-Govela *et al.*, 2015b; Mennah-Govela and Bornhorst, 2016b, 2017; Dalmau *et al.*, 2017). However, there is no study about gastric disintegration of confectionary products. Also, digestion study on rare sugar containing products has not been studied at all. Since low calorie foods are very trending topic, gastric disintegration is thought to be an important topic to study.

Hypothesis of the thesis study can be described as follows

Since soy proteins and sugar could affect the gelation behaviour of pectin, addition of soy protein D-Allulose to a pectin jelly will change the physical properties and digestion behaviour of the formulated confectionary products.

CHAPTER 2

MATERIAL AND METHOD

2.1. Materials

The materials that were used to prepare soft candy are as follows; High-Methoxyl Pectin, Sucrose, D-Allulose, Glucose Syrup (DE=42), Soy Protein Isolate, Citric Acid Monohydrate (60% w/v). High methoxyl pectin from sugar beet pulp with DE of 55% was used in this study which was kindly provided by Kervan Gıda A.Ş (İstanbul, Turkey). Soy protein isolate (SPI) with a protein content of over 90% was purchased from Alfsasol, Turkey. 42 DE glucose syrup was also provided by Kervan Gıda A.Ş. (İstanbul, Turkey). Sucrose (Keskinkılıç Gıda Sanayi ve Ticaret A.Ş., Gebze, Kocaeli) was bought from a local market in Ankara. D-Allulose (Santiva Inc, Downers Grove, IL, USA) was used as the rare sugar source. Citric acid monohydrate (ACS reagent ≥99.0%, Sigma- Aldrich Chemical Co., Saint Louis, MO, USA) was used to obtain high methoxyl pectin gels.

The materials that were used to prepare saliva and gastric juices are as follows; Mucin from porcine stomach (Sigma-Aldrich Chemical Co., Saint Louis, MO, USA), α-amylase (from *Bacillus subtilis*, MP Biomedicals, catalogue number 100447, activity of 160,000 BAU/g, Santa Ana, CA, U.S.A.), pepsin (from porcine gastric mucosa, MP Biomedicals, Solon, OH, USA, measured activity of 242 U/mg), NaCl (Sigma-Aldrich Chemical Co., Saint Louis, MO, USA), KCl (Sigma-Aldrich Chemical Co., Saint Louis, MO, USA), $NaHCO_3$(Sigma-Aldrich Chemical Co., Saint Louis, MO, USA)

2.2. Methods

2.2.1. Preparation of Pectin-based Soft Candies

All the ingredients are listed in previous section. First, glucose syrup was weighed into a beaker and then soy protein and water were added. The solution in the beaker was mixed by using a high shear homogenizer (ULTRA-TURRAX, WiseTisHG-15D, Wertheim, Germany) at 9,000 rpm for 1 minute. In another beaker, sucrose and D-Allulose were weighed. Then, on top of this mixture pectin was added. In this beaker, pectin and sugar mixture were dry mixed to prevent pectin aggregation during cooking. Following weighing, first mixture was put into oil bath and oil bath was set to 127.5 ^{o}C. Afterwards, the dry mix was added into the first prepared mixture and constantly stirred. When the temperature of the whole mixture reached 109 ^{o}C, citric acid solution (60% w/v) was added and the mixture was immediately poured into 3x3x3 cm cubic moulds. Flow chart of the candy production is given in Figure 2.1.

Soft candies were kept for 24 hours to set crosslinks. Experimental design of the study and the formulation of pectin based soft candies are given in Table 2.1. and 2.2 respectively.

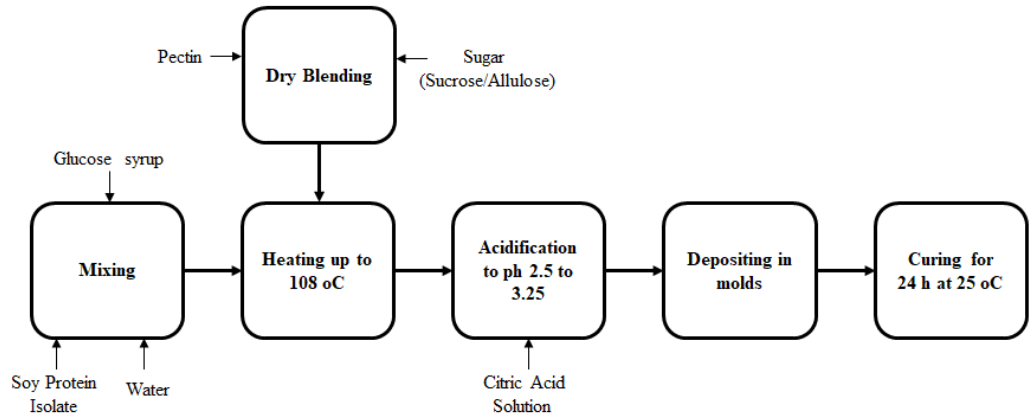

Figure 2.1. *Flow chart of pectin based soft candies*

Table 2.1. *Experimental Design of Soft Candies*

FACTORS	**LEVELS**
Gelling Agent Type	Pectin
Gelling Agent Concentration	Pectin (4%)
Soy Protein Concentration	0%, 1%
D-Allulose Concentration	0%, 15%, 25%, 35%
Sucrose Concentration	35%, 20%, 10%, 0%
Digestion Time	0, 10, 20, 30, 45, 60, 75, 90,120 min.

**D-Allulose and Sucrose concentration summation must be 35%.*

Table 2.2. *Formulation of Pectin based soft candies*

	Soy Protein	**Water**	**Sucrose**	**D-Allulose**
S0_SA_035	0	17	0	35
S0_SA_1025	0	17	10	25
S0_SA_2015	0	17	20	15
S0_SA_350	0	17	35	0
S1_SA_035	1	16	0	35
S1_SA_1025	1	16	10	25
S1_SA_2015	1	16	20	15
S1_SA_350	1	16	35	0

*All candy formulations contain 4% pectin, 40% glucose syrup, 4% Citric acid solution

2.2.2. Preparation of Simulated Saliva

Saliva was prepared by following the method of Mennah-Govela *et al (2015)*. Mucin, α-amylase, NaCl, KCl and $NaHCO_3$ were mixed in deionized water in the amounts given in Table 2.3. After mixing, pH was set to 7 by using 0.01 N NaOH (Sigma-Aldrich, Mo., USA).

Table 2.3. *Composition of Simulated Saliva and Simulated Gastric Fluid*

Ingredient	**Simulated Saliva**	**Simulated Gastric Fluid**
Mucin	1 g/L	1.5 g/L
NaCl	0.117 g/L	7.8 g/L
KCl	0.149 g/L	-
$NaHCO_3$	2.100 g/L	-
α-amylase	1.180 g/L	-
Pepsin	-	1 g/L

2.2.3. Preparation of Simulated Gastric Juice

Simulated Gastric Fluid (SGF) was also prepared by the method described by Mennah-Govela, Bornhorst and Singh (Mennah-Govela *et al.*, 2015b). All the ingredients shown in Table 2.3 (mucin, NaCl, and pepsin from porcine pancreas) were mixed in deionized water. Following mixing, pH was adjusted to 1.8 by using 3N HCl. Pepsin was added to the mixture just before digestion starts since pepsin activity starts when pH is very low and the optimum working temperature for pepsin is 37ºC. *In vitro* digestion experiments were conducted for 120 min.

2.2.4. Digestion conditions

To simulate gastric and oral digestion conditions, the following preparations were made. Approximately 35 grams of sample was prepared (3x3x3 cm-cubes) and placed it into 250 ml beaker. According to Bornhorst study, 0.2ml/g sample saliva was added into the beaker and shaken for 30 seconds to simulate oral digestion conditions (Mennah-Govela and Bornhorst, 2016a). Then, gastric juice (5ml/g sample) which was kept at 37^{o}C was immediately added to the beaker. The beaker was placed into shaken water bath (37^{o}C, 100 rpm) for 120 min (Mennah-Govela and Bornhorst, 2016a). Brix measurements were taken at 10, 20,30,45,60,75,90,120th min of the digestion. After digestion, samples were analysed for textural analysis, moisture content and pH.

Figure 2.2. *Schematic representation of oral and gastric digestion of the pectin-based sample*

2.3. Characterization of soft candies

To characterize the physical and chemical properties of the soft candies, the following experiments were done before and after digestion;

- Textural measurements,
- Brix measurements during digestion,
- Nuclear Magnetic Resonance (NMR) Relaxometry,
- Moisture content measurements,
- Scanning Electron Microscopy experiments (SEM).
- pH

The following experiments were only done to characterize the soft candy product; hence, they were only conducted before digestion:

- Colour,
- Water activity,
- X-Ray Diffraction
- Fast Field Cycling (FFC) NMR.

Scanning Electron Microscopy experiments were done to investigate morphological properties. X-Ray Diffraction experiments were conducted to explore the crystallization of the soft candies. In addition, to observe change in molecular dynamics on soft candy product, Fast Field Cycling NMR Relaxometry experiment were conducted.

2.3.1. Texture Profile Analysis

Hardness of the soft candies were measured by using Texture Analyser (Brookfield Ametek CT3, TA18 probe, Middleboro, MA, USA) . Load was set to 0.05N, number of cycles was two and test speed was adjusted to 1 mm/s. Measurements were conducted before and after digestion. Before digestion, the sample volume was 3*3*3 cm^3; however, after digestion, the samples were shrunken. In addition to hardness, cohesiveness and springiness were also recorded but since some of the data were not meaningfull, only hardness values were reported in this study.

2.3.2. Brix Measurements

Brix values of the gastric juice (following the saliva) were measured with respect to time by a hand refractometer (HANNA, HI96801, USA) while digestion is in progress. Each measurement was taken every 10 minutes of the 1^{st} hour, and every 15 minutes of 2^{nd} hour. Change in brix of the juice were associated as loss of soluble solid content of the soft candies.

2.3.3. Moisture Content

Moisture content of the candy samples before and after digestion were measured using a vacuum oven (DAIHAN, Germany) was used. The temperature was set to 70^{o}C and pressure was reduced to 0.1 MPa. Samples were kept in the oven for 3 hours to reach the equilibrium. Each sample was weighed before and after the drying to determine the moisture loss. Moisture content of the samples were calculated as follows;

$$Moisture\ Content(\%) = \frac{Initial\ Weight - Final\ Weight}{Initial\ Weight} * 100$$

2.3.4. Water Activity

Water activity of the samples were measured using an Aqualab 4TE instrument (METER Group, Pullman, WA, USA). Measurement was only done before digestion for characterization purposes. Temperature of the samples were around room temperature of 25 ^{o}C

2.3.5. Colour

Colour of the samples were only measured before digestion. Measurement was performed using Konica Minolta Spectrophotometer (CM-5, Japan).CIELAB method was used to interpret the data. L indicates brightness and (+) L means lighter, (-) L means darker while (+) a^* means red, (-) a^* means green and finally (+) b^* means yellow, (-) a^* means blue. Also, ΔE values were also evaluated to see differences between samples. Non-soy sucrose only sample (S0_SA_350) was selected as reference. Calculation of ΔE is as follows;

$$\Delta E = \sqrt{(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2}$$

2.3.6. pH

pH of the samples was determined by using a wireless pH-meter (Hanna Lab, FC2022, USA) which is appropriate for semi-solid food samples. pH of the samples was measured before and after digestion at room temperature.

2.3.7. Nuclear Magnetic Resonance Relaxometry Experiments

2.3.7.1. Time Domain NMR

Time Domain Nuclear Magnetic Resonance Relaxometry (Spin Track, Russia) experiments were performed at Middle East Technical University Food Engineering Laboratory. A 0.5 Tesla (20.34 MHz) system (Spin Track GmbH, Kircheim/Teck, Germany) was used for the experiments. CPMG (Carr-Purcell-Meiboom-Gill) sequence was used to measure T_2 relaxation times. A representative T_2 decay curve is shown in Fig. 2.2. Echo time and was set to 40 μs and the number of echoes changed between 400 to 900. Relaxation delay of 300 ms was used for all measurements. To explore the multi compartments in the samples XPFit (Alango Technologies LTD., Israel) software was used.

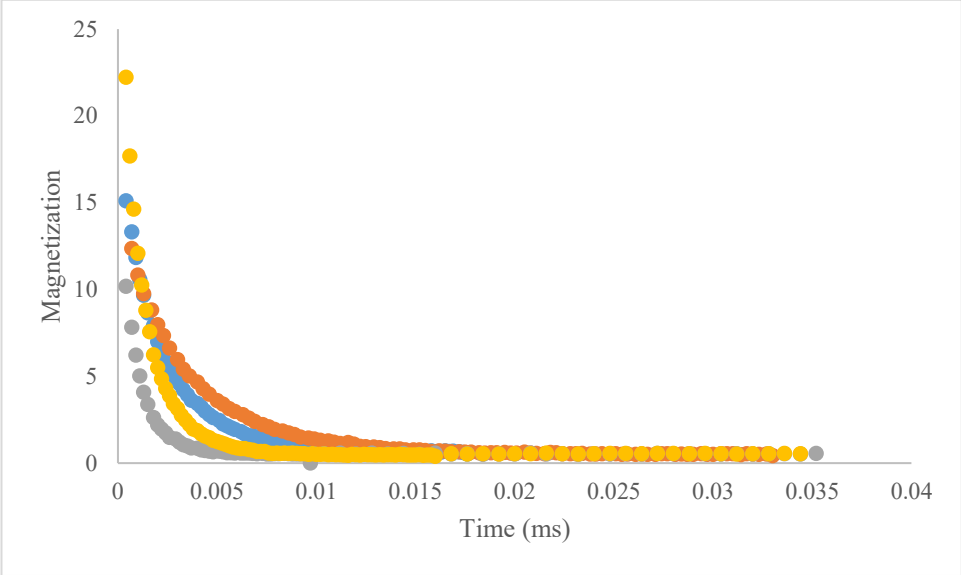

Figure 2.3. *CMPG decay curve of pectin based candies (S0_SA_035 (blue), S1_SA_035 (orange), S0_SA_350 (gray), S1_SA_350 (yellow))*

2.3.7.2. Fast Field Cycling (FFC) NMR Relaxometry

Fast Field Cycling Nuclear Magnetic resonance relaxometry experiments were conducted in the NMR Laboratory of Complex Fluids, NMR and Surfaces group of the centre of Physics and Engineering of Advanced Materials-CeFEMA at Instituto Superior Tecnico (IST) in Lisbon, Portugal. Fast Field Cycling experiments were conducted by using a home-developed equipment which operates at 0.215 Tesla with 3 ms switching times. Spin-Lattice relaxation times (T_1) were measured by 10 scans per measurement with a polarization time of 500 ms. The frequency changed between 1.5 to 300 MHz. Output of FFC experiment is known as the NMR dispersion curve and it is given in given as in Fig 2.3. The curve shows the dependence of T_1 relaxation time with respect to frequency (or magnetic field strength).

2.3.8. X-Ray Diffraction

The experiment was conducted in Middle East Technical University Central Laboratory using an X-Ray Diffractometer (Rigaku Ultima IV, Japan). Copper electrode was used, and the voltage value was 40 kV and ampere value were set to 30 mA. By 2θ scanning method and fixed grazing angle (minimum 0.1°), stronger signals were obtained. The samples were prepared as a thin layer film (1cm*1cm*7mm).

An example diffractogram showing the areas is given in Figure 2.4. Red line was used to calculate the crystalline area and green line was used to calculate the overall area of the samples.

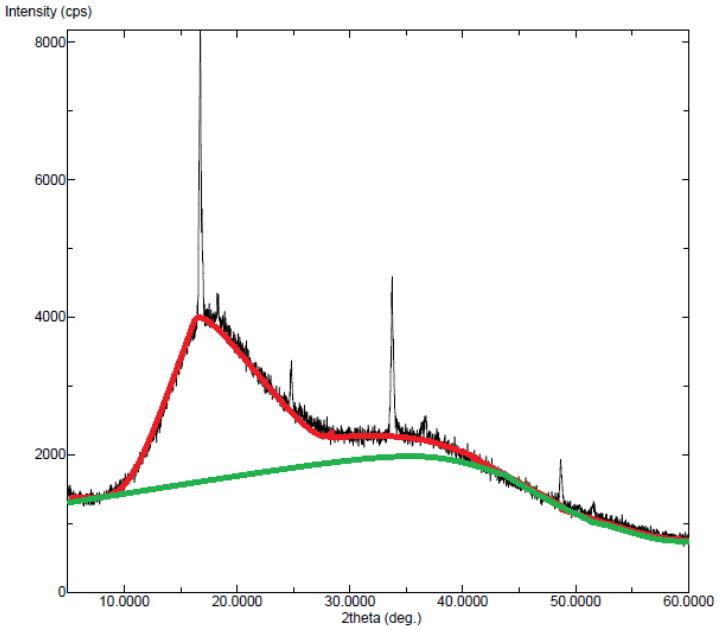

***Figure 2.4**. Example of X-Ray Diffractogram showing crystalline and total areas*

2.3.9. Scanning Electron Microscopy (SEM)

SEM analysis was conducted in METU Metallurgical and Materials Engineering Department. First, samples were freeze dried for 2 days and then samples were covered by Gold-Palladium alloy by HUMMLE VII Sputter Coating Device. Morphological differences were observed for different samples via JSM-6400 Scanning Electron Microscope (JEOL, Equipped with NORAN system 6 X-ray Microanalysis system and semaphore digitizer, Westhorst, NL). Magnification is same for all samples and it is 100X and the accelerating rate of electron beam is 5 kV.

