USE OF NON-CONVENTIONAL TIME DOMAIN (TD) NMR APPROACHES FOR CHARACTERISATION OF GELATIN BASED SOFT CANDIES

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USE OF NON-CONVENTIONAL TIME DOMAIN (TD) NMR APPROACHES FOR CHARACTERISATION OF GELATIN BASED SOFT CANDIES

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ABSTRACT

USE OF NON-CONVENTIONAL TIME DOMAIN (TD) NMR APPROACHES FOR CHARACTERISATION OF GELATIN BASED SOFT CANDIES

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TD-NMR technique mostly involves the use of T_1 (spin-lattice) and T_2 (spin-spin) relaxation times to explain the changes occurring in food systems. However, these relaxation times are affected from many factors and might not always be the best indicators to work with in food related TD-NMR studies. In this thesis, to our knowledge, the *non-conventional* TD-NMR approaches of *Solid Echo/Magic Sandwich Echo and Spin Diffusion* in food systems were used for the 1st time. As the system of interest, soft confectionery gels were selected due to the simplicity of their composition and the flexibility of preparing standardized formulations. Two problems have been identified related with the confectionery gels. And in that regard, the thesis was structured in 2 parts. In the first part of the study, soft confectionary gelatin gels were formulated and conventional (T_1, T_2) and non-conventional (SE, MSE and Spin Diffusion) TD-NMR experiments were performed. Corn syrups differing in their glucose/fructose compositions were used to prepare the soft candies and five different syrup types were used. Hardness, ^{o}Brix and water activity (a_w) were also measured as the complementary experiments to NMR. Relaxation times changed with syrup type ($p<0.05$) but not an obvious trend was detected. On the other hand, SE/MSE experiments which were performed to calculate the crystallinity

of the samples yielded valuable results. Samples prepared with fructose had the lowest crystallinity values ($p<0.05$) as expected due to the higher solubility of fructose compared to glucose. Spin Diffusion experiments were performed by using *Goldman-Shen* pulse sequence and the interface thickness (*d*) was calculated after a detailed data analysis. Interface thickness showed a wide range of variation ($p<0.05$) thus it was proposed as an indicator for differentiating the candies prepared with different syrups.

In the 2nd part of the study, gelatin-based candies were prepared with the same formulations by just one syrup type but by using bovine and porcine gelatin as the polymer sources. Water activity and ^{o}Brix values were measured. T_1 times did not differ ($p>0.05$) whereas T_2 times were found to be higher for the bovine gels despite their lower water activity. This time, crystallinity values calculated from SE/MSE did not show differences wrt to gelatin source ($p>0.05$). Interface thickness values calculated from SD experiments showed that porcine gelatin-based candies were more stable compared to bovine samples ($p<0.05$).

Results showed that non-conventional NMR approaches had high potential to be utilized in food systems for quality control purposes.

Keywords: Soft gel; gelatin; confectionery; TD-NMR; crystallinity; relaxation times; spin diffusion; Magic Sandwich Echo; Solid Echo

ÖZ

JELATİN TABANLI YUMUŞAK ŞEKERLEMELERİN KARAKTERİZASYONUNDA GELENEKSEL OLMAYAN ZAMANSAL ALANDA NMR YAKLAŞIMLARININ KULLANILMASI

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Zamansal alanda Nükleer Manyetik Rezonans tekniği, gıda sistemlerinde meydana gelen değişiklikleri açıklamak için çoğunlukla T_1 (spin-latis) ve T_2 (spin-spin) relaksasyon zamanlarının kullanılmasını içerir. Bununla birlikte, bu relaksasyon zamanları birçok faktörden etkilenir ve gıda ile ilgili zamansal alanda NMR analizlerinde her zaman çalışmak için en iyi göstergeler olmayabilir. Bu tezde, gıda sistemlerinde Katı hal ekosu / Sihirli sandviç eko ve Spin Difüzyonu gibi geleneksel olmayan zamansal alanda NMR yaklaşımlarının kullanımı literatürde ilk defa araştırılmıştır. İlgili sistem olarak yumuşak şekerleme jelleri, bileşimlerinin basitliği ve standartlaştırılmış formülasyonları hazırlama esnekliği nedeniyle seçilmiştir. Şekerlemeler ile ilgili iki problem tespit edilmiştir. Ve bu bağlamda tez iki bölüm olarak hazırlanmıştır. Çalışmanın ilk bölümünde jelatinden yapılan yumuşak şekerleme jelleri formüle edilmiştir ve konvansiyonel (T_1, T_2) ve konvansiyonel olmayan (SE, MSE ve Spin Difüzyon) NMR deneyleri yapılmıştır. Yumuşak şekerlerin hazırlanmasında glikoz / fruktoz bileşimleri farklı olan 5 mısır şurubu kullanılmıştır. Sertlik, ^{o}Brix ve su aktivitesi (a_w) de tamamlayıcı deneyler olarak yapılmıştır. Relaksasyon zamanlarının şurup türü ile değiştiği ancak belirgin bir

eğilim göstermediği tespit edilmiştir ($p<0.05$). Öte yandan numunelerin kristalinitelerini hesaplamak için yapılan Katı hal ekosu / Sihirli sandviç eko deneyleri önemli sonuçlar vermiştir. Fruktoz ile hazırlanan numunelerinin, fruktozun glikoza kıyasla daha yüksek çözünürlüğünden dolayı en düşük kristallinite değerlerine ($p<0.05$) sahip olduğu görülmüştür. Spin Difüzyon deneyleri, *Goldman-Shen* puls dizisi kullanılarak gerçekleştirilmiştir ve arayüz kalınlığı (d), detaylı bir veri analizi sonrasında hesaplanmıştır. Arayüz kalınlığı geniş bir varyasyon gösterdiği için ($p<0.05$), farklı şuruplarla hazırlanan şekerleri ayırt etmek için bir gösterge olarak önerilmiştir.

Çalışmanın ikinci bölümünde aynı formülasyonla ancak kaynak olarak sığır ve domuz jelatini kullanılarak tek bir mısır şurubu tipi ile hazırlanan jelatin bazlı şekerler hazırlanmıştır. Su aktivitesi ve ^{o}Brix değerleri ölçülmüştür. T_1 süreleri farklılık göstermezken ($p> 0.05$), T_2 süreleri daha düşük su aktivitesine rağmen sığır jellerinde daha yüksek bulunmuştur. Çalışmanın bu bölümünde, SE / MSE'den hesaplanan kristalinite değerleri jelatin kaynağına göre farklılık göstermemiştir ($p>0.05$). SD deneylerinden hesaplanan arayüz kalınlık değerleri, domuz jelatin bazlı şekerlerin sığır numunelerine kıyasla daha stabil olduğunu göstermiştir ($p<0.05$).

Sonuçlar, geleneksel olmayan NMR yaklaşımlarının, kalite kontrol amacıyla gıda sistemlerinde kullanılma potansiyeline sahip olduğunu göstermiştir.

Anahtar Kelimeler: Yumuşak jel; jelatin; şekerleme; Zamansal alanda NMR; kristallik; relaksasyon zamanları; spin difüzyon; Katı hal ekosu; Sihirli sandviç eko

Dedicated to rockets

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TABLE OF CONTENTS

LIST OF TABLES

TABLES

LIST OF FIGURES

FIGURES

LIST OF ABBREVIATIONS

BC	: Bovine based candies
CPMG	: Carr-Purcell-Meiboom-Gill
DE	: Dextrose equivalence
FID	: Free induction decay
HF-NMR	: High field nuclear magnetic resonance
LF-NMR	: Low field nuclear magnetic resonance
MSE	: Magic sandwich echo
NMR	: Nuclear magnetic resonance
PC	: Porcine based candies
RF	: Radiofrequency
SD	: Spin diffusion
SE	: Solid echo
SFC	: Solid fat content
TD NMR	: Time domain nuclear magnetic resonance
TE	: Echo delay time
TR	: Repetition Time

CHAPTER 1

INTRODUCTION

1.1 Gelatin-based candies

Confectionery gel market has a huge share in the confectionary industry. The global jellies and gummy market size was valued at USD 13.9 billion in 2018 (Strouse, 2019). Gelatin based candies are very popular products which are mostly consumed by children and teenagers. However, their production method and style differs according to the taste of different nationalities (Edwards, 2001).They are composed of sucrose and glucose syrups, gelling agent such as gelatin or pectin, coloring, acid and flavoring agents (Delgado & Bañón, 2018). Flavor and sweetness are important parameters for candy industry. Sugar is added to the product not only for the textural properties but also to prevent microbial growth (Efe et al., 2019). The presence of various bonds within these confectionary products result in them being considered as complex gel systems (Pocan et al., 2019). Contrary to a simple gel system, soft candies contain less moisture and mostly carbohydrates that have low molecular weight such as sucrose and corn syrups (DeMars & Ziegler, 2001).