2.3.10. Statistical Analysis

Experiments were carried out with duplicates and/or triplicates. All experimental data were analysed to compare whether there is significant change between samples by ANOVA (analysis of variance) by Minitab V17 (Minitab Inc., Coventry, UK). Tukey's comparison test was used at 95% of confidence interval. Assumptions of ANOVA were checked before analysis and outliers were removed from the data set if necessary.

CHAPTER 3

RESULTS AND DISCUSSION

3.1. Texture Profile Analysis

Texture is a very significant quality indicator for confectionery products. Usually, a firm and chewy structure is expected in a commercial soft candy. So, the main aim in this study is to obtain a hard and chewy structure for pectin-based candies. In this study, hardness measurements were reported as stated in the previous section.

To obtain high methoxyl pectin gels, sugar is needed to dehydrate the environment and prevent pectin-water interaction. Also, acidic environment is needed to decrease the negative charge of the pectin so that repulsion between pectin molecules are eliminated and pectin-pectin interaction is favoured (Thakur *et al.*, 1997). These are the main factors that affect pectin gel strength and they can be associated with the hardness of the samples. Table 3.1. shows the hardness values of the pectin based soft candies before and after digestion.

Statistical results of the experiments are given in the Appendix. ANOVA and t-test results of hardness values are given Table A.1-A.3. ANOVA was conducted considering the factors of; D-Allulose content (0-35%) and the presence of soy protein (0, 1). For before and after digestion experiments separate ANOVA were conducted and to explore the effect of digestion, t-test was performed for the difference of the means of each sample at 95% confidence level.

As will be seen in the Appendix tables, when the main effects were explored, both D-Allulose content and the presence of soy protein and their interactions were found to be significant ($p<0.05$). Addition of soy protein increased the hardness values of the samples.

For candies without soy protein; generally, hardness increased as D-Allulose concentration increased up to 25%; however, for only D-Allulose containing ones, hardness decreased and came back to the same hardness value of sucrose only containing one. Therefore, it was concluded that sucrose and D-Allulose containing ones showed synergistic effect and increased hardness more than only sucrose or D-Allulose containing ones.

Soy protein addition to the system generally increased the hardness of the samples significantly ($p<0.05$). This might be due to possible pectin-soy gelation mechanism. To explain this further, a separate experiment was conducted; 4% pectin and 1% soy protein solutions were prepared without sugar and one set of the solutions was heated up to 109^{o}C for 3 hours (same conditions for the candies) and the other one was not heated. To understand what changes occurred in the solutions with heating, NMR Relaxometry experiments were performed. T_2 relaxation times were measured and a multiexponential model was tried to be fit to the data for the heated/unheated solutions. Results showed that there was only 1 component in the heated samples and 2 components in the unheated samples. The meaning of these components will further be explained in the NMR section. At that point it is sufficient to say that these 2 components were different proton pools. In the absence of sugar, heating resulted in a more homogenous solution thus 1 component was observed which could further indicate that soy protein also had role on the gelation of pectin during heating and this effect resulted in increase on the hardness of the samples. The presence of less components in the polymer mixture might be associated with a strong pectin-soy protein interaction. In the literature, there are few examples of implying that there could form a crosslink between pectin and soy protein (Lam *et al.*, 2007; Piazza *et al.*, 2009; Jaramillo *et al.*, 2011; Sessler *et al.*, 2013).

The addition of a soy protein to the candy matrix also brings Maillard reaction to the scene. Due to corn syrup present at a fix concentration in all matrices, reducing sugar was present in all formulations and with D-Allulose addition concentration of reducing

sugar increased further. A study revealed that Maillard reaction which took place in the presence of egg white and galactomannan was an effective method to obtain firmer gels with higher strength (Matsudomi *et al.*, 2002). Since in this study, soy protein isolate was used, Maillard reaction definitely occurred and this might have contributed to overall hardness of the candies.

Although the soy protein containing samples were higher in hardness due to soy-pectin gelation enhancement, when the hardness of the soy protein containing candies were investigated with respect to D-allulose concentration it was observed that addition of D-Allulose increased the hardness values of the candies. This result was not expected since D-Allulose was not very good at water binding (Ikeda *et al.*, 2011; Pocan *et al.*, 2019) and there was possibly still free water in the gel matrix which could also hinder the gelation ability of pectin. As it was explained before, sugar was added to the HMP solutions to dehydrate the system so, possible pectin-water interaction which generally lead to weak gel structure was eliminated. Since D-Allulose could not eliminate water in the system and therefore could not reduce water-pectin interaction, D-Allulose containing candies were expected to have a softer texture than sucrose containing candies. However, another mechanism existed in the candies and that was Maillard Browning reaction. Since the addition of soy protein promoted Maillard browning reactions and it was known that D-allulose participated in Maillard reaction more compared to other monosaccharides (O'Charoen *et al.*, 2014). Thus, as Maillard occurred more, higher gel strength was obtained.

Gastric digestion experiments were performed at simulated gastric fluids followed by exposing the samples to simulated saliva. Temperature was kept at 37 ^{o}C. As this was a model study, effect of chewing and peristaltic movement was neglected. This is how digestion has been studied in many researches (Bornhorst and Singh, 2012; Kaur *et al.*, 2014; Mennah-Govela *et al.*, 2015b; Mennah-Govela and Bornhorst, 2017). In this study, the goal was to see how the digestion environment would affect these different

formulations. Since the temperature was 37 ^{o}C, and samples were containing significant amount of sugar, decrease in size of the candies was observed.

ANOVA was conducted separately for the digested samples and when the main effects were examined (Table A.2) it was seen that soy protein addition did not result in a difference ($p>0.05$). That was reasonable, as being a protein, it was expected to be affected from the pepsin enzyme and the higher hardness values observed before digestion were no longer present due to digestion ($p>0.05$). On the other hand, effect of D-Allulose concentration was still significant ($p<0.05$), but the trend changed compared to 'before digestion' results. Sucrose only containing formulations were found to have higher hardness values than the D-Allulose only containing ones ($p<0.05$) as will be explained later for soy protein containing candies.

Hardness values of before and after digestion were compared by the differences of means using t test at 95 % significance level (Table A.3). After 2 hours of gastric digestion, hardness value of the candies generally decreased. This decrease was significant for non-soy protein containing candies except for non-soy sucrose only containing formulation. It was also significant for 1% soy protein containing candies except for sucrose only and D-Allulose only containing ones. During digestion, sugar is expected to dissolve, and soy protein is digested in the gastric juice. As a result, weakness in the gel structure were observed and softer gels were obtained. However, sucrose containing candies hardness values in the absence and presence of soy protein did not significantly change. As explained before, effect of soy protein was found to be insignificant due to the digestion of the protein. The reason for the 'no change in sucrose only formulation' could be explained as follows. Sucrose formed a more compact crosslink structure than D-Allulose since D-Allulose let pectin to interact with water due to its low water binding capacity (Ikeda *et al.*, 2011). Therefore, D-Allulose gel structure was easily broken; however, sucrose did not let this happen and its gel structure was not destroyed easily since sucrose did not let pectin-water interaction in the first place.

Table 3.1. Hardness of candies before and after digestion

	Hardness (N)	
	Before Digestion	*After Digestion*
S0_SA_035	11.65±0.41 ^{d,A}	13.02±1.21 ^{b,B}
S0_SA_1025	15.45±1.22 ^{ab,A}	13.10±0.75 ^{b,B}
S0_SA_2015	17.14±0.76 ^{bc,A}	11.53±0.75 ^{ab,B}
S0_SA_350	11.48+0.11 ^{d,A}	12.36±0.68 ^{b,A}
S1_SA_035	19.22±0.66 ^{cd,A}	17.75±0.39^{b,A}
S1_SA_1025	20.05±0.43 ^{bc,A}	13.72±0.40 ^{b,B}
S1_SA_2015	15.11±0.46 ^{a,A}	9.74±0.68 ^{b,B}
S1_SA_350	13.05±1.08 ^{a,A}	11.48±2.04 ^{a,A}

Small letters (a-d) means that they differ significantly for each sample in the same column; Capital letters (A-B) means that they differ significantly for each sample in the same row.

3.2. Brix Measurements

Brix measurements of the samples were conducted during the 2 hours of gastric digestion. Figure 3.1. shows how brix changed during digestion. Brix values increased with time. This was expected since sugar in the matrix was dissolving and soy protein was being digested. For non-soy containing candies, increase in brix did not significantly change as for soy protein containing candies (Table 3.2.). Brix means soluble dry matter in a matrix. Although the ratio of D-Allulose / sucrose in the formulations were different, the total sugar content did not change, so it was normal for each sample to have the same increase. Soy protein containing candies had higher brix values than non-soy protein containing candies since soy protein was introduced in these formulations as an additional soluble dry matter.

When the behaviour of the Brix values was investigated with respect to time, it was observed that increase exhibited as Fickian like diffusion. Since there was also erosion taking place on the gels, moving boundaries existed and it was not easy to model the erosion through a classical mathematical approach. Also, for food disintegration in stomach, Power law model was suggested some researchers (Siegel *et al.*, 1988). A

model study was carried out with raw carrots and power law assumption was found to be good fit (Kong and Singh, 2008a). This approach has been used in many hydrogel studies to monitor diffusion (Ozel *et al.*, 2017c). Also, in the literature, there are lots of studies which used the power law assumption to food samples (Siegel *et al.*, 1988; Singh, 2007; Fabek, 2011; Mohos, 2017; Ozel *et al.*, 2017c). When power law model was applied to the disintegration of soft candies, it was seen that data fitted well to the power law model (R^2>0.95). Thus, a Power Law's approach was followed.

To make a comparison between the samples power law constants were calculated. Power Law equation is given as follows:

$$M_C/M_\infty = k * t^n \quad \text{.....(Eqn. 1)}$$

After logarithmic transformation;

$$\ln\left(M_c/M_\infty\right) = lnk + n * lnt \quad \text{.....(Eqn. 2)}$$

'k' can be considered as dissolution rate constant and n is the power law index. n indicates dissolution mechanism of the candies. Dissolution mechanism of the candies changes for different n values. If n is equal to 0.5, dissolution is diffusion controlled. If n is equal to 1, it means dissolution is swelling controlled. If n is between those, it means dissolution is controlled by both mechanisms meaning anomalous transport (Kim *et al.*, 2003; Siepmann and Peppas, 2012). M_c and M_∞ are the concentrations at time 't' and at equilibrium respectively M_c and M_∞ are the concentrations at time 't' and at equilibrium respectively.

M_∞ values were calculated considering Bornhorst's study as the reference (Mennah-Govela and Bornhorst, 2016a). In that study, for 20g of sample 100 ml digestion juice was used. Digestion experiments were also performed at this ratio in this study. So, if all of the sample were let to dissolve in gastric juice at equilibrium the brix value that can be reached was calculated to be 20g/ml. Therefore, 20 g/ml was used as an estimate for the equilibrium value of M_∞.

Table 3.3. shows the flow behaviour index of the samples. Dissolution rate constant gives information about how fast dissolution occur and samples disintegrates. As can be seen from the Table 3.3, dissolution rate constants of the non-soy protein containing candies were not different than %1 soy protein containing candies ($p>0.05$). Addition of soy protein did not significantly change the dissolution rate constant of each sample as can be seen in Table 3.3. As was explained in texture analysis part (Section 1), effect of soy protein was found to be insignificant during digestion since soy protein was digested in simulated gastric juice so, its effect was lost on the soft candy samples. Also, From Table 3.2. it can be seen that higher brix value at the end of 2 hours of digestion was obtained for soy protein containing candies. This means that at the same dissolution rate, soy protein isolate containing candies can reach higher brix values than non-soy protein isolate containing ones. This can be interpreted as follows, soy protein containing candies can easily dissolve and reach higher brix value so, soy protein containing confectionery gives feeling of satiety more.

***Table 3.2**. Maximum brix value after 2 hours of digestion for each sample*

Sample name	***Brix at 120 min***
S0_SA_035	8.00 ± 0.28^{c}
S0_SA_1025	8.35 ± 0.07^{c}
S0_SA_2015	7.45 ± 0.49^{c}
S0_SA_350	9.05 ± 0.21^{bc}
S1_SA_035	11.90 ± 0.28^{a}
S1_SA_1025	12.40 ± 0.48^{a}
S1_SA_2015	10.80 ± 0.48^{ab}
S1_SA_350	11.95 ± 0.21^{a}

Small letters (a-d) means that they differ significantly for each sample in the same column

Table 3.3. *Power Law index and Dissolution Constant*

	k	n	R^2
S0_SA_035	0.049 ± 0.0003^{c}	0.428 ± 0.007^{b}	0.981
S0_SA_1025	0.050 ± 0.0008^{c}	0.436 ± 0.002^{b}	0.981
S0_SA_2015	0.060 ± 0.0007^{a}	0.364 ± 0.008^{c}	0.979
S0_SA_350	0.052 ± 0.0020^{bc}	0.447 ± 0.003^{b}	0.992
S1_SA_035	0.054 ± 0.0004^{bc}	0.503 ± 0.006^{a}	0.998
S1_SA_1025	0.053 ± 0.0020^{bc}	0.509 ± 0.011^{a}	0.995
S1_SA_2015	0.056 ± 0.0023^{ab}	0.440 ± 0.006^{b}	0.974
S1_SA_350	0.052 ± 0.0011^{bc}	0.508 ± 0.004^{a}	0.996

Small letters (a-d) means that they differ significantly for each sample in the same column

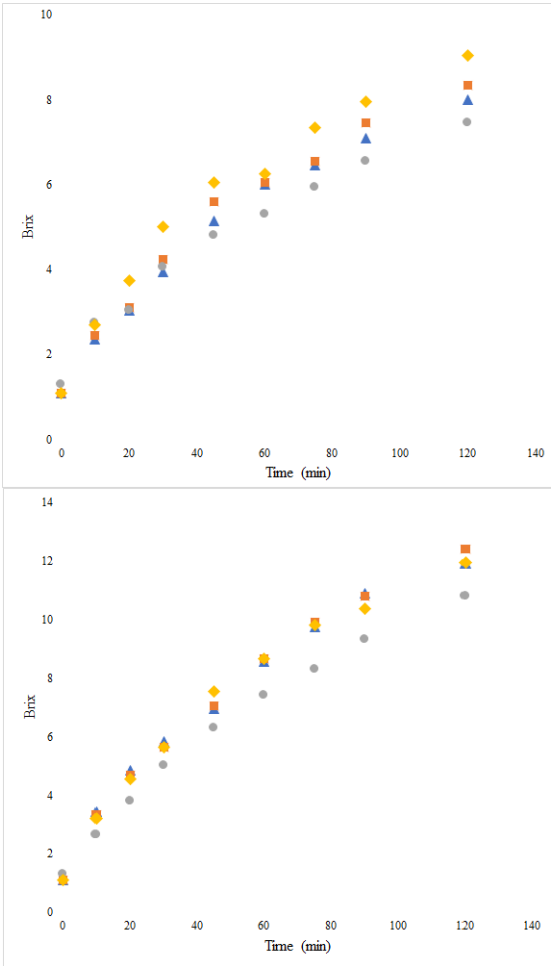

Figure 3.1. Brix values vs time (a) %0 soy protein, (b) 1% soy protein SA_035 (triangular), SA_1025 (square), SA_2015 (circle), SA_350 (pyramid)

3.3. Moisture Content

Water is the most essential ingredient in food products. It is closely related to the shelf life and the quality of the product. Moisture content of the food samples is an important factor and it affects microbial spoilage and also the physical appearance. Soft candies' moisture content were found on the range of 8-22% (Ergun *et al.*, 2010). In this study, moisture content of the candies changed between 7-19% depending on the formulation. D-Allulose is known as a humectant and its solubility is almost as high as other six carbon aldoses and ketoses. Although its solubility is high, water binding ability of the D-Allulose is known to be lower than sucrose (Fukada *et al.*, 2010; Ikeda *et al.*, 2011). This told us that that D-Allulose containing systems could have more free water in the system and thus tendency to evaporate during cooking was much higher.

Both D-Allulose concentration and the presence of soy protein and their interaction had significant effect on the moisture content of samples ($p<0.05$, Table A.5). Soy protein containing formulations were found to have higher moisture content than non-soy samples ($p<0.05$).

Moisture content results showed that as sucrose was replaced by D-Allulose, moisture content decreased for non-soy protein containing candies. So, sucrose containing candies had higher moisture content than D-Allulose containing samples and this was significant as can be seen in Table 3.4 ($p<0.05$). This can be explained with the fact that D-Allulose samples had more free water in the system because of its low water binding capacity and during candy making process, D-Allulose containing candies might have lost more water than sucrose containing ones. And this resulted in lower moisture content with increasing D-Allulose concentration.

On the other hand, the story was different for soy protein containing samples. Soy protein addition caused increase in moisture content at all D-Allulose concentrations. This result was not surprising since proteins have an ability to bind water extensively

as they absorb water and swell (Jideani, 2012). Moreover, soy proteins are hydrophilic in nature so they have tendency to absorb and/or retain water (Wolf, 1970). Moreover, soy protein isolate contains higher protein content so, it has the highest water binding capacity of all other soy protein products (Jideani, 2012). At lower concentrations, since SPI could not form a gel, they could only form soy protein aggregates and they just swell. Anyway, due to high water binding ability, higher moisture content was expected when soy protein isolate was added to pectin gel system. Moreover, as it will be explained later in Nuclear Magnetic Resonance (Section 3.7.1) and partially explained in texture analysis part, T_2 relaxation time of the heated samples were found to higher than not heated ones. This was explained that soy protein might have helped the gelation of pectin which meant there was possibly crosslink formation between pectin and soy protein so, some water was possibly entrapped in the gel network. This could also have contributed to the increase in the moisture content.