Industrial production of soft candies such as gummies and jellies vary according to the type of the hydrocolloid used. In the case of gelatin, sweeteners including syrups and sucrose are mixed and cooked while the gelatin is mixed with water and heated separately. After this, these solutions are mixed and cooked further till the desired moisture content is obtained. Then, they are deposited in molds and stored (Hartel et

al., 2018). Figure 1.1 shows a schematic for the industrial processing of these candies.

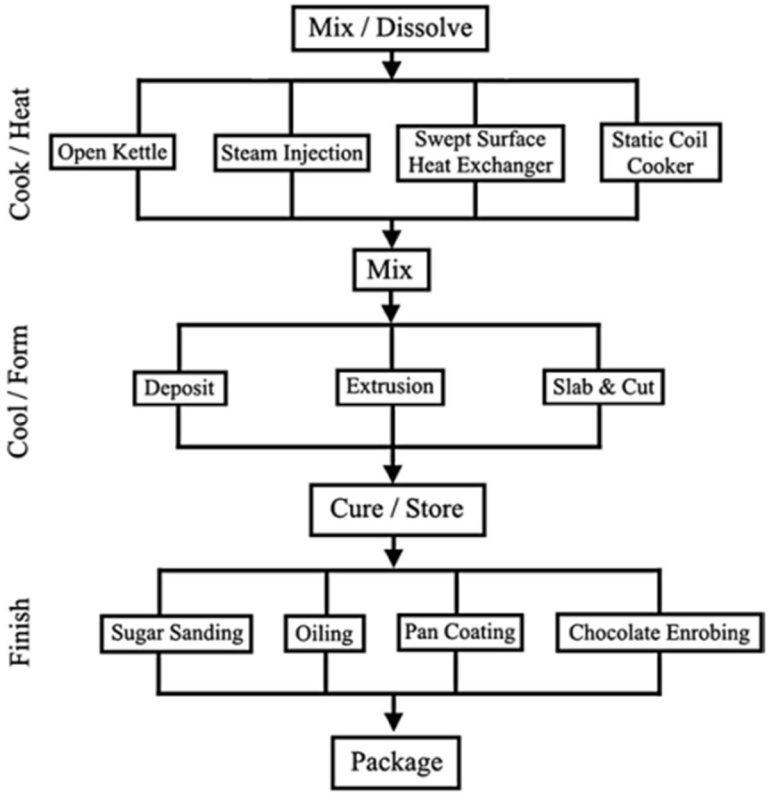

Figure 1.1. Schematic Representation of Gelatin-based Candy Production (Hartel et al. , 2018)

1.2. Ingredients of Gelatin-based Candies

1.2.1. Gelatin

Gelatin is a solid polymer which is flavorless, translucent, nontoxic, and natural. Collagen which is derived from animal bones, skins, and white connective tissue is partially hydrolyzed to produce gelatin (Kariduraganavar et al., 2014).

Triple helix is the native form of collagen which is held together by the interchain hydrogen bonding. Gelatin molecules, when dissolved in water at 37°C, exists as disordered and separate chains (coils). These chains revert to an ordered, helical like form as in the collagen upon cooling, forming a thermoreversible gel with an infinite network of hydrogen-bonded gelatin molecules (Marfil et al., 2012).

Gelatin gives desirable characteristics form to soft candies with its unique molecular characteristics. Physical properties of soft candies can be determined by the nature of the gelatin network (Hofberger, 2018). It gives springiness and transparency to the gels (Marfil et al., 2012).