Moisture content of soy protein containing samples showed an interesting behaviour with allulose addition. Sucrose only containing sample had lower moisture content than allulose only sample. This was the reverse case observed in non-soy samples. Although soy protein concentration was not sufficient for gel formation, Maillard reaction definitely occurred in the samples with proteins. Moreover, hardness values showed that soy protein samples were harder compared to non-soy ones. Gelation properties of soy proteins could also have been improved with Maillard reaction resulting in entrapped water in the network causing higher moisture values. Maillard reaction being faster with D-Allulose (O'Charoen *et al.*, 2014) and D-Allulose sample having higher moisture content and higher texture confirmed the results.

After 2 hours of digestion, significant increase in the moisture content was observed for candies for all samples ($p<0.05$). Since pectin gel structure strongly depended on the sugar, dissolution of sugar loosened the gel structure and more water infused into the structure. Therefore, moisture content of the candies increased.

When the ANOVA results were investigated between samples after digestion (Table A.6) it was observed that soy protein containing samples had higher moisture content ($p<0.05$). D-Allulose concentration and protein-sugar concentration interaction was also significant. Proteins are digested in gastric environment. Due to this vulnerable gel structure gastric juice could migrate to the sample and lead to higher moisture content. In addition, during digestion sugar was dissolved and caused gel matrix to become weaker. Except the non-soy and sucrose only sample, all non-soy samples had lower moisture content ($p<0.05$). High water binding ability of sucrose compared to allulose could explain the higher moisture content on 'non soy sucrose only samples'. For protein containing samples allulose concentration did not have a significant effect on the moisture content after the digestion ($p>0.05$).

Table 3.4. *Moisture content of the pectin based candies before and after digestion*

	Moisture Content (%)	
	Before Digestion	*After Digestion*
S0_SA_035	$9.467\pm0.005^{e,A}$	$10.767\pm0.011^{d,A}$
S0_SA_1025	$7.853\pm0.008^{e,A}$	$12.3211\pm0.002^{cd,B}$
S0_SA_2015	$9.796\pm0.011^{de,A}$	$15.0034\pm0.008^{bc,B}$
S0_SA_350	$11.713\pm0.005^{cd,A}$	$17.3577\pm0.016^{ab,B}$
S1_SA_035	$15.141\pm0.009^{a,A}$	$17.7099\pm0.003^{ab,B}$
S1_SA_1025	$14.370\pm0.005^{ab,A}$	$16.5132\pm0.019^{ab,B}$
S1_SA_2015	$14.904\pm0.005^{a,A}$	$19.0332\pm0.007^{a,B}$
S1_SA_350	$12.845\pm0.004^{bc,A}$	$15.8796\pm0.001^{abc,B}$

Small letters (a-d) means that they differ significantly for each sample in the same column; Capital letters (A-B) means that they differ significantly for each sample in the same row

3.4. Water activity

Water activity is an important parameter for the confectionary products due to shelf life, quality and sensory attributes. Adding sugar in a food product is generally considered as an efficient way to protect it since it reduces the water activity and disables the growth of microorganisms. Water activity of the confectionary products is usually at a range of 0.50-0.80 (Fontana, 1995; Ergun *et al.*, 2010).

Table A.8 gives the ANOVA results for the water activity of samples. Both soy protein addition, D-allulose concentration and their interactions were found to be significant ($p<0.05$). Soy addition resulted in lower water activity values for sucrose only containing samples ($p<0.05$). Table 3.5. shows water activity of the pectin based soft candies. As can be seen from the Table, for non soy protein containing samples, sucrose containing candies had higher a_w than D-Allulose containing ones. Fructose and glucose have lower activity than sucrose at the same concentrations (Mohos, 2017). Sucrose is a disaccharide and D-allulose is a monosaccharide. It is known that molecular weight is an important parameter for a_w. It is known that molecules with low molecular weight reduces water activity more (Ergun *et al.*, 2010). Despite the fact that sucrose has higher hydration ability than D-allulose, molecular weight effect seemed to be dominant effect and 'non soy sucrose only containing' had the highest a_w among all samples. Therefore, this result was expected.

At 1% soy protein concentration, the situation was reversed. D-Allulose containing candies had higher water activity than sucrose containing ones. There might be soy protein isolate interference to the gelation mechanism of pectin. As mentioned in moisture content section, soy protein isolate had excellent water binding capacity. So, for the gel system with sucrose, soy protein isolate could have shown this property and help to reduce water activity by reducing the amount of available water. However, if moisture content results were compared, it would be seen that D-Allulose containing ones had lower moisture content than sucrose containing ones. Since moisture contents were not the same, it was hard to compare their water activity results. Moreover,

moisture content and water activity results were not correlated. Measuring water activity for the soft candy product was quite challenging to interpret.

Table 3.5. *Water Activity of the pectin based soft candies*

Water activity (a_w)	
	Before Digestion
S0_SA_035	0.57±0.0004[d]
S0_SA_1025	0.57±0.0003[d]
S0_SA_2015	0.60±0.0004[c]
S0_SA_350	0.72±0.0005[a]
S1_SA_035	0.64±0.0006[b]
S1_SA_1025	0.64±0.0004[b]
S1_SA_2015	0.57±0.0006[d]
S1_SA_350	0.56±0.0086[d]

Small letters (a-d) means that they differ significantly for each sample in the same column

3.5. Colour

Colour is an important parameter for food products since it can affect consumers' perception and also it can be related to product quality. Colour of the food samples can be associated with enzymatic/ non enzymatic browning reactions, gelatinization or crystallization. In this study, CIELAB method was used to observe the colour differences of samples. Also, ΔE values were also evaluated to see differences between samples. ΔE indicates that the larger the ΔE, the greater the difference between the colours compared (Özcan, 2008; Mennah-Govela and Bornhorst, 2017)

Soy protein containing candies had an opaque appearance while no soy protein candies had a translucent appearance.

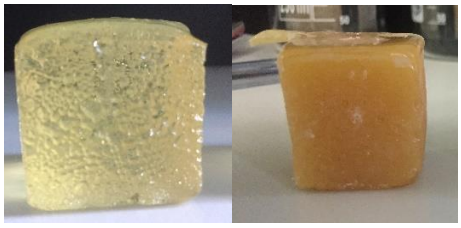

Figure *3.2*. *Physical appearance of pectin-based soy candies with soy (right) without soy (left)*

Results clearly showed that as sucrose was replaced by D-Allulose, lightness values (L*) decreased and also a* value which was indication of redness increased significantly for all soy protein isolate concentrations ($p<0.05$). D-Allulose containing candies were darker and redder than sucrose containing candies. D-Allulose has more tendency to participate in Maillard Browning reactions (Sun *et al.*, 2008; O'Charoen *et al.*, 2014). For non-soy protein containing candies, even though there was no amino acid group supplied, still browner colour was clearly observed in D-Allulose containing candies. This can be explained by caramelization reactions. While preparing the candies, temperature was kept at 109^{o}C (Oshima *et al.*, 2014). Although moisture contents were not that low for caramelization to occur, darker colours obtained in non-protein samples prepared with D-Allulose showed that there was some caramelization occurring in the samples.

When the main effects were examined (Table A.10.1) in ANOVA results for L* values it was seen that soy protein addition did not change L* values significantly ($p>0.05$). But D-allulose concentration was significant ($p<0.05$). For a* and b* values addition of soy and D-Allulose concentration were both significant ($p<0.05$). Among the soy containing samples, 'only sucrose containing samples showed the highest L* value and the lowest a* value. This can also be explained by the fact that even small addition of D-Allulose into the gel matrix for especially intermediate concentrations of sugars

(SA_1025 and SA_2015), it contributed significantly to the Maillard reactions. Table 3.6. shows L*, a*, b* value of each sample.

ΔE results showed comparison based on the sample S0_SA_350. D-Allulose containing ones showed higher ΔE values. For soy protein containing candies, sucrose only containing candies showed significant ΔE value. It is indicated that if ΔE value is greater than 2, samples can be easily differentiated by the consumer (Mennah-Govela and Bornhorst, 2017). Therefore, soy protein and non-soy protein sucrose only containing candies can be easily differentiated.

Table 3.6. *CIELAB constants of the soft candies*

	L*	**a***	**b***	**ΔE**
S0_SA_035	45.31 ± 0.08^{d}	1.04 ± 0.01^{a}	11.38 ± 0.03^{a}	11.19 ± 0.08^{c}
S0_SA_1025	44.54 ± 0.20^{e}	0.86 ± 0.01^{b}	6.36 ± 0.05^{b}	12.05 ± 0.20^{ab}
S0_SA_2015	47.41 ± 0.01^{c}	0.26 ± 0.01^{g}	12.88 ± 0.04^{c}	9.58 ± 0.03^{d}
S0_SA_350	56.24 ± 0.16^{a}	0.11 ± 0.01^{g}	9.16 ± 0.01^{f}	-
S1_SA_035	47.35 ± 0.25^{c}	5.81 ± 0.11^{c}	14.32 ± 0.35^{d}	11.75 ± 0.39^{bc}
S1_SA_1025	47.92 ± 0.08^{c}	4.50 ± 0.02^{d}	9.21 ± 0.06^{f}	9.40 ± 0.09^{d}
S1_SA_2015	45.415 ± 0.15^{d}	4.94 ± 0.04^{e}	13.42 ± 0.11^{e}	12.59 ± 0.17^{a}
S1_SA_350	52.68 ± 0.16^{b}	1.72 ± 0.01^{f}	10.31 ± 0.01^{g}	4.07 ± 0.13^{e}

Small letters (a-d) means that they differ significantly for each sample in the same column

3.6. pH

pH of the pectin-based candies was measured before and after digestion. pH is an important parameter for HMP gels since gel network is only achieved by lowering the pH. Generally HMP gels are formed at a pH value between 2.8-3.4 (DeMars and Ziegler, 2001; Lam *et al.*, 2007; Sessler *et al.*, 2013). pH value of the candy product is given in Table 3.7.

Soy protein addition, and D-allulose concentration and their interactions was found to be significant both before and after digestion (Table A11, A12). For non-soy samples intermediate concentrations of D-allulose were found to be same ($p>0.05$) and had higher pH than only allulose and sucrose containing samples ($p<0.05$). This behaviour was also observed in texture results. Sucrose and D-Allulose when they were together, showed synergistic effect and they increased the hardness of the samples. Since pectin gel structure depends on the acidic conditions, hardness is expected to be correlated with pH value. Pearson correlation analysis showed that there was correlation and it was significant ($R=0.929$, $p<0.05$) (Table A.14). Higher pH value was associated with increased hardness and this was explained by the synergistic effect.

At 1% soy protein isolate concentration, changing the sugar type did not significantly affect the pH value of the samples. All samples had approximately the same pH value ($p>0.05$). Protein containing samples had lower pH than the non-protein ones ($p<0.05$). Presence of protein might have affected the protonation state of the pectin. Soy protein isolate might have not let pectin to be protonated thus in the gel matrix and consequently pH could have become lower due to higher concentrations of H^+.

After 2 hours of digestion, for non-soy protein containing samples pH values decreased significantly ($p<0.05$). This decrease was reasonable since sucrose and D-Allulose was dissolving and causing gel structure to deteriorate slowly so gastric juice might easily diffuse into sample. Therefore, decrease in overall pH was observed. On the other hand, at 1% soy protein concentration, pH of the samples increased. During digestion proteins are hydrolysed to amino acids and amino acids are zwitter ions. Depending

on the pKa, amino and carboxylic acid groups could be protonated or unprotonated. At the low pH of the gastric juice, H^+ concentration on the gastric juice could decrease due to the protonation of the free amino acids obtained through hydrolysis.

Table 3.7. pH of the pectin-based candies before and after digestion

	pH	
	Before Digestion	*After Digestion*
S0_SA_035	$3.06\pm0.01^{bc,A}$	$2.93\pm0.01^{c,B}$
S0_SA_1025	$3.39\pm0.01^{a,A}$	$2.93\pm0.01^{c,B}$
S0_SA_2015	$3.37\pm0.01^{a,A}$	$2.92\pm0.01^{c,B}$
S0_SA_350	$3.10\pm0.01^{b,A}$	$2.93\pm0.01^{c,B}$
S1_SA_035	$3.04\pm0.04^{bc,A}$	$3.03\pm0.04^{b,A}$
S1_SA_1025	$3.00\pm0.02^{c,A}$	$3.12\pm0.01^{a,B}$
S1_SA_2015	$3.04\pm0.02^{bc,A}$	$3.15\pm0.01^{a,B}$
S1_SA_350	$2.98\pm0.04^{c,A}$	$3.13\pm0.01^{a,B}$

Small letters (a-d) means that they differ significantly for each sample in the same column; Capital letters (A-B) means that they differ significantly for each sample in the same row

3.7. Nuclear Magnetic Resonance Relaxometry Measurement

3.7.1. Time Domain NMR Relaxometry

NMR relaxometry is a non-destructive and non-invasive method and it enables to estimate proton pools present in gel matrices (Ozel *et al.*, 2017a). T_2 is known as spin-spin relaxation time which is the time constant that characterizes the rate at which the transverse magnetization (M_{xy}) decays (Oztop *et al.*, 2010; Kirtil and Oztop, 2016). T_2 times were the main subject of the NMR relaxometry experiments for this study. T_2 relaxation time gives information about moisture content, formation of new proton pools by interaction in the surroundings and the changes on the state of the water in a system (Kirtil *et al.*, 2014; Pocan *et al.*, 2019). In literature, it can be clearly seen that food systems generally shows a multicomponent relaxation behaviour (Oztop *et al.*, 2010; Kirtil *et al.*, 2014, 2017a,b; Efe *et al.*, 2019; Pocan *et al.*, 2019). The T_2 results

in this study was discussed both from a multicompartmental and noncompartmental perspective.

3.7.1.1. Multicompartmental approach

In this study, Global Least Square Analysis through XPFit software were conducted and T_2 relaxation times and % contribution of (Relative Area) of the possible proton pools were calculated as seen in Table 3.8. % contribution of the proton pools gives an idea about the contribution of that component to the overall gel matrix. In this study, 2 components were observed for the candies. These components are also referred to as 'peaks' in the relaxation spectrum. An example relaxation signal and its corresponding spectrum is given in Figure 3.3.

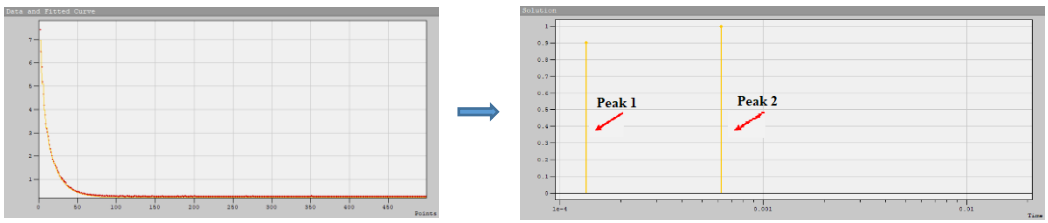

Figure 3.3*. Example of Relaxation signal (left) and corresponding spectrum (right)*

Each peak represents different proton pools. First proton pool with very short relaxation times (0.7-2.5 ms) was attributed to protons that were tightly associated with the solid fractions in the sample. (Ozel *et al.*, 2017c; Pocan *et al.*, 2019). The second proton pool with longer T_2 (>1.5 ms) was associated with the entrapped water in the gel matrix.

T_2 value of the peak 1 was lower than peak 2 for all candy formulations. Therefore, the first peak was attributed to non-exchangeable protons from the solid compartment (Pocan *et al.*, 2019). Candy formulations had too much solid (75% sugar and 5% pectin and soy protein), and this eventually led to solid-solid interactions in the system. In HMP gels, sugar is added so that the possible pectin-water interactions are eliminated, and pectin molecules are let to interact with each other to form a strong gel. So, this peak could be related to protons associated with this pectin-pectin

interaction. Echo time used in the study were 40 us. If shorter echo times or FID - CPMG or Hahn Echo sequences were used, sugar-sugar interaction could have been observed as well.

As mentioned earlier, the second peak had the longest T_2 value and was attributed to water entrapped in the gel system. Here water was not entirely in free form due to high amount of sugar dissolved. However, this compartment still had a long T_2 compared to the 1st peak since mobility of water ions were still higher than the other compartment. In a different study, investigation of water uptake behaviour of whey protein hydrogels was studied. The highest T_2 value was again attributed to water entrapment in gel matrix. Considering pectin based soft candies as a composite gel, the similar results was expected in this study (Ozel *et al.*, 2017c).

Addition of soy protein to the sucrose containing candy changed the number of proton pools as can be seen from the Table 3.8. Before the addition of soy protein into the system, only one component was observed but after the addition, system had 2 components. This was explained with the gelation effect of sucrose and D-Allulose being different on pectin. While sucrose had more ability to make H-bond with water and did not allow pectin to interact with water, D-Allulose was not very capable of making H-bond with water due to its low water binding capacity and it could have let pectin to bond with water which is not wanted in HMP. So, this might explain why sucrose had one component while D-Allulose had two components before soy addition. After soy protein isolate addition, sucrose containing ones showed two peaks the thus number of compartments increased, while D-Allulose containing ones still showed two peaks. Addition of SPI to sucrose containing ones could have created new interaction between soy and water or soy and pectin. However, D-Allulose containing ones already had two components and addition of SPI may have changed the interactions but not the compartment number. Soy addition might have resulted in pectin-protein interaction and this prevented water-pectin interaction and eliminated or overlapped with the compartment coming from pectin-water and the new peak

might be attributed to the soy protein-water interactions. That's why still two peaks may appear. To understand this mechanism more 2D-NMR Relaxometry experiments could have been performed which can differentiate the same T_2 (overlapping) compartments with respect to their T_1s.

3.7.1.2. Mono-compartmental approach

In addition to multicompartmental approach, one compartment analysis of T_2 relaxation times were also obtained to explain SPI addition and rare sugar effect on candies. As can be seen from Table 3.9, D-Allulose containing candy formulations had higher overall T_2 values for all soy protein concentrations. Sucrose could be the responsible element for this because sucrose was more active to bind water in the system and reduced the mobility more than D-Allulose did. As mentioned before, water binding of D-Allulose ability was lower than sucrose. Secondly, sucrose as being a disaccharide and consisting of fructose and glucose had more H-bonding ability.

Addition of the soy protein to the system dd not have an impact on T_2 values when mono exponential T_2 results were considered.

After 2 hours of digestion, significant increase in T_2 (mono-exponential relaxation time) was observed as can be seen from Figure 3.4. ($p<0.05$). In the gastric juice, pectin gel network might have been broken due to sugar dissolving and this led to a softer matrix. This let gastric juice to diffuse into the system and resulted in increase in T_2 value. The acidic nature of gastric juice with more protons (H^+) was also a definite reason for the increase in T_2. Also, in gastric juice soy protein was digested. However, all protein was not expected to be digested since there may be possible soy protein and pectin gel network and it could be more resistant to digestion since the peptide bonds could not be accessible due to possible crosslinking with pectin.

Table 3.8. *Average T_2 (spin-spin relaxation time) and percent relative areas (RA) of each compartment for pectin based soft candies*

Peak	Sample	T_2 (ms)	% Contribution
Peak 1	**s0_SA_035**	1.50 ± 0.0007^{ab}	38.65 ± 0.13^{b}
	s0_SA_1025	1.00 ± 0.0000^{b}	32.10 ± 0.02^{b}
	s0_SA_2015	2.50 ± 0.0007^{a}	100.00 ± 0.00^{a}
	s0_SA_350	2.00 ± 0.0000^{a}	100.00 ± 0.00^{a}
	s1_SA_035	1.00 ± 0.0000^{b}	32.40 ± 0.05^{b}
	s1_SA_1025	0.78 ± 0.0001^{b}	64.80 ± 0.13^{ab}
	s1_SA_2015	0.89 ± 0.00007^{b}	49.30 ± 0.03^{b}
	s1_SA_350	0.72 ± 0.00004^{b}	60.25 ± 0.02^{b}
Peak 2	**s0_SA_035**	4.00 ± 0.0014^{ab}	61.35 ± 0.13^{a}
	s0_SA_1025	4.00 ± 0.0000^{ab}	67.90 ± 0.02^{a}
	s0_SA_2015	-	-
	s0_SA_350	-	-
	s1_SA_035	4.50 ± 0.0007^{a}	67.60 ± 0.08^{a}
	s1_SA_1025	1.50 ± 0.0007^{c}	35.20 ± 0.13^{a}
	s1_SA_2015	3.00 ± 0.0000^{abc}	50.70 ± 0.03^{a}
	s1_SA_350	2.00 ± 0.0000^{bc}	39.75 ± 0.02^{a}

Small letters (a-d) means that they differ significantly for each sample in the same column

Table 3.9. Mono-exponential transverse relaxation times (T_2) of pectin based soft candies

	T_2 (ms)	
	Before Digestion	*After Digestion*
S0_SA_035	$4.13\pm0.46^{a,A}$	$5.71\pm0.43^{abc,B}$
S0_SA_1025	$2.98\pm0.24^{b,A}$	$6.17\pm0.13^{ab,B}$
S0_SA_2015	$2.46\pm0.20^{bc,A}$	$4.66\pm0.79^{bc,B}$
S0_SA_350	$1.52\pm0.17^{cd,A}$	$3.49\pm0.38^{c,B}$
S1_SA_035	$4.20\pm0.64^{a,A}$	$7.07\pm0.61^{a,B}$
S1_SA_1025	$2.19\pm0.21^{bcd,A}$	$6.07\pm0.16^{abc,B}$
S1_SA_2015	$2.04\pm0.07^{bcd,A}$	$5.09\pm0.23^{bc,B}$
S1_SA_350	$1.45\pm0.20^{d,A}$	$3.92\pm0.72^{bc,B}$

Small letters (a-d) means that they differ significantly for each sample in the same column; Capital letters (A-B) means that they differ significantly for each sample in the same row

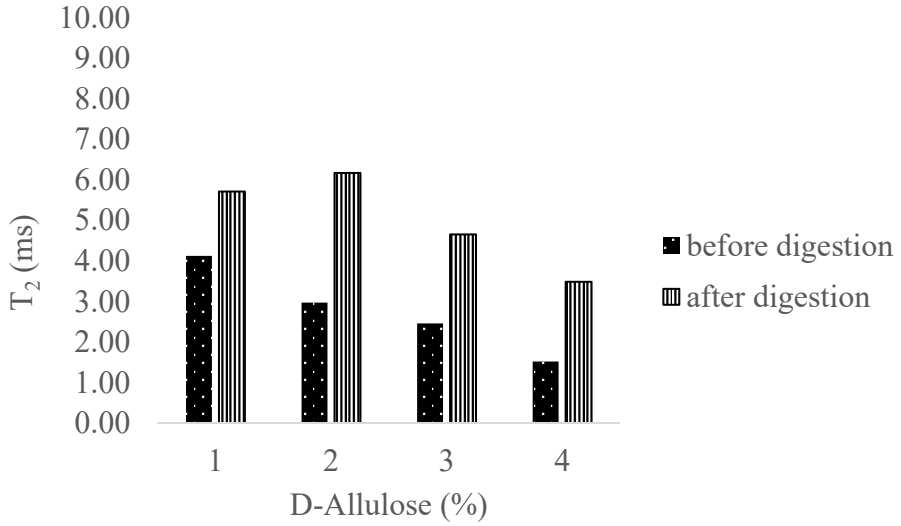

Figure 3.4. *Effect of digestion on mono-exponential spin-spin relaxation time of 0% soy protein containing candies*

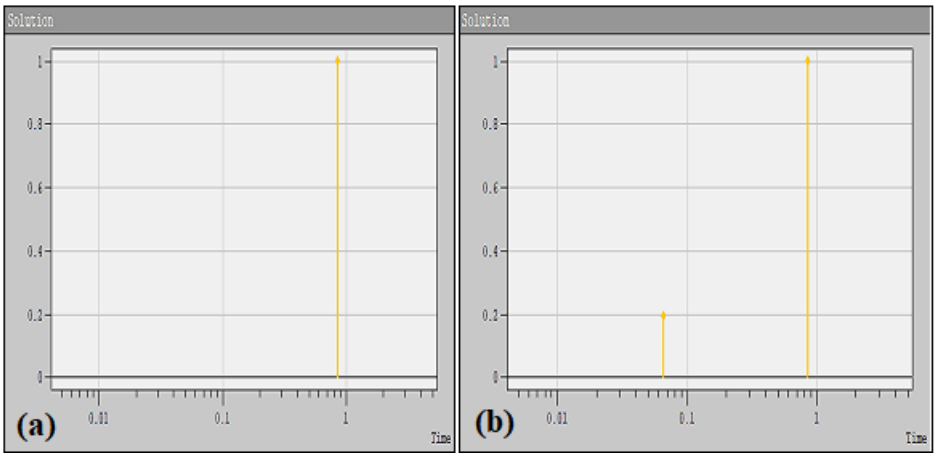

Figure 3.5*. Global Least Square Analysis of the 4% pectin and 1% soy protein solutions. (a)Heated (b)Not Heated*

3.7.2. Fast Field Cycling (FFC) NMR Relaxometry Experiments

Fast field cycling NMR relaxometry is a technique based on measuring T_1 longitudinal relaxation times over a wide range of frequencies using a single instrument. The magnet is capable of switching the magnetic field very fast. FFC is getting more attention in the medical, chemical and physical research areas and recently it has been

started to be used for food applications. Application on food is very limited and it has only been used for identification or quality assessment purposes so far (Baroni *et al.*, 2009; Capitani *et al.*, 2014; Steele *et al.*, 2016; Ladd-Parada *et al.*, 2019; Uguz *et al.*, 2019). In a study, molten and cooled cocoa butter was examined through FFC-NMR and it was clearly observed that crystallization of cocoa butter at different temperatures could be monitored. There was an increase in T_1 values and this was explained by solidification of the cocoa butter and this resulted in crystallization (Ladd-Parada *et al.*, 2019). Since the candy formulations were used in this study and they are prone to crystallization, same attribute was also expected.

In this study, at different frequencies, different T_1 values were obtained. Figure 3.6. shows how T_1 curves were changing for different candy formulations.

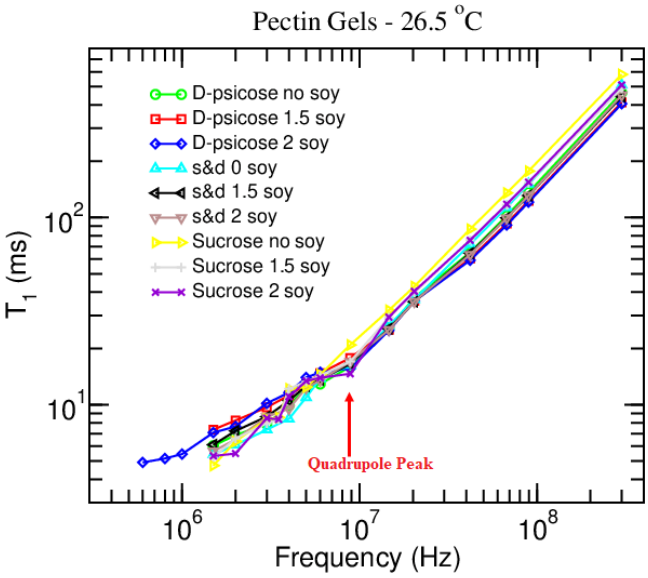

Figure 3.6. *Effect of D-Allulose and soy protein concentration on T1 at different frequency*

It was observed that as D-Allulose concentration increased, T_1 values increased at frequencies below 9 MHz; however, at higher frequencies, as D-Allulose concentration increased, T_1 times decreased. At lower frequencies, change in molecular dynamics occur in the polymer system and it is easier to discriminate compared to higher frequencies (Pasin and Ferrante, 2017). T_1 is strongly related to

water content (Ozel *et al.*, 2017b; Pocan *et al.*, 2019). Sucrose containing candies had lower T_1 values than D-Allulose containing candies. D-Allulose was not able to bind water as sucrose does so system had more free water than sucrose containing ones. Because of that, D-Allulose containing ones were expected to have higher water content and longer T_1 times. As frequency gets higher, this gets the other way around meaning that sucrose containing ones had longer T_1 values. The reason for that is as frequency gets higher, differentiation of the samples got difficult and resulted in different results. At around 9 MHz, curve changed its slope and got larger.

Another result obtained was, as soy protein concentration increased, T_1 value increased at low frequency values but at higher frequencies it was vice versa. The reason for that could be as frequency increases, water in hydration layers around proteins became more restricted and this led to significantly shorter T_1 values (Kimmich and Anoardo, 2004). In addition to these, at around 9 MHz, soy protein containing candies showed another peak. This is known as the quadrupole peak and indicates that the sample contained protein in the formulation (Steele, 2018).

It was concluded that D-Allulose promoted polymer water interaction in the gel matrix. Longer relaxation time was due to relaxation of bulk water (Chávez and Halle, 2006). By FFC analysis, it was clearly seen that D-Allulose containing ones have longer T_1 and so, higher amount of bulk water. However, polymer-water interactions in pectin based soft candies were not sufficient and as explained before the aim of adding of sugar to the pectin gels is for dehydrating the system and to promote pectin-pectin interaction. D-Allulose did not meet this expectation.

Moreover, the obtained T_1 curves were fitted for Renormalized Rouse model since it was more applicable for polymer nanocomposites. The formulated candies can also be considered as a polymeric material and Rouse model constants are valid in such a case. There is a critical polymer specific molecular weight, denoted as M_c. Rouse model is used to project chain dynamics below M_c whereas Renormalized Rouse model is used to reflect entanglements above M_c. Rouse model is mainly used where polymer chains

can easily form homogeneous viscous medium while in Renormalized Rouse model polymer chain dynamics is not dominated because of neighboring chains confinement (Kimmich and Anoardo, 2004). In this study, neighboring elements were thought to be important, since pectin-pectin and sugar-sugar interaction had a huge impact on pectin structure. That's why Renormalized Rouse model was selected. Renormalized Rouse model fitting curve can be seen in Figure 3.7. and Renormalized Rouse model constant which were obtained by using Fitteia software can be seen in Table 3.10. Fitteia implementation of the Rouse model is as follows;

$$T_1(\nu) = A \begin{cases} \nu^{p_1}, & \nu \geq \nu_M \\ (\nu_M)^{p_1 - p_2}\ \nu^{p_2}, & \nu_m \leq \nu < \nu_M \\ (\nu_M)^{p_1 - p_2}\ (\nu_m)^{p_3 - p_2}\ \nu^{p_2}, & \nu < \nu_m \end{cases}$$

where

Constant	Description	Range
f	υ, frequency (υ=$\omega_L/2\pi$), unit: Hz	0 to 10^9 Hz
a_1	A, amplitude for f>f0, unit: $s^{(p1+1)}$	~10^5 $s^{(p1+1)}$
f_0	υ_M, higher characteristic frequency: unit: Hz	~10^6 to 10^7 Hz
f_1	υ_m, lowest characteristic frequency: unit: Hz	~10^4 to 10^5 Hz
p_1	exponent of the hight frequency regime: unit: -	0.25-1
p_2	exponent of the intermediate frequency regime: unit: -	0.3-0.75
p_3	exponent of the low frequency regime: unit: -	0-0.5

Interpretation of the constants requires a deep knowledge. In this study, FFC experiments were conducted just to see whether candies could be differentiated or not. Fitting of the NMRD profile was also performed and some constants were found. Interpretations of these constants were not considered in the scope of this thesis and is expected to be discussed in further study.

Table 3.10. *Renormalized Rouse Model constants of the samples*

Sample name	a	f_0	f_1	p_1	p_2	p_3
S0_SA_350	3.61E-06	1.54E+07	1	9.67E-01	8.28E-01	1
S1.5_SA_350	6.21E-06	8.86E+06	1	9.30E-01	6.97E-01	1
S2_SA_350	3.57E-06	8.86E+06	1	9.62E-01	6.84E-01	1
S0_SA_2015	5.62E-06	4.09E+06	1	9.34E-01	4.84E-01	1
S1.5_SA_2015	5.07E-06	1.08E+07	1	9.34E-01	5.72E-01	1
S2_SA_2015	4.42E-06	1.22E+07	1	9.41E-01	6.33E-01	1
S0_SA_035	4.35E-06	9.19E+06	1	9.44E-01	5.72E-01	1
S1.5_SA_035	7.52E-06	1.17E+07	1	9.09E-01	5.04E-01	1
S2_SA_035	8.02E-06	1.10E+07	1	9.05E-01	4.88E-01	1

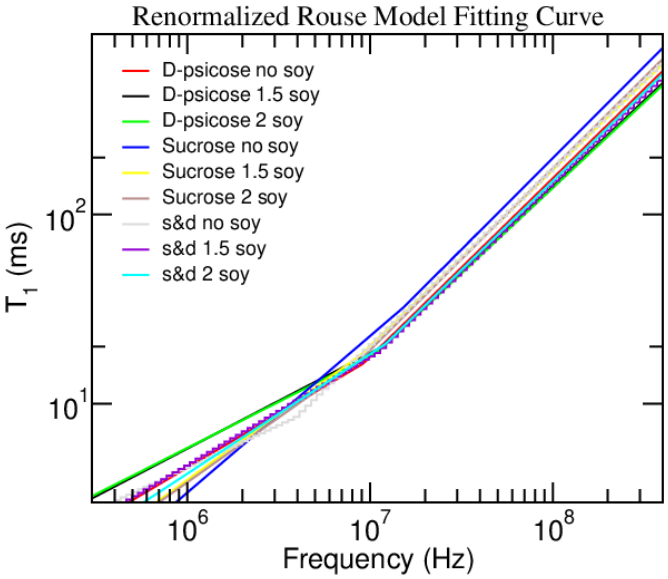

Figure 3.7. Renormalized Rouse Model Fitting Curve for all candy formulations

3.8. X-Ray Diffraction

X-ray diffraction analysis was conducted on 0^{th} and 28^{th} days to understand crystallization tendency of the formulated soft candies. It is an important criterion to guess the shelf life of a product. In these experiments, only sucrose and only D-Allulose containing ones were analysed. The experiment was only done before the digestion since crystallization behaviour was only important for characterization purposes. Results stated that at the beginning, all the samples were at amorphous state. After 28^{th} days, D-Allulose containing ones showed a partially crystalline behaviour; however, sucrose containing ones show only amorph behaviour. This result was not expected since in the literature there were similar experiments which was conducted with gelatine based candy and it was stated that D-Allulose had more tendency to retard crystallization while sucrose did not (Pocan *et al.*, 2019). Moreover, in the literature, there were numerous studies showing that sucrose had a distinct crystal region and had tendency to crystallize easily (Palmer *et al.*, 1956; Chinachoti and Steinberg, 1986; Leinen and Labuza, 2006).