Isoelectric point (pI) is considered as an important property of proteins. The net charge on proteins is zero at this pH, and thus it is easier to precipitate them while they are at this point. This isoelectric point is denoted as pI by analogy of the pH scale, and acid-processed gelatin has a pI between 6.3-9.5 while alkali-processed gelatin has a pI between 4.5-5.2 (Edwards, 2001).

Generally, bovine and porcine gelatin types are used in formulation that are obtained from cattle and pigs respectively (Edwards, 2001). Differences of bovine and porcine gelatin come from their amino acid composition. Porcine gelatin has higher amounts of glycine, proline and arginine amino acids (Gilsenan & Ross-Murphy, 2000). Porcine gelatin is more stable than bovine gelatin since these amino acids have significant role in secondary structure formation (Jiang, 2015). In addition, the different composition of the amino acids is responsible for having different gelling behavior and melting points of gelatins (Gilsenan & Ross-Murphy, 2000).

Confectionery industry uses significant amount of gelatin for the production of soft candies and marshmallow. However, since porcine gelatin is not preferred by a large population group due to religious beliefs, alternatives to gelatin are sought for. In Turkey, use of porcine gelatin in food products is not allowed. However, since gelatin production does not occur at a level that satisfies the demand of the industry (Cebi et al., 2019), most of the gelatin is imported from other countries and since this increases the cost, there is significant tendency to adulterate the formulations by using porcine gelatin. In that regard, there is an essential necessity for building reliable and strong methods detect the source of commercial gelatin and detect the source of gelatin as an ingredient in food products (Cebi et al., 2019). Polymerase chain reaction (PCR) based, electrophoretic, chromatographic and spectroscopic techniques such as FTIR have been used for this purpose (Cebi et al., 2019; Eryilmaz et al., 2017; Hameed et al., 2018; Hassan et al., 2018).

1.2.2. Corn syrups

Starch is hydrolyzed with acid to obtain glucose syrup (Edwards, 2001). In order to control the process, a Fehling's titration is carried to assume that a proportion of the syrup is dextrose. The obtained syrup is also known as maltose, glucose or fructose syrup depending on the composition. Corn can be hydrolyzed to produce maltose syrup or if the hydrolysis is further increased; glucose monomers are formed and glucose syrup is obtained. To obtain a sweeter syrup, glucose is further isomerized by enzymes and fructose syrups are obtained. Although it can be produced by any source of corn, glucose syrup is mostly made from maize starch, potato starch (Edwards, 2001).

Dextrose equivalence (DE) is the term used to characterize the corn syrups. It is a parameter that shows the reducing sugar content of the syrup. DE 100 refers to the

presence of only reducing sugars in the mix, while DE 0 shows the absence of reducing sugars (Burey et al., 2009). There are various syrups present in the market having different DE values. DE values are also used as indicators for the application group of the syrup. For instance for fruit juices and soft drinks syrup which has 48 DE value is used while 37 DE value syrup is used for jellies and gums (*Sunar Mısır*, 2020).

Since glucose syrup is cheaper than sucrose, it is preferred by the manufacturers as the sugar source. Corn syrup, as being a starch hydrolysate and still containing some oligosaccharide remnants, is much less prone to crystallization. In addition to cost, crystallization retarding property is also an important attribute of corn syrup (Özen et al., 2011). For soft candies, syrups have an important role to control the crystallization of sucrose (Gabarra & Hartel, 1998). This also makes it a popular food ingredient in confectionery products.

However, since either glucose or maltose is not sweet as sucrose (Moskowitz, 1970) corn syrup is usually not used alone as the sweetness source (Edwards, 2001). As long as fructose syrup is not used, sucrose is also added to the formulations. In addition to its sweetness contribution, syrups are also used for texture and stability. They can be used to control water activity and help to provide better texture. Soft candies can generally contain more syrups than sucrose (Burey et al., 2009).

1.2.3. Sucrose

Sucrose is used as main sweetening agent in soft candies (Efe et al., 2019). Sucrose is a nonreducing disaccharide. It is composed of glucose (dextrose) and fructose which are reducing monosaccharides (Edwards, 2001). There is glycosidic linkage

between C1 of glucose and C2 of fructose (Hartel et al., 2018). Sugar beet or sugar cane are used for extraction of sucrose (Edwards, 2001).