Presence of narrow and higher peak areas are usually related to more crystalline regions (Leinen and Labuza, 2006). From Figure 3.8. at the first day, D-Allulose containing ones showed higher intensity and narrower relative areas while sucrose containing ones had wider and broader areas. From Figure 3.9., at the 28^{th} day, D-Allulose containing ones showed crystal peaks for both non soy and %1 soy protein formulations. The results were different than expected and it was explained by the pectin gelation mechanism being different for different types of sugar. D-Allulose formulations exhibited crystallization. This might be due to low water binding capacity of the D-Allulose. Since it had lower ability to bind water, pectin gel mechanism was not really fully satisfied. The reason of sugar addition to HMP is to prevent pectin making H-bond with water. However, in case of D-Allulose, due to low water binding ability, pectin might have bonded with water and gel network was not strong enough. Also, in TD-NMR experiments, multicompartmental analysis showed that D-Allulose

containing candies had two peaks before and after addition of soy protein isolate while sucrose containing ones only had one peak before the addition of soy protein isolate. This result indicated that sucrose containing ones had a compact gel structure by showing one peak and sucrose did not let pectin to make H-bond with water as expected; however, D-Allulose containing ones formed less stable gel network and showed two peak meaning that D-Allulose might have let pectin to make H-bond with water. This leads to a less stable gel network and as a result crystallization was observed easily.

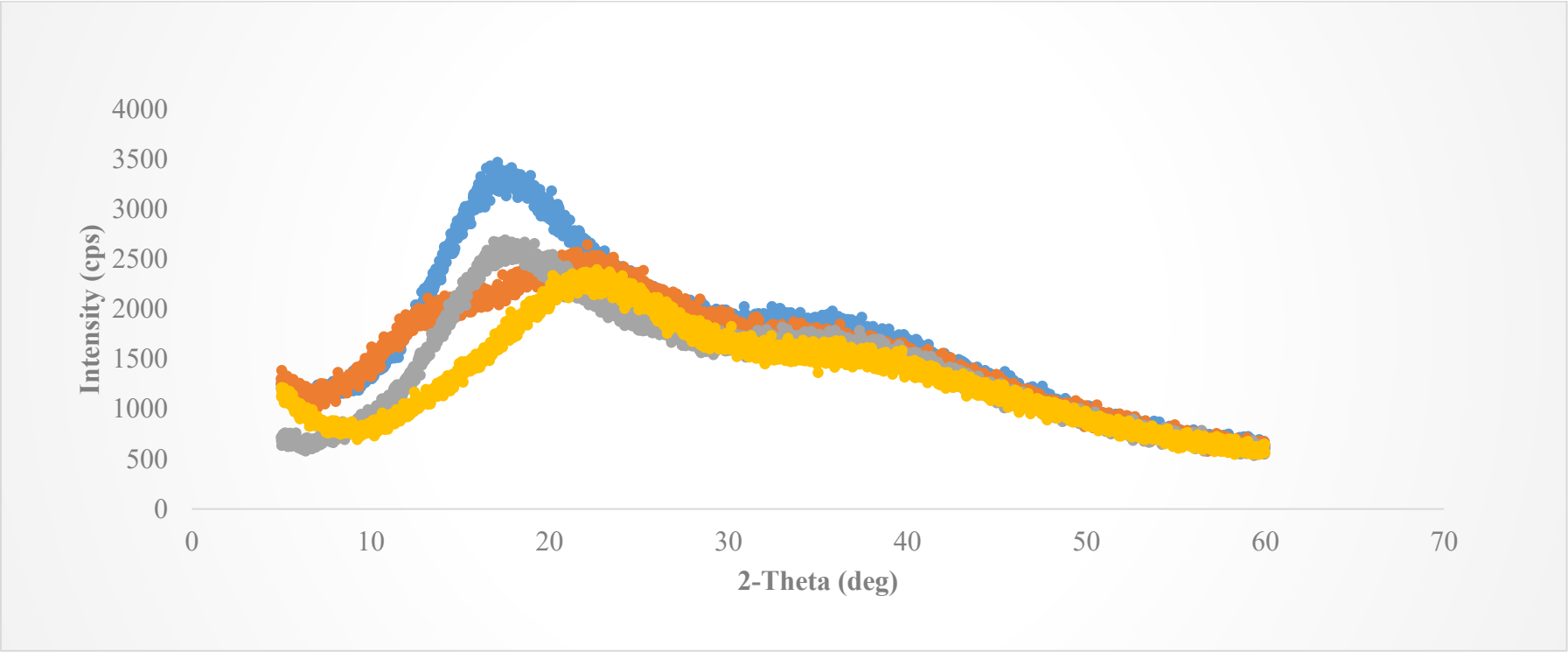

Figure 3.8. *X-Ray diffraction pattern of pectin based soft candies at the first day ( ) S1_SA_035 ( ) S0_SA_035 ( ) S1_SA_350 ( ) S0_SA_350*

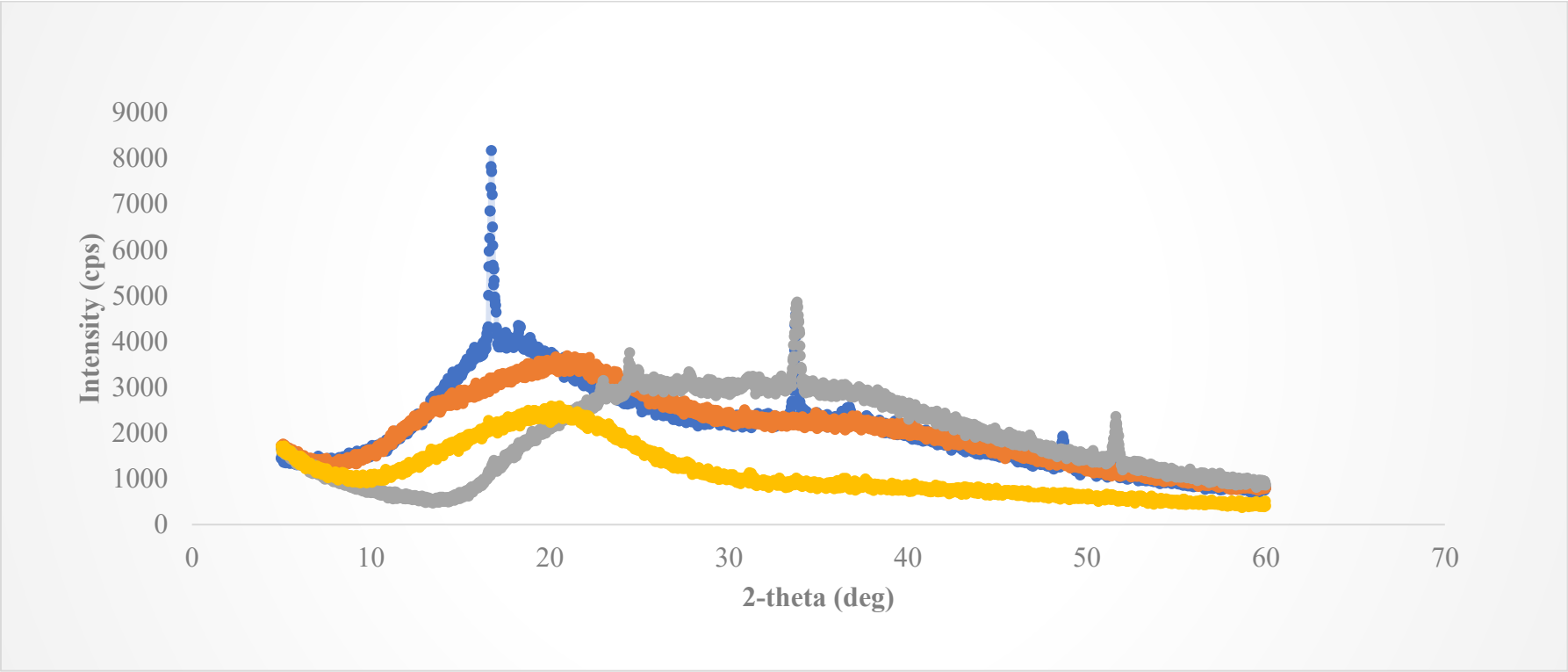

Figure 3.9. *X-Ray diffraction pattern of pectin based soft candies at 28th day (—) S1_SA_035 (—) S0_SA_035 (—) S1_SA_350 (—) S0_SA_35*

3.9. Scanning Electron Microscopy

Scanning Electron Microscopy (SEM) analysis is important to observe morphological differences. It can give an idea about clusters and smooth surface of the gel matrix. In this study, SEM experiments were performed before and after digestion and Figure 3.10. shows the effect of digestion and sugar type for non-soy protein containing candies while Figure 3.11. shows 1% soy protein containing candies.

As can be seen in Figures 3.10-11 D-Allulose addition to the samples caused smoother surface; however, substitution of D-Allulose with sucrose created a more branched and rough structure. Even intermediate concentrations of D-Allulose and sucrose created branched structure, so sucrose was found to be the responsible ingredient for that morphology.

SEM experiments were also conducted on pectin based soft candy, starch based soft candy and hard candy formulations by other researchers (Gu *et al.*, 2015). It was also seen in the study that pectin based soft candies had a more branched structure than the starch based soft candies at a magnification of 2500x. The reason for that was the presence of sucrose in the pectin-based candies.

Soy addition to the system also caused a smoother surface but over the surface new holes were detected. This was explained by the foaming ability of soy protein (Shao and Kao, 2014) and while preparing candies soy protein showed this effect significantly. Foaming property of soy protein isolate is what makes soy protein isolate functional when they are used in confectionary products (Jideani, 2012). Due to this ability, bubbles were formed and while setting candies for 24 hours after cooking, these bubbles raised up to the surface.

After 2 hours of digestion, SEM images showed that candies had smoother surface for both non-soy and 1% soy protein containing candies. The apparent reason for that is

sugar was dissolving at that temperature so, sucrose lost its ability to create the branched structure. Soy protein was digested in the gastric medium as being a protein but even after digestion some pores still existed. That was an indication that it may not be fully digested. In addition, as explained before, there might have been a gel network between pectin and soy protein and this gel network could not easily be broken and let the soy protein digested. This possible network between soy protein and pectin was also discussed in the texture profile analysis part which proved that after heating of pectin and soy protein for 3 hours a more homogenous structure was present. This can be a clue that soy protein had a role for pectin gelation mechanism. Moreover, in the NMR relaxometry section, T_2 values of heated and unheated solutions were mentioned and it was stated that T_2 of heated solution was lower which meant that pectin and soy protein possibly created a gel network that reduced the mobility of the water resulting in shorter T_2 values.

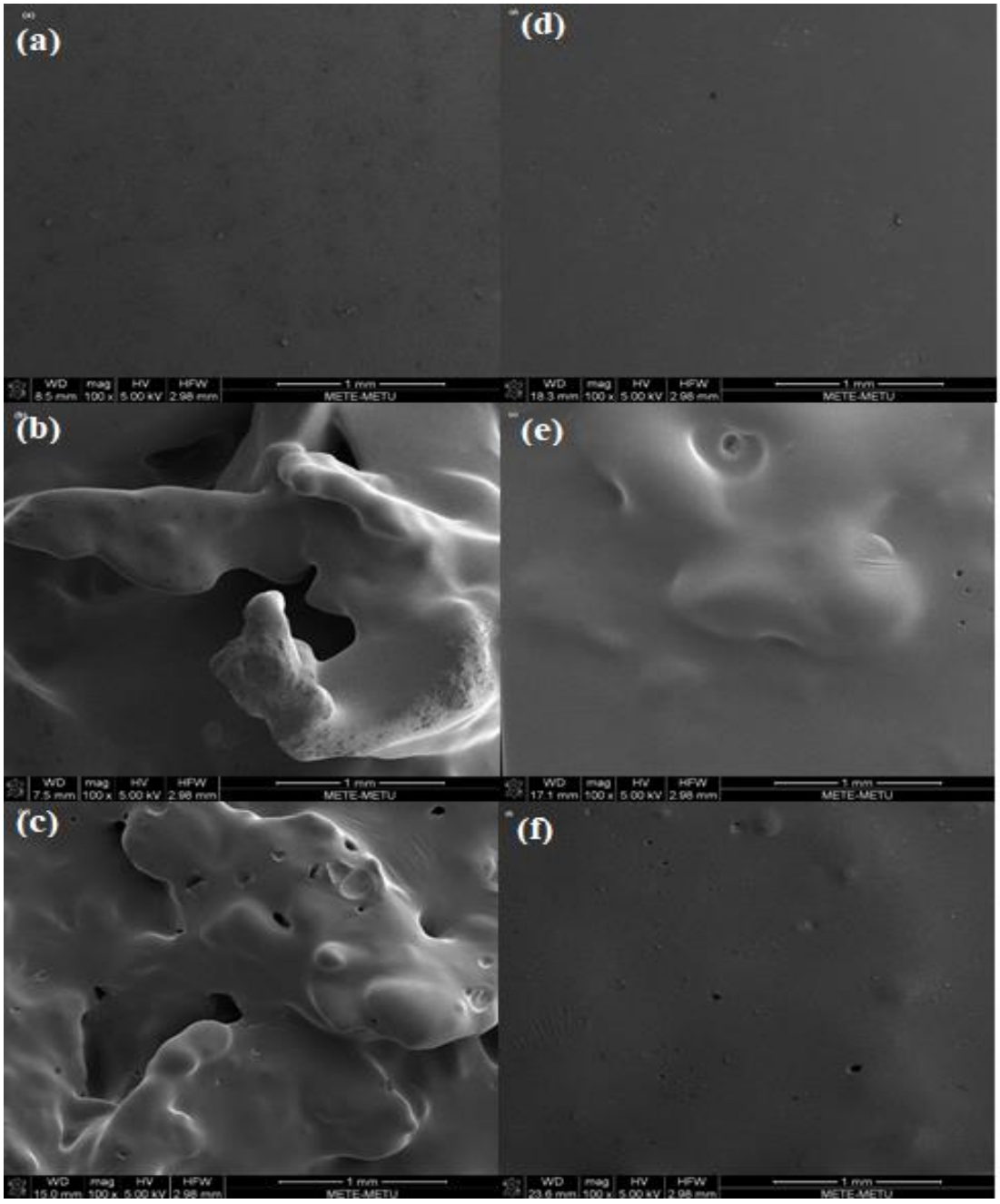

Figure 3.10. *Scanning Electron Micrographs (SEM) of %0 soy protein containing pectin-based candies (a)SA_0/35 (b)SA_10/25 (c)35/0 (d)-(f)after digestion respectively*

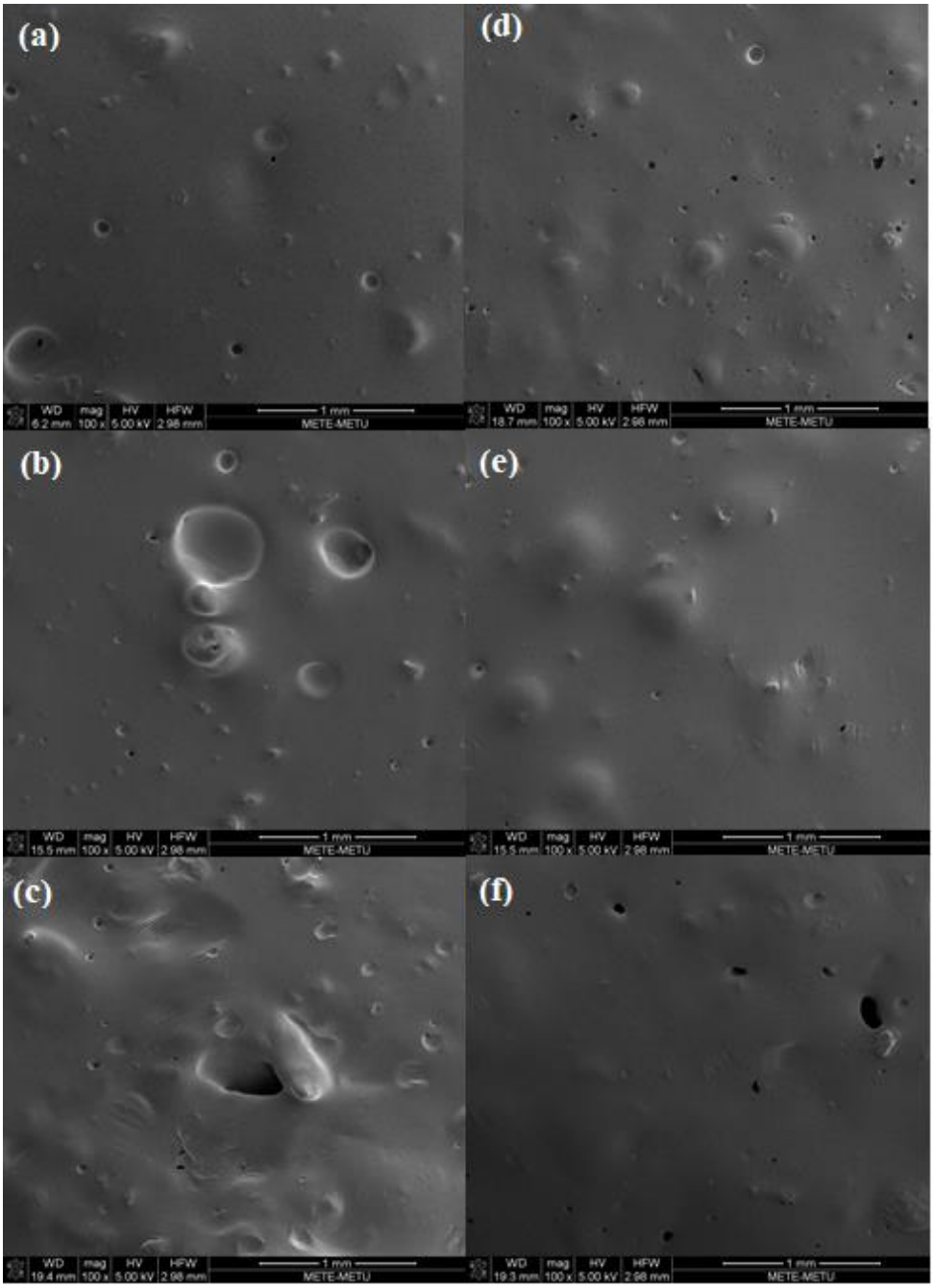

Figure 3.11. *Scanning Electron Micrographs (SEM) of %1 soy protein containing pectin-based candies (a)SA_0/35 (b)SA_10/25 (c)35/0 (d)-(f)after digestion respectively*

CHAPTER 4

CONCLUSION AND RECOMENDATION

In this thesis study, characterization and digestion behavior of the pectin-based candies were studied. Soy protein isolate concentrations in the formulations changed from 0% to 1% while D-Allulose were used at concentrations of 0%, 15%, 25%, 35%. For characterization purposes, texture profile analysis, pH, color, moisture content, water activity, brix measurements were conducted. Moreover, to explain different proton pools present in samples, Nuclear Magnetic Resonance (NMR) T_2 Relaxation experiments were performed. Scanning electron microscopy and X-ray Diffraction experiments were also conducted to characterize structural and crystallization changes respectively. As an additional advanced characterization technique, FFC NMR relaxometry experiments were performed.

Texture profile analysis results showed that hardness also increased with increasing SPI concentration. Also, for non-soy protein containing candies, there might be a synergistic effect between D-Allulose and sucrose so that the hardness of the samples increased at intermediate concentrations. Moreover, pH results before the digestion also pointed out the same fact so this might actually be the case for the formation of a pectin-based gel network system.

This study revealed that there might be a pectin-soy protein interaction in the gel matrix. Moisture contents of the soy protein containing candies were higher than non-soy protein ones. The so-called soy protein-pectin gel matrix retained water inside and this resulted in an increase on the moisture. When TD-NMR experiments were

explored it was seen that number of proton compartments increased when soy protein was added to the system. This result also indicated that there was another network formed between SPI and pectin that entrapped some mobile protons as TD-NMR was generally used as an indicator for the protons status in a matrix.

Since D-Allulose is known to be more susceptible to Maillard Reaction, brownish color was observed more in those samples. Lightness values decreased as a* values increased. Even sucrose and D-Allulose were used together, brownish color was clearly observed.

The most interesting result was from the X-ray diffraction experiments. In the literature, it was proved that D-Allulose had the ability to retard crystallization. However, in pectin gel network, D-Allulose promoted crystallization since after 28th days, sucrose containing candies showed no crystal peaks whereas D-Allulose containing ones showed those peaks.

To sum up, D-Allulose replacement with sucrose changed the characteristic gel mechanism of pectin since D-Allulose had lower water binding ability. So, D-Allulose was found to be inefficient to dehydrate the system. As a result, pectin interacted with water and a weak gel structure was formed. Aside from these, digestion behavior and dissolution constant of the D-Allulose containing ones and sucrose containing ones are seemed to be the same. Addition of soy protein generated another crosslinking between pectin and soy protein so, this affected the overall system. More porous medium, higher moisture content, and higher pH values were obtained. Moreover, dissolution behavior of the samples was analyzed, and higher dissolution constant observed at S0_SA_2015. Finally, FFC experiment was conducted and it was seen that FFC NMR relaxometry is a promising tool to differentiate pectin-based soft candies. In addition to all these, TD-NMR Relaxometry was shown to be a successful tool for characterizing the pectin-based confectionery products.

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APPENDICES

A. ANOVA TABLES

Table A.1. Analysis of Varience for Hardness values of pectin based soft candies before digestion

```
Factor      Type   Levels  Values
D-Allulose  fixed       4  0; 15; 25; 35
soy         fixed       2  0; 1


Analysis of Variance for Hardness, using Adjusted SS for Tests

Source          DF   Seq SS   Adj SS  Adj MS      F      P
D-Allulose       3   98.887  109.255  36.418  26.45  0.000
soy              1   64.078   66.355  66.355  48.19  0.000
D-Allulose*soy   3   89.337   89.337  29.779  21.63  0.000
Error           21   28.914   28.914   1.377
Total           28  281.216


S = 1.17340   R-Sq = 89.72%   R-Sq(adj) = 86.29%


Grouping Information Using Tukey Method and 95.0% Confidence

D-Allulose  N  Mean  Grouping
15          7  17.1  A
25          7  16.5  A
 0          7  15.9  A
35          8  12.3    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

soy   N  Mean  Grouping
1    14  17.0  A
0    15  13.9    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

D-Allulose  soy  N  Mean  Grouping
 0          1    3  20.2  A
15          1    3  18.9  A
25          0    3  17.1  A B
```

```
25          1    4  15.8    B C
15          0    4  15.4    B C
35          1    4  13.0      C D
 0          0    4  11.7        D
35          0    4  11.5        D

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

Table A.2. Analysis of Varience for Hardness values of pectin based soft candies after digestion

```
Factor       Type   Levels  Values
D-Allulose   fixed       4  0; 15; 25; 35
Soy Protein  fixed       2  0; 1


Analysis of Variance for Hardness (N), using Adjusted SS for Tests

Source                  DF   Seq SS  Adj SS  Adj MS     F      P
D-Allulose               3   33.631  44.712  14.904  5.99  0.010
Soy Protein              1    2.047   1.967   1.967  0.79  0.391
D-Allulose*Soy Protein   3   42.578  42.578  14.193  5.70  0.012
Error                   12   29.860  29.860   2.488
Total                   19  108.116


S = 1.57745   R-Sq = 72.38%   R-Sq(adj) = 56.27%


Grouping Information Using Tukey Method and 95.0% Confidence

D-Allulose  N  Mean  Grouping
 0          5  14.9  A
15          5  12.8  A B
35          6  11.9    B
25          4  10.6    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

Soy
Protein   N  Mean  Grouping
1        10  12.9  A
0        10  12.3  A

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

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

            Soy
D-Allulose  Protein  N  Mean  Grouping
 0          1        2  17.8  A
15          0        2  13.1  A B
15          1        3  12.6    B
35          0        3  12.4    B
 0          0        3  12.1    B
25          0        2  11.5    B
35          1        3  11.5    B
25          1        2   9.7    B

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

Table A.3. Analysis of Varience for Digestion effect on each pectin-based soft candies sample

1. Digestion effect on S0_SA_035

```
Factor     Type   Levels  Values
digestion  fixed       2  0; 1


Analysis of Variance for mc_s0_sa_035, using Adjusted SS for Tests

Source     DF  Seq SS  Adj SS  Adj MS     F      P
digestion   1  1.3013  1.3013  1.3013  6.71  0.049
Error       5  0.9700  0.9700  0.1940
Total       6  2.2713


S = 0.440459   R-Sq = 57.29%   R-Sq(adj) = 48.75%


Unusual Observations for mc_s0_sa_035

Obs  mc_s0_sa_035      Fit  SE Fit  Residual  St Resid
  6       11.5650  12.3550  0.2543   -0.7900     -2.20 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95.0% Confidence

digestion  N  Mean  Grouping
1          3  12.4  A
0          4  11.5    B

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

2. Digestion effect on S0_SA_1025

```
Factor     Type   Levels  Values
digestion  fixed       2  0; 1


Analysis of Variance for mc_s0_sa_1025, using Adjusted SS for Tests

Source     DF  Seq SS  Adj SS  Adj MS      F      P
digestion   1  37.767  37.767  37.767  65.91  0.004
Error       3   1.719   1.719   0.573
Total       4  39.486


S = 0.756979   R-Sq = 95.65%   R-Sq(adj) = 94.20%


Grouping Information Using Tukey Method and 95.0% Confidence

digestion  N  Mean  Grouping
0          3  17.1  A
1          2  11.5    B

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

3. Digestion effect on S0_SA_2015

```
Factor     Type   Levels  Values
digestion  fixed       2  0; 1


Analysis of Variance for mc_s0_sa_2015, using Adjusted SS for Tests

Source     DF   Seq SS  Adj SS  Adj MS      F      P
digestion   1   7.3477  7.3477  7.3477  10.98  0.030
Error       4   2.6769  2.6769  0.6692
Total       5  10.0245


S = 0.818056   R-Sq = 73.30%   R-Sq(adj) = 66.62%


Grouping Information Using Tukey Method and 95.0% Confidence

digestion  N  Mean  Grouping
0          4  15.4  A
1          2  13.1    B

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

4. Digestion effect on S0_SA_350

```
Factor     Type   Levels  Values
digestion  fixed       2  0; 1


Analysis of Variance for mc_s0_sa_350, using Adjusted SS for Tests

Source     DF  Seq SS  Adj SS  Adj MS     F      P
digestion   1  2.5071  2.5071  2.5071  5.07  0.087
Error       4  1.9779  1.9779  0.4945
Total       5  4.4850


S = 0.703195   R-Sq = 55.90%   R-Sq(adj) = 44.87%


Grouping Information Using Tukey Method and 95.0% Confidence

digestion  N  Mean  Grouping
1          2  13.0  A
0          4  11.7  A

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

5. Digestion effect on S1_SA_035

```
Factor     Type   Levels  Values
digestion  fixed       2  0; 1


Analysis of Variance for mc_s1_sa_035, using Adjusted SS for Tests

Source     DF  Seq SS  Adj SS  Adj MS     F      P
digestion   1   6.647   6.647   6.647  3.14  0.151
Error       4   8.454   8.454   2.114
Total       5  15.101


S = 1.45382   R-Sq = 44.01%   R-Sq(adj) = 30.02%


Grouping Information Using Tukey Method and 95.0% Confidence

digestion  N  Mean  Grouping
0          3  13.6  A
1          3  11.5  A

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

6. Digestion effect on S1_SA_1025

```
Factor     Type   Levels  Values
digestion  fixed       2  0; 1


Analysis of Variance for mc_s1_sa_1025, using Adjusted SS for Tests

Source     DF  Seq SS  Adj SS  Adj MS       F      P
digestion   1  34.669  34.669  34.669  116.36  0.002
Error       3   0.894   0.894   0.298
Total       4  35.563


S = 0.545833   R-Sq = 97.49%   R-Sq(adj) = 96.65%


Grouping Information Using Tukey Method and 95.0% Confidence

digestion  N  Mean  Grouping
0          3  15.1  A
1          2   9.7    B

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

7. Digestion effect on S1_SA_2015

```
Factor     Type   Levels  Values
digestion  fixed       2  0; 1


Analysis of Variance for mc_s1_sa_2015, using Adjusted SS for Tests

Source     DF  Seq SS  Adj SS  Adj MS       F      P
digestion   1  40.227  40.227  40.227  228.85  0.004
Error       2   0.352   0.352   0.176
Total       3  40.579


S = 0.419263   R-Sq = 99.13%   R-Sq(adj) = 98.70%


Grouping Information Using Tukey Method and 95.0% Confidence

digestion  N  Mean  Grouping
0          2  20.1  A
1          2  13.7    B

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

8. Digestion effect on S1_SA_350

```
Factor     Type   Levels  Values
digestion  fixed       2  0; 1


Analysis of Variance for mc_s1_sa_350, using Adjusted SS for Tests

Source     DF  Seq SS  Adj SS  Adj MS     F      P
digestion   1   6.864   6.864   6.864  2.08  0.245
Error       3   9.899   9.899   3.300
Total       4  16.763


S = 1.81650   R-Sq = 40.95%   R-Sq(adj) = 21.26%


Grouping Information Using Tukey Method and 95.0% Confidence

digestion  N  Mean  Grouping
0          3  20.2  A
1          2  17.8  A

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

Table A.4. Analysis of Varience for Brix values of pectin-based soft candies during digestion

```
Factor    Type   Levels  Values
DAllulose  fixed       4  0; 15; 25; 35
soy       fixed       2  0; 1


Analysis of Variance for brix, using Adjusted SS for Tests

Source          DF    Seq SS    Adj SS   Adj MS      F      P
DAllulose        3     9.192     9.192    3.064   0.36  0.780
soy              1   123.210   123.210  123.210  14.58  0.000
DAllulose*soy    3    10.771    10.771    3.590   0.42  0.736
Error          136  1149.620  1149.620    8.453
Total          143  1292.793


S = 2.90742   R-Sq = 11.07%   R-Sq(adj) = 6.50%


Grouping Information Using Tukey Method and 95.0% Confidence


DAllulose   N  Mean  Grouping
```

```
 0        36   6.2  A
35        36   5.9  A
15        36   5.8  A
25        36   5.5  A

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

soy   N  Mean  Grouping
1    72   6.8  A
0    72   5.0    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

DAllulose  soy   N  Mean  Grouping
15         1    18   7.1  A
 0         1    18   7.0  A
35         1    18   7.0  A
25         1    18   6.1  A
 0         0    18   5.5  A
25         0    18   5.0  A
35         0    18   4.8  A
15         0    18   4.6  A

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

Table A.4.1. Analysis of Varience for Brix values of pectin-based soft candies at 120 min.

```
Factor    Type   Levels  Values
Dpsicose  fixed       4  0; 15; 25; 35
soy       fixed       2  0; 1


Analysis of Variance for brix, using Adjusted SS for Tests

Source         DF   Seq SS   Adj SS   Adj MS       F      P
Dpsicose        3   1.6475   1.6475   0.5492    2.26  0.158
soy             1  50.4100  50.4100  50.4100  207.88  0.000
Dpsicose*soy    3   3.8200   3.8200   1.2733    5.25  0.027
Error           8   1.9400   1.9400   0.2425
Total          15  57.8175


S = 0.492443   R-Sq = 96.64%   R-Sq(adj) = 93.71%
```

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

Dpsicose  N  Mean  Grouping
 0        4  10.5  A
35        4  10.0  A
15        4   9.9  A
25        4   9.6  A

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

soy  N  Mean  Grouping
1    8  11.8  A
0    8   8.2    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

Dpsicose  soy  N  Mean  Grouping
15        1    2  12.4  A
35        1    2  11.9  A
 0        1    2  11.9  A
25        1    2  10.8  A B
 0        0    2   9.0    B C
25        0    2   8.3      C
35        0    2   8.0      C
15        0    2   7.4      C

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

Table A.4.2. Analysis of Varience for Power law model constants of pectin-based soft candies

1. ANOVA for power law index (n) of pectin based soft candies

```
Factor     Type   Levels  Values
soy        fixed       2  0; 1
d-psicose  fixed       4  0; 15; 25; 35


Analysis of Variance for n, using Adjusted SS for Tests

Source         DF     Seq SS     Adj SS     Adj MS       F      P
soy             1  0.0202208  0.0202208  0.0202208  418.66  0.000
d-psicose       3  0.0149448  0.0149448  0.0049816  103.14  0.000
```

```
soy*d-psicose   3  0.0001326  0.0001326  0.0000442    0.92  0.476
Error           8  0.0003864  0.0003864  0.0000483
Total          15  0.0356846


S = 0.00694973   R-Sq = 98.92%   R-Sq(adj) = 97.97%


Grouping Information Using Tukey Method and 95.0% Confidence

soy  N  Mean  Grouping
1    8   0.5  A
0    8   0.4    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

d-psicose  N  Mean  Grouping
 0         4   0.5  A
25         4   0.5  A
35         4   0.5  A
15         4   0.4    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

soy  d-psicose  N  Mean  Grouping
1    25         2   0.5  A
1     0         2   0.5  A
1    35         2   0.5  A
0     0         2   0.4    B
1    15         2   0.4    B
0    25         2   0.4    B
0    35         2   0.4    B
0    15         2   0.4      C

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

2. ANOVA for dissolution constant (k) for pectin based soft candies

```
Factor     Type   Levels  Values
soy        fixed       2  0; 1
d-psicose  fixed       4  0; 15; 25; 35


Analysis of Variance for k, using Adjusted SS for Tests
```

Source	DF	Seq SS	Adj SS	Adj MS	F	P
soy	1	0.0000041	0.0000041	0.0000041	2.02	0.193
d-psicose	3	0.0001230	0.0001230	0.0000410	20.04	0.000
soy*d-psicose	3	0.0000518	0.0000518	0.0000173	8.44	0.007
Error	8	0.0000164	0.0000164	0.0000020		
Total	15	0.0001953				

S = 0.00143053 R-Sq = 91.62% R-Sq(adj) = 84.28%

Grouping Information Using Tukey Method and 95.0% Confidence

soy	N	Mean	Grouping
1	8	0.1	A
0	8	0.1	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

d-psicose	N	Mean	Grouping
15	4	0.1	A
0	4	0.1	B
35	4	0.1	B
25	4	0.1	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

soy	d-psicose	N	Mean	Grouping
0	15	2	0.1	A
1	15	2	0.1	A B
1	35	2	0.1	B C
1	25	2	0.1	B C
1	0	2	0.1	B C
0	0	2	0.1	B C
0	25	2	0.0	C
0	35	2	0.0	C

Means that do not share a letter are significantly different.