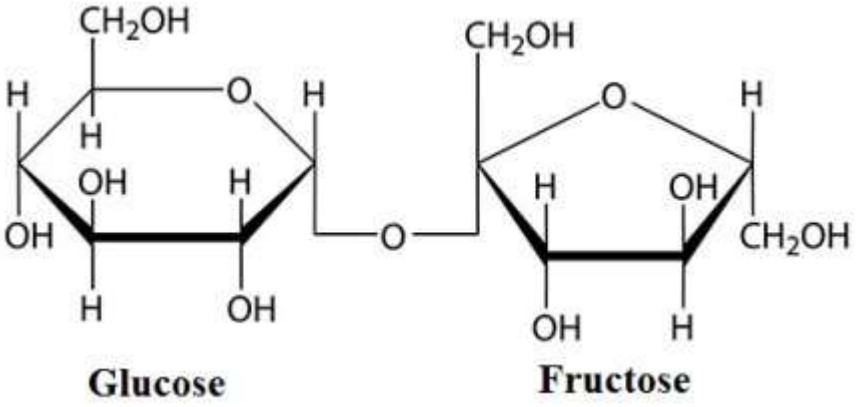

Figure 1.2. Chemical Structure of Sucrose

1.3. TD-NMR Relaxometry

Gorter, a Dutch physicist, was the first scientist who came up with the idea of Nuclear Magnetic Resonance (NMR) in 1936. However, he did not obtain any successful results by using NMR with lithium fluoride. In the following years, American researchers Felix Bloch and Edward Purcell, studied NMR to investigate the working principle of it. Later, they were awarded the Nobel Prize in Physics in 1952 (Princeton University, 2018). Thanks to developed technologies, the quality of the NMR studies has increased and many of them were awarded Nobel Prize over the 21^{th} century (Reddy, 2004).

Today, scientists are working with NMR in many aspects such as the molecular structure of the compounds and dynamics of homogeneous components. The nuclear core's inherent magnetic dipole moment is responsible for the basics of NMR. This

moment is generated by a spin due to the angular momentum of atomic nucleus in the ground states (Försterling, 2009).

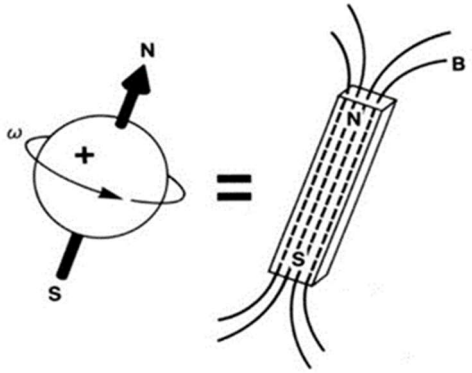

Figure 1.3. The charged nucleus rotating with angular frequency ω with the spin rotation axis (Hashemi, 2010)

When NMR is run and the sample is put in a magnetic field, spins are aligned with the direction of the magnetic field and start to precess at a frequency that is proportional to the magnetic field strength. This precession frequency is known as the *Larmour frequency*. Following the application of a radiofrequency pulse generated by the amplifier, the spins are excited which is in fact the process that triggers the NMR signal.

The precessing frequency is also affected from the type of the nucleus in addition to the magnetic field strength. Apart from that, the chemical environment of the compound has a crucial effect on the frequency. For example, when two different tryptophan protons of amide compounds in a native protein were checked, it was seen that they absorb energy at different frequencies because of their different chemical environments (Polje, 1987). Lastly, the configurational location in the magnetic field affects the NMR frequency. Indeed, if the magnetic field inside of the NMR was not uniform everywhere, the chance of having a meaningful result at the end would be less. In order to find the area where the magnetic field is highest, the

most homogenous spot should be found. This specific area is called as the 'sweet spot' of the magnet (Miyazawa, 1985).

In proton NMR, hydrogen nucleus that is a single and positively charged proton is examined. It is a known fact that the net magnetic field in the nucleus is created if there is an odd number of protons like hydrogen nucleus (^{1}H). This is also one of the reasons why ^{1}H or ^{13}C are utilized in NMR as they have unpaired protons. With the effect of external magnetic field, unpaired protons line up and RF pulse at the corresponding frequency is applied to the sample for a while and removed and a signal is generated. This obtained signal provides the data to be investigated (Hans J. Reich, 2017).