Table A.5. Analysis of Varience for moisture content values of pectin-based soft candies before digestion

```
Factor      Type   Levels  Values
D-Allulose  fixed       4  0; 15; 25; 35
soy         fixed       2  0; 1


Analysis of Variance for moisture, using Adjusted SS for Tests

Source          DF     Seq SS     Adj SS     Adj MS       F      P
D-Allulose       3  0.0006491  0.0006491  0.0002164    4.50  0.018
soy              1  0.0127389  0.0127389  0.0127389  264.81  0.000
D-Allulose*soy   3  0.0025672  0.0025672  0.0008557   17.79  0.000
Error           16  0.0007697  0.0007697  0.0000481
Total           23  0.0167248


S = 0.00693577   R-Sq = 95.40%   R-Sq(adj) = 93.38%


Unusual Observations for moisture

Obs  moisture       Fit    SE Fit  Residual  St Resid
  7  0.110103  0.097960  0.004004  0.012143      2.14 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95.0% Confidence

D-Allulose  N  Mean  Grouping
15          6   0.1  A
35          6   0.1  A
 0          6   0.1  A
25          6   0.1    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

soy   N  Mean  Grouping
1    12   0.1  A
0    12   0.1    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

D-Allulose  soy  N  Mean  Grouping
```

```
35          1    3   0.2  A
15          1    3   0.1  A
25          1    3   0.1  A B
 0          1    3   0.1    B C
 0          0    3   0.1      C D
15          0    3   0.1        D E
35          0    3   0.1          E
25          0    3   0.1          E

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

Table A.6. Analysis of Varience for moisture content values of pectin-based soft candies after digestion

```
Factor      Type   Levels  Values
D-Allulose  fixed       4  0; 15; 25; 35
soy         fixed       2  0; 1


Analysis of Variance for moisture, using Adjusted SS for Tests

Source          DF     Seq SS     Adj SS     Adj MS      F      P
D-Allulose       3  0.0044467  0.0031900  0.0010633   8.70  0.004
soy              1  0.0063447  0.0039368  0.0039368  32.23  0.000
D-Allulose*soy   3  0.0039516  0.0039516  0.0013172  10.78  0.002
Error           10  0.0012216  0.0012216  0.0001222
Total           17  0.0159646


S = 0.0110524   R-Sq = 92.35%   R-Sq(adj) = 86.99%


Unusual Observations for moisture

Obs  moisture       Fit    SE Fit  Residual  St Resid
  8  0.184946  0.184946  0.011052  0.000000         * X
 11  0.187068  0.165132  0.006381  0.021936      2.43 R

R denotes an observation with a large standardized residual.
X denotes an observation whose X value gives it large leverage.


Grouping Information Using Tukey Method and 95.0% Confidence

D-Allulose  N  Mean  Grouping
 0          3   0.2  A
15          5   0.2  A
25          5   0.1    B
35          5   0.1    B

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

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

soy   N  Mean  Grouping
1    10   0.2  A
0     8   0.1    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

D-Allulose  soy  N  Mean  Grouping
15          1    3   0.2  A
 0          0    1   0.2  A B
35          1    2   0.2  A B
25          1    3   0.2  A B
 0          1    2   0.2  A B C
15          0    2   0.2    B C
25          0    2   0.1      C D
35          0    3   0.1        D

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

Table A.7. Analysis of Varience for digestion effect on moisture content of each pectin-based soft candies

1. Digestion effect on S0_SA_035

```
Factor     Type   Levels  Values
digestion  fixed       2  0; 1


Analysis of Variance for mc_s0_sa_035, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS     F      P
digestion   1  0.0002228  0.0002228  0.0002228  2.23  0.233
Error       3  0.0003003  0.0003003  0.0001001
Total       4  0.0005232


S = 0.0100056   R-Sq = 42.59%   R-Sq(adj) = 23.45%


Grouping Information Using Tukey Method and 95.0% Confidence

digestion  N  Mean  Grouping
1          2   0.1  A
0          3   0.1  A
```

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

a. T-test for digestion effect on moisture content of S0_SA_035

```
Two-sample T for Moisture  before vs moisture after

                  N     Mean    StDev  SE Mean
Moisture  before  3  0.09468  0.00509   0.0029
moisture after    2   0.1083   0.0158    0.011


Difference = mu (Moisture  before) - mu (moisture after)
Estimate for difference:  -0.01363
95% CI for difference:  (-0.04269; 0.01544)
T-Test of difference = 0 (vs not =): T-Value = -1.49  P-Value = 0.233  DF =
3
Both use Pooled StDev = 0.0100
```

2. Digestion effect on S0_SA_1025

```
Factor     Type   Levels  Values
digestion  fixed       2  0; 1


Analysis of Variance for mc_s0_sa_1025, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS      F      P
digestion   1  0.0023953  0.0023953  0.0023953  53.59  0.005
Error       3  0.0001341  0.0001341  0.0000447
Total       4  0.0025294


S = 0.00668591   R-Sq = 94.70%   R-Sq(adj) = 92.93%


Grouping Information Using Tukey Method and 95.0% Confidence

digestion  N  Mean  Grouping
1          2   0.1  A
0          3   0.1    B

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

3. Digestion effect on S0_SA_2015

```
Factor     Type   Levels  Values
digestion  fixed       2  0; 1


Analysis of Variance for mc_s0_sa_2015, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS      F      P
digestion   1  0.0032541  0.0032541  0.0032541  33.52  0.010
Error       3  0.0002912  0.0002912  0.0000971
Total       4  0.0035453


S = 0.00985226   R-Sq = 91.79%   R-Sq(adj) = 89.05%


Grouping Information Using Tukey Method and 95.0% Confidence

digestion  N  Mean  Grouping
1          2   0.2  A
0          3   0.1    B

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

4. Digestion effect on S0_SA_350

```
Factor     Type   Levels  Values
digestion  fixed       2  0; 1


Analysis of Variance for mc_s0_sa_350, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS      F      P
digestion   1  0.0038230  0.0038230  0.0038230  36.64  0.009
Error       3  0.0003131  0.0003131  0.0001044
Total       4  0.0041360


S = 0.0102152   R-Sq = 92.43%   R-Sq(adj) = 89.91%


Grouping Information Using Tukey Method and 95.0% Confidence

digestion  N  Mean  Grouping
1          2   0.2  A
0          3   0.1    B

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

5. Digestion effect on S1_SA_035

```
Factor     Type   Levels  Values
digestion  fixed       2  0; 1


Analysis of Variance for mc_s1_sa_035, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS      F      P
digestion   1  0.0007918  0.0007918  0.0007918  12.82  0.037
Error       3  0.0001853  0.0001853  0.0000618
Total       4  0.0009771


S = 0.00785947   R-Sq = 81.03%   R-Sq(adj) = 74.71%


Grouping Information Using Tukey Method and 95.0% Confidence

digestion  N  Mean  Grouping
1          2   0.2  A
0          3   0.2    B

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

6. Digestion effect on S1_SA_1025

```
Factor     Type   Levels  Values
digestion  fixed       2  0; 1


Analysis of Variance for mc_s1_sa_1025, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS     F      P
digestion   1  0.0006862  0.0006862  0.0006862  2.55  0.208
Error       3  0.0008064  0.0008064  0.0002688
Total       4  0.0014926


S = 0.0163951   R-Sq = 45.97%   R-Sq(adj) = 27.97%


Grouping Information Using Tukey Method and 95.0% Confidence

digestion  N  Mean  Grouping
1          2   0.2  A
0          3   0.1     B

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

7. Digestion effect on S1_SA_2015

```
Factor     Type   Levels  Values
digestion  fixed       2  0; 1


Analysis of Variance for mc_s1_sa_2015, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS      F      P
digestion   1  0.0018607  0.0018607  0.0018607  45.53  0.007
Error       3  0.0001226  0.0001226  0.0000409
Total       4  0.0019833


S = 0.00639269   R-Sq = 93.82%   R-Sq(adj) = 91.76%


Grouping Information Using Tukey Method and 95.0% Confidence

digestion  N  Mean  Grouping
1          2   0.2  A
0          3   0.1    B

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

8. Digestion effect on S1_SA_350

```
Factor     Type   Levels  Values
digestion  fixed       2  0; 1


Analysis of Variance for mc_s1_sa_350, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS      F      P
digestion   1  0.0011049  0.0011049  0.0011049  93.15  0.002
Error       3  0.0000356  0.0000356  0.0000119
Total       4  0.0011405


S = 0.00344405   R-Sq = 96.88%   R-Sq(adj) = 95.84%


Grouping Information Using Tukey Method and 95.0% Confidence

digestion  N  Mean  Grouping
1          2   0.2  A
0          3   0.1    B

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

Table A.8. Analysis of Varience for water activity values of pectin-based soft candies before digestion

```
Factor    Type   Levels  Values
Allulose  fixed       4  0; 15; 25; 35
soy       fixed       2  0; 1


Analysis of Variance for aw, using Adjusted SS for Tests

Source        DF     Seq SS     Adj SS     Adj MS        F      P
Allulose       3  0.0058973  0.0058973  0.0019658   210.65  0.000
soy            1  0.0008194  0.0008194  0.0008194    87.81  0.000
Allulose*soy   3  0.0358717  0.0358717  0.0119572  1281.33  0.000
Error          8  0.0000747  0.0000747  0.0000093
Total         15  0.0426631


S = 0.00305481   R-Sq = 99.83%   R-Sq(adj) = 99.67%


Unusual Observations for aw

Obs        aw       Fit    SE Fit   Residual  St Resid
 15  0.552200  0.558250  0.002160  -0.006050     -2.80 R
 16  0.564300  0.558250  0.002160   0.006050      2.80 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95.0% Confidence

Allulose  N  Mean  Grouping
 0        4   0.6  A
35        4   0.6    B
25        4   0.6    B
15        4   0.6      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

soy  N  Mean  Grouping
0    8   0.6  A
1    8   0.6    B

Means that do not share a letter are significantly different.


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

```
Allulose  soy  N  Mean  Grouping
 0        0    2   0.7  A
35        1    2   0.6    B
25        1    2   0.6    B
15        0    2   0.6      C
25        0    2   0.6        D
35        0    2   0.6        D
15        1    2   0.6        D
 0        1    2   0.6        D

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

Table A.9. Correlation for Moisture content vs water activity

```
Pearson correlation of moisture (%) and aw = 0.232
P-Value = 0.581
```

Table A.10. Analysis of Varience for colour values of pectin-based soft candies before digestion

1. ANOVA for L values of pectin based soft candies

```
Factor      Type   Levels  Values
D-Allulose  fixed       4  0; 15; 25; 35
soy         fixed       2  0; 1


Analysis of Variance for L, using Adjusted SS for Tests

Source          DF   Seq SS   Adj SS  Adj MS        F      P
D-Allulose       3  198.642  198.642  66.214  2801.97  0.000
soy              1    0.005    0.005   0.005     0.19  0.672
D-Allulose*soy   3   32.235   32.235  10.745   454.70  0.000
Error            8    0.189    0.189   0.024
Total           15  231.071


S = 0.153725   R-Sq = 99.92%   R-Sq(adj) = 99.85%


Grouping Information Using Tukey Method and 95.0% Confidence

D-Allulose  N  Mean  Grouping
 0          4  54.5  A
15          4  46.4    B
35          4  46.3    B
25          4  46.2    B

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

Grouping Information Using Tukey Method and 95.0% Confidence

soy	N	Mean	Grouping
0	8	48.4	A
1	8	48.3	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

D-Allulose	soy	N	Mean	Grouping
0	0	2	56.2	A
0	1	2	52.7	B
25	1	2	47.9	C
15	0	2	47.4	C
35	1	2	47.4	C
15	1	2	45.4	D
35	0	2	45.3	D
25	0	2	44.5	E

Means that do not share a letter are significantly different.

2. ANOVA for a* values of pectin based soft candies

Factor	Type	Levels	Values
D-Allulose	fixed	4	0; 15; 25; 35
soy	fixed	2	0; 1

Analysis of Variance for a, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
D-Allulose	3	13.497	13.497	4.499	2726.59	0.000
soy	1	54.022	54.022	54.022	32740.91	0.000
D-Allulose*soy	3	6.474	6.474	2.158	1307.98	0.000
Error	8	0.013	0.013	0.002		
Total	15	74.007				

S = 0.0406202 R-Sq = 99.98% R-Sq(adj) = 99.97%

Unusual Observations for a

Obs	a	Fit	SE Fit	Residual	St Resid	
9	5.73000	5.80500	0.02872	-0.07500	-2.61	R
10	5.88000	5.80500	0.02872	0.07500	2.61	R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95.0% Confidence

```
D-Allulose  N  Mean  Grouping
35          4   3.4  A
25          4   2.7    B
15          4   2.6    B
 0          4   0.9      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

soy  N  Mean  Grouping
1    8   4.2  A
0    8   0.6    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

D-Allulose  soy  N  Mean  Grouping
35          1    2   5.8  A
15          1    2   4.9    B
25          1    2   4.5      C
 0          1    2   1.7        D
35          0    2   1.0          E
25          0    2   0.9            F
15          0    2   0.3              G
 0          0    2   0.1              G

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

3. ANOVA for b* values of pectin based soft candies

```
Factor      Type   Levels  Values
D-Allulose  fixed       4  0; 15; 25; 35
soy         fixed       2  0; 1


Analysis of Variance for b, using Adjusted SS for Tests

Source          DF  Seq SS  Adj SS  Adj MS        F      P
D-Allulose       3  79.797  79.797  26.599  1451.51  0.000
soy              1  14.025  14.025  14.025   765.35  0.000
D-Allulose*soy   3   4.372   4.372   1.457    79.53  0.000
Error            8   0.147   0.147   0.018
Total           15  98.341


S = 0.135370   R-Sq = 99.85%   R-Sq(adj) = 99.72%
```

Unusual Observations for b

Obs	b	Fit	SE Fit	Residual	St Resid
9	14.0700	14.3200	0.0957	-0.2500	-2.61 R
10	14.5700	14.3200	0.0957	0.2500	2.61 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95.0% Confidence

D-Allulose	N	Mean	Grouping
15	4	13.1	A
35	4	12.9	A
0	4	9.7	B
25	4	7.8	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

soy	N	Mean	Grouping
1	8	11.8	A
0	8	9.9	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

D-Allulose	soy	N	Mean	Grouping
35	1	2	14.3	A
15	1	2	13.4	B
15	0	2	12.9	C
35	0	2	11.4	D
0	1	2	10.3	E
25	1	2	9.2	F
0	0	2	9.2	F
25	0	2	6.4	G

Means that do not share a letter are significantly different.

Table A.11. Analysis of Varience for pH of pectin-based soft candies before digestion

```
Factor    Type   Levels  Values
Allulose  fixed       4  0; 15; 25; 35
soy       fixed       2  0; 1


Analysis of Variance for pH, using Adjusted SS for Tests

Source        DF    Seq SS    Adj SS    Adj MS       F      P
Allulose       3  0.093250  0.093250  0.031083   54.06  0.000
soy            1  0.189225  0.189225  0.189225  329.09  0.000
Allulose*soy   3  0.090725  0.090725  0.030242   52.59  0.000
Error          8  0.004600  0.004600  0.000575
Total         15  0.377800


S = 0.0239792   R-Sq = 98.78%   R-Sq(adj) = 97.72%


Grouping Information Using Tukey Method and 95.0% Confidence

Allulose  N  Mean  Grouping
15        4   3.2  A
25        4   3.2  A
35        4   3.0    B
 0        4   3.0    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

soy  N  Mean  Grouping
0    8   3.2  A
1    8   3.0    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

Allulose  soy  N  Mean  Grouping
25        0    2   3.4  A
15        0    2   3.4  A
 0        0    2   3.1    B
35        0    2   3.1    B C
35        1    2   3.0    B C
15        1    2   3.0    B C
25        1    2   3.0      C
 0        1    2   3.0      C

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

Table A.12. Analysis of Varience for pH of pectin-based soft candies after digestion

```
Factor    Type   Levels  Values
Allulose  fixed       4  0; 15; 25; 35
soy       fixed       2  0; 1


Analysis of Variance for pH, using Adjusted SS for Tests

Source        DF    Seq SS    Adj SS    Adj MS       F      P
Allulose       3  0.007319  0.007319  0.002440    7.97  0.009
soy            1  0.131406  0.131406  0.131406  429.08  0.000
Allulose*soy   3  0.008619  0.008619  0.002873    9.38  0.005
Error          8  0.002450  0.002450  0.000306
Total         15  0.149794


S = 0.0175   R-Sq = 98.36%   R-Sq(adj) = 96.93%


Unusual Observations for pH

Obs       pH      Fit   SE Fit  Residual  St Resid
  9  3.00000  3.03000  0.01237  -0.03000     -2.42 R
 10  3.06000  3.03000  0.01237   0.03000      2.42 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95.0% Confidence

Allulose  N  Mean  Grouping
15        4   3.0  A
25        4   3.0  A
 0        4   3.0  A
35        4   3.0    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

soy  N  Mean  Grouping
1    8   3.1  A
0    8   2.9    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

Allulose  soy  N  Mean  Grouping
```

```
15        1    2   3.1  A
 0        1    2   3.1  A
25        1    2   3.1  A
35        1    2   3.0    B
25        0    2   2.9      C
 0        0    2   2.9      C
35        0    2   2.9      C
15        0    2   2.9      C

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

Table A.13. Analysis of Varience for digestion effect on pH of each pectin-based soft candies

1. Digestion effect on S0_SA_035

```
Factor     Type   Levels  Values
digestion  fixed       2  0; 1


Analysis of Variance for ph_s0_sa_035, using Adjusted SS for Tests

Source     DF    Seq SS    Adj SS    Adj MS       F      P
digestion   1  0.018225  0.018225  0.018225  145.80  0.007
Error       2  0.000250  0.000250  0.000125
Total       3  0.018475


S = 0.0111803   R-Sq = 98.65%   R-Sq(adj) = 97.97%


Grouping Information Using Tukey Method and 95.0% Confidence

digestion  N  Mean  Grouping
0          2   3.1  A
1          2   2.9    B

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

2. Digestion effect on S0_SA_1025

```
Factor     Type   Levels  Values
digestion  fixed       2  0; 1


Analysis of Variance for ph_s0_sa_1025, using Adjusted SS for Tests

Source     DF   Seq SS   Adj SS   Adj MS        F      P
digestion   1  0.21160  0.21160  0.21160  1058.00  0.001
Error       2  0.00040  0.00040  0.00020
Total       3  0.21200


S = 0.0141421   R-Sq = 99.81%   R-Sq(adj) = 99.72%


Grouping Information Using Tukey Method and 95.0% Confidence

digestion  N  Mean  Grouping
0          2   3.4  A
1          2   2.9    B

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

3. Digestion effect on S0_SA_2015

```
Factor     Type   Levels  Values
digestion  fixed       2  0; 1


Analysis of Variance for ph_s0_sa_2015, using Adjusted SS for Tests

Source     DF   Seq SS   Adj SS   Adj MS        F      P
digestion   1  0.20250  0.20250  0.20250  4050.00  0.000
Error       2  0.00010  0.00010  0.00005
Total       3  0.20260


S = 0.00707107   R-Sq = 99.95%   R-Sq(adj) = 99.93%


Grouping Information Using Tukey Method and 95.0% Confidence

digestion  N  Mean  Grouping
0          2   3.4  A
1          2   2.9    B

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

4. Digestion effect on S0_SA_350

```
Factor     Type   Levels  Values
digestion  fixed       2  0; 1


Analysis of Variance for ph_s0_sa_350, using Adjusted SS for Tests

Source     DF    Seq SS    Adj SS    Adj MS       F      P
digestion   1  0.030625  0.030625  0.030625  245.00  0.004
Error       2  0.000250  0.000250  0.000125
Total       3  0.030875


S = 0.0111803   R-Sq = 99.19%   R-Sq(adj) = 98.79%


Grouping Information Using Tukey Method and 95.0% Confidence

digestion  N  Mean  Grouping
0          2   3.1  A
1          2   2.9    B

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

5. Digestion effect on S1_SA_035

```
Factor     Type   Levels  Values
digestion  fixed       2  0; 1


Analysis of Variance for ph_s1_sa_035, using Adjusted SS for Tests

Source     DF    Seq SS    Adj SS    Adj MS     F      P
digestion   1  0.000025  0.000025  0.000025  0.02  0.910
Error       2  0.003050  0.003050  0.001525
Total       3  0.003075


S = 0.0390512   R-Sq = 0.81%   R-Sq(adj) = 0.00%


Grouping Information Using Tukey Method and 95.0% Confidence

digestion  N  Mean  Grouping
0          2   3.0  A
1          2   3.0  A

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

6. Digestion effect on S1_SA_1025

```
Factor     Type   Levels  Values
digestion  fixed       2  0; 1


Analysis of Variance for ph_s1_sa_1025, using Adjusted SS for Tests

Source     DF    Seq SS    Adj SS    Adj MS      F      P
digestion   1  0.015625  0.015625  0.015625  48.08  0.020
Error       2  0.000650  0.000650  0.000325
Total       3  0.016275


S = 0.0180278   R-Sq = 96.01%   R-Sq(adj) = 94.01%


Grouping Information Using Tukey Method and 95.0% Confidence

digestion  N  Mean  Grouping
1          2   3.1  A
0          2   3.0    B

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

7. Digestion effect on S1_SA_2015

```
Factor     Type   Levels  Values
digestion  fixed       2  0; 1


Analysis of Variance for ph_s1_sa_2015, using Adjusted SS for Tests

Source     DF    Seq SS    Adj SS    Adj MS      F      P
digestion   1  0.012100  0.012100  0.012100  48.40  0.020
Error       2  0.000500  0.000500  0.000250
Total       3  0.012600


S = 0.0158114   R-Sq = 96.03%   R-Sq(adj) = 94.05%


Grouping Information Using Tukey Method and 95.0% Confidence

digestion  N  Mean  Grouping
1          2   3.1  A
0          2   3.0    B

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

8. Digestion effect on S1_SA_350

```
Factor     Type   Levels  Values
digestion  fixed       2  0; 1


Analysis of Variance for ph_s1_sa_350, using Adjusted SS for Tests

Source     DF    Seq SS    Adj SS    Adj MS      F      P
digestion   1  0.021025  0.021025  0.021025  22.73  0.041
Error       2  0.001850  0.001850  0.000925
Total       3  0.022875


S = 0.0304138   R-Sq = 91.91%   R-Sq(adj) = 87.87%


Grouping Information Using Tukey Method and 95.0% Confidence

digestion  N  Mean  Grouping
1          2   3.1  A
0          2   3.0    B

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

Table A.14. Correlation between pH and Hardness of the pectin based soft candies

```
Pearson correlation of ph and hardness = 0.929
P-Value = 0.017
```

Table A.15. Analysis of Varience for Global Least Square Analysis (T2) of pectin-based soft candies after digestion

1. ANOVA Result for Peak 1

```
Factor      Type   Levels  Values
D-allulose  fixed       4  0; 15; 25; 35
Soy         fixed       2  0; 1


Analysis of Variance for Peak 1, using Adjusted SS for Tests

Source          DF  Seq SS  Adj SS  Adj MS      F      P
D-allulose       3  1.3291  1.3291  0.4430   3.44  0.072
Soy              1  3.2671  3.2671  3.2671  25.40  0.001
D-allulose*Soy   3  1.2641  1.2641  0.4214   3.28  0.080
Error            8  1.0289  1.0289  0.1286
Total           15  6.8890
```

```
S = 0.358617   R-Sq = 85.07%   R-Sq(adj) = 72.00%


Grouping Information Using Tukey Method and 95.0% Confidence

D-allulose  N  Mean  Grouping
15          4   1.7  A
 0          4   1.4  A
35          4   1.3  A
25          4   0.9  A

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

Soy  N  Mean  Grouping
0    8   1.8  A
1    8   0.8    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

D-allulose  Soy  N  Mean  Grouping
15          0    2   2.5  A
 0          0    2   2.0  A B
35          0    2   1.5  A B
35          1    2   1.0    B
25          0    2   1.0    B
15          1    2   0.9    B
25          1    2   0.8    B
 0          1    2   0.7    B

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

2. ANOVA results for peak 2

```
Factor      Type   Levels  Values
D-allulose  fixed       4  0; 15; 25; 35
Soy         fixed       2  0; 1


Analysis of Variance for Peak 2, using Adjusted SS for Tests

Source          DF   Seq SS   Adj SS  Adj MS      F      P
D-allulose       3  25.2500  25.2500  8.4167  22.44  0.000
Soy              1   2.2500   2.2500  2.2500   6.00  0.040
D-allulose*Soy   3  17.2500  17.2500  5.7500  15.33  0.001
Error            8   3.0000   3.0000  0.3750
```

```
Total           15  47.7500


S = 0.612372   R-Sq = 93.72%   R-Sq(adj) = 88.22%


Unusual Observations for Peak 2

Obs   Peak 2      Fit   SE Fit  Residual  St Resid
  1  3.00000  4.00000  0.43301  -1.00000     -2.31 R
  2  5.00000  4.00000  0.43301   1.00000      2.31 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95.0% Confidence

D-allulose  N  Mean  Grouping
35          4   4.2  A
25          4   2.8    B
15          4   1.5    B C
 0          4   1.0      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

Soy  N  Mean  Grouping
1    8   2.7  A
0    8   2.0    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

D-allulose  Soy  N  Mean  Grouping
35          1    2   4.5  A
25          0    2   4.0  A B
35          0    2   4.0  A B
15          1    2   3.0  A B C
 0          1    2   2.0    B C D
25          1    2   1.5      C D
15          0    2  -0.0        D
 0          0    2  -0.0        D

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

3. ANOVA results for Relative Area (RA) of peak 1

```
Factor      Type   Levels  Values
D-allulose  fixed       4  0; 15; 25; 35
Soy         fixed       2  0; 1


Analysis of Variance for RA peak 1, using Adjusted SS for Tests

Source          DF   Seq SS  Adj SS  Adj MS      F      P
D-allulose       3   4739.8  4739.8  1579.9  11.97  0.003
Soy              1   2634.3  2634.3  2634.3  19.96  0.002
D-allulose*Soy   3   2701.4  2701.4   900.5   6.82  0.014
Error            8   1056.0  1056.0   132.0
Total           15  11131.4


S = 11.4889   R-Sq = 90.51%   R-Sq(adj) = 82.21%


Grouping Information Using Tukey Method and 95.0% Confidence

D-allulose  N  Mean  Grouping
 0          4  75.0  A
15          4  74.2  A
25          4  50.9  A B
35          4  33.8    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

Soy  N  Mean  Grouping
0    8  71.3  A
1    8  45.6    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

D-allulose  Soy  N   Mean  Grouping
 0          0    2  100.0  A
15          0    2  100.0  A
25          1    2   55.2  A B
 0          1    2   50.0    B
15          1    2   48.4    B
25          0    2   46.6    B
35          0    2   38.7    B
35          1    2   29.0    B

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

4. ANOVA results for Relative Area (RA) of peak 1

```
Factor      Type   Levels  Values
D-allulose  fixed       4  0; 15; 25; 35
Soy         fixed       2  0; 1


Analysis of Variance for RA peak 2, using Adjusted SS for Tests

Source          DF  Seq SS  Adj SS  Adj MS      F      P
D-allulose       3  3740.5  3740.5  1246.8   9.78  0.005
Soy              1  2520.0  2520.0  2520.0  19.76  0.002
D-allulose*Soy   3  2653.1  2653.1   884.4   6.93  0.013
Error            8  1020.3  1020.3   127.5
Total           15  9933.9


S = 11.2933   R-Sq = 89.73%   R-Sq(adj) = 80.74%


Grouping Information Using Tukey Method and 95.0% Confidence

D-allulose  N  Mean  Grouping
35          4  61.8  A
25          4  45.9  A B
15          4  25.8    B
 0          4  25.0    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

Soy  N  Mean  Grouping
1    8  52.2  A
0    8  27.1    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

D-allulose  Soy  N  Mean  Grouping
35          1    2  62.3  A
35          0    2  61.3  A
15          1    2  51.6  A
 0          1    2  50.0  A
25          0    2  47.0  A
25          1    2  44.8  A
15          0    2   0.0    B
 0          0    2  -0.0    B

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

Table A.16. Analysis of Varience for monoexponential tranverse relaxation times (T2) of pectin-based soft candies before digestion

```
Factor     Type   Levels  Values
soy        fixed       2  0; 1
D-psicose  fixed       4  0; 15; 25; 35


Analysis of Variance for T2, using Adjusted SS for Tests

Source         DF   Seq SS   Adj SS  Adj MS      F      P
soy             1   0.4536   0.5212  0.5212   3.67  0.072
D-psicose       3  27.7521  26.7148  8.9049  62.69  0.000
soy*D-psicose   3   0.6261   0.6261  0.2087   1.47  0.258
Error          17   2.4148   2.4148  0.1420
Total          24  31.2467


S = 0.376894   R-Sq = 92.27%   R-Sq(adj) = 89.09%


Unusual Observations for T2

Obs       T2      Fit   SE Fit  Residual  St Resid
 14  3.47510  4.20207  0.18845  -0.72697     -2.23 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95.0% Confidence

soy   N  Mean  Grouping
0    12   2.8  A
1    13   2.5  A

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

D-psicose  N  Mean  Grouping
35         7   4.2  A
25         5   2.6    B
15         5   2.2    B
 0         8   1.5      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

soy  D-psicose  N  Mean  Grouping
```

```
1    35          4   4.2  A
0    35          3   4.1  A
0    25          3   3.0     B
0    15          3   2.5     B C
1    25          2   2.2     B C  D
1    15          2   2.0     B C  D
0     0          3   1.5        C  D
1     0          5   1.4           D

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

Table A.17. Analysis of Varience for monoexponential tranverse relaxation times (T2) of pectin-based soft candies after digestion

```
Factor     Type   Levels  Values
D-psicose  fixed       4  0; 15; 25; 35
soy        fixed       2  0; 1


Analysis of Variance for T2, using Adjusted SS for Tests

Source          DF   Seq SS   Adj SS  Adj MS      F      P
D-psicose        3  25.4903  27.7684  9.2561  17.49  0.000
soy              1   2.7098   2.3918  2.3918   4.52  0.057
D-psicose*soy    3   3.3701   3.3701  1.1234   2.12  0.155
Error           11   5.8213   5.8213  0.5292
Total           18  37.3915


S = 0.727468   R-Sq = 84.43%   R-Sq(adj) = 74.52%


Grouping Information Using Tukey Method and 95.0% Confidence

D-psicose  N  Mean  Grouping
35         5   6.8  A
25         4   6.1  A B
15         4   4.9     B C
 0         6   3.7        C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

soy   N  Mean  Grouping
1    10   5.7  A
0     9   5.0  A

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

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

D-psicose  soy  N  Mean  Grouping
35         1    2   7.1  A
25         0    2   6.2  A B
25         1    2   6.1  A B C
35         0    3   5.7  A B C
15         1    2   5.1    B C
15         0    2   4.7    B C
 0         1    4   3.9    B C
 0         0    2   3.5      C

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

Table A.18. Analysis of Varience for digestion effect on T2 of each pectin-based soft candies

1. Digestion effect on S0_SA_035

```
Factor     Type   Levels  Values
digestion  fixed       2  0; 1


Analysis of Variance for T2_s0_sa_035, using Adjusted SS for Tests

Source     DF  Seq SS  Adj SS  Adj MS      F      P
digestion   1  3.7701  3.7701  3.7701  19.02  0.012
Error       4  0.7929  0.7929  0.1982
Total       5  4.5630


S = 0.445227   R-Sq = 82.62%   R-Sq(adj) = 78.28%


Grouping Information Using Tukey Method and 95.0% Confidence

digestion  N  Mean  Grouping
1          3   5.7  A
0          3   4.1    B

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

2. Digestion effect on S0_SA_1025

```
Factor     Type   Levels  Values
digestion  fixed       2  0; 1


Analysis of Variance for T2_s0_sa_1025, using Adjusted SS for Tests

Source     DF  Seq SS  Adj SS  Adj MS       F      P
digestion   1  12.250  12.250  12.250  272.91  0.000
Error       3   0.135   0.135   0.045
Total       4  12.385


S = 0.211867   R-Sq = 98.91%   R-Sq(adj) = 98.55%


Grouping Information Using Tukey Method and 95.0% Confidence

digestion  N  Mean  Grouping
1          2   6.2  A
0          3   3.0    B

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

3. Digestion effect on S0_SA_2015

```
Factor     Type   Levels  Values
digestion  fixed       2  0; 1


Analysis of Variance for T2_s0_sa_2015, using Adjusted SS for Tests

Source     DF  Seq SS  Adj SS  Adj MS      F      P
digestion   1  5.8006  5.8006  5.8006  24.61  0.016
Error       3  0.7070  0.7070  0.2357
Total       4  6.5076


S = 0.485446   R-Sq = 89.14%   R-Sq(adj) = 85.51%


Grouping Information Using Tukey Method and 95.0% Confidence

digestion  N  Mean  Grouping
1          2   4.7  A
0          3   2.5    B

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

4. Digestion effect on S0_SA_350

```
Factor     Type   Levels  Values
digestion  fixed       2  0; 1


Analysis of Variance for T2_s0_sa_350, using Adjusted SS for Tests

Source     DF  Seq SS  Adj SS  Adj MS     F      P
digestion   1  4.6532  4.6532  4.6532  7.11  0.076
Error       3  1.9629  1.9629  0.6543
Total       4  6.6161


S = 0.808890   R-Sq = 70.33%   R-Sq(adj) = 60.44%


Grouping Information Using Tukey Method and 95.0% Confidence

digestion  N  Mean  Grouping
1          2   3.5  A
0          3   1.5    B

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

5. Digestion effect on S1_SA_035

```
Factor     Type   Levels  Values
digestion  fixed       2  0; 1


Analysis of Variance for T2_s1_sa_035, using Adjusted SS for Tests

Source     DF  Seq SS  Adj SS  Adj MS      F      P
digestion   1  18.033  18.033  18.033  25.63  0.007
Error       4   2.814   2.814   0.704
Total       5  20.847


S = 0.838793   R-Sq = 86.50%   R-Sq(adj) = 83.13%


Grouping Information Using Tukey Method and 95.0% Confidence

digestion  N  Mean  Grouping
1          2   7.9  A
0          4   4.2    B

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

6. Digestion effect on S1_SA_1025

```
Factor     Type   Levels  Values
digestion  fixed       2  0; 1


Analysis of Variance for T2_s1_sa_1025, using Adjusted SS for Tests

Source     DF  Seq SS  Adj SS  Adj MS       F      P
digestion   1  15.084  15.084  15.084  429.93  0.002
Error       2   0.070   0.070   0.035
Total       3  15.154


S = 0.187307   R-Sq = 99.54%   R-Sq(adj) = 99.31%


Grouping Information Using Tukey Method and 95.0% Confidence

digestion  N  Mean  Grouping
1          2   6.1  A
0          2   2.2    B

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

7. Digestion effect on S1_SA_2015

```
Factor     Type   Levels  Values
digestion  fixed       2  0; 1


Analysis of Variance for T2_s1_sa_2015, using Adjusted SS for Tests

Source     DF  Seq SS  Adj SS  Adj MS       F      P
digestion   1  9.2988  9.2988  9.2988  334.79  0.003
Error       2  0.0556  0.0556  0.0278
Total       3  9.3544


S = 0.166659   R-Sq = 99.41%   R-Sq(adj) = 99.11%


Grouping Information Using Tukey Method and 95.0% Confidence

digestion  N  Mean  Grouping
1          2   5.1  A
0          2   2.0    B

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

8. Digestion effect on S1_SA_350

```
Factor     Type   Levels  Values
digestion  fixed       2  0; 1


Analysis of Variance for T2_s1_sa_350, using Adjusted SS for Tests

Source     DF  Seq SS  Adj SS  Adj MS      F      P
digestion   1  13.552  13.552  13.552  55.85  0.000
Error       7   1.699   1.699   0.243
Total       8  15.251


S = 0.492612   R-Sq = 88.86%   R-Sq(adj) = 87.27%


R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95.0% Confidence

digestion  N  Mean  Grouping
1          4   3.9  A
0          5   1.4    B

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