1.3.1. Spin-Lattice Relaxation (T_1) Time

In NMR studies, the relaxation times are determined while examining the behavior of a material. One of them is known as Spin-Lattice Relaxation (T_1) or also named as longitudinal relaxation time. Spin-lattice relaxation time (T_1) is the time required for the spins to realign along the longitudinal z axis. Indeed, T_1 is the time for the spins to give the energy back that is taken by RF pulse to the surrounding lattice. At the end, spins will be at the equilibrium state. This state is known as relaxation in which the spins are in their lowest energy state (Kirtil & Oztop, 2016).

As stated earlier, in an NMR experiment, an RF pulse is applied. After the application of the RF pulse, the longitudinal magnetization vector is flipped into x-y plane while the M_{xy} precesses within x-y plane as it oscillates around z-axis with all protons rotating in phase (Figure 1.4).

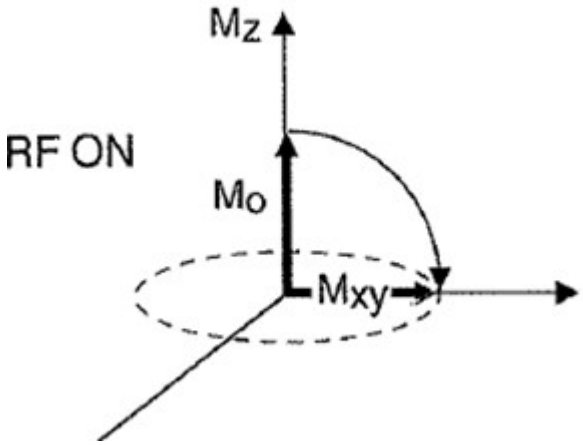

Figure 1.4. Flipping of the longitudinal magnetization vector into the x-y plane (Hashemi, 2010)

After RF pulse is turned off, the spins come back to their equilibrium state and get out of phase with each other. As a result, x-y component of the magnetization vector (M_{xy}) decreases immediately. Moreover, z component of the magnetization vector (M_z) recovers slowly along the z-axis (Figure 1.5) (Kirtil & Oztop, 2016).

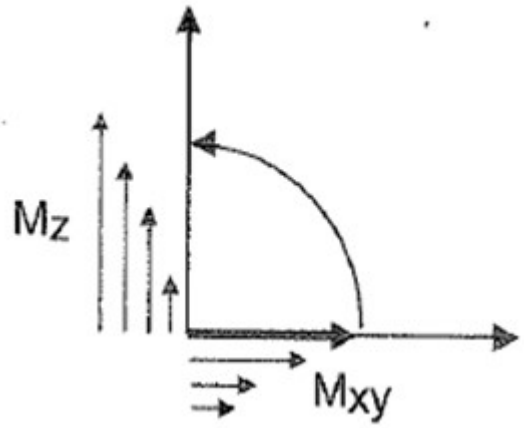

Figure 1.5. Recovery of longitudinal component while transverse magnetization vector decays (Hashemi, 2010)

In conclusion, the rate of recovering to initial M_o of M_z component is characterized by T_1 (Figure 1.6).

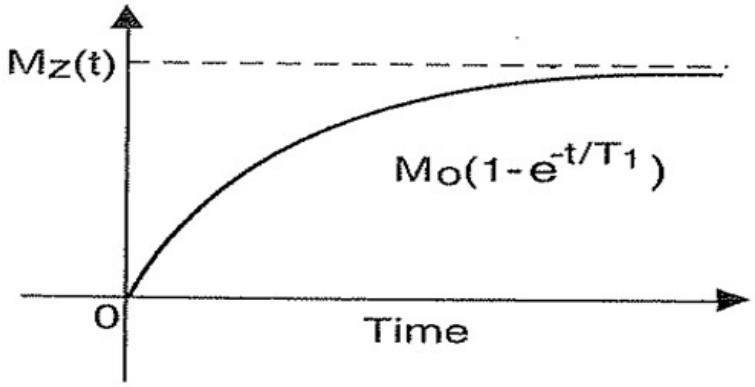

Figure 1.6. Recovery longitudinal magnetization (Hashemi, 2010).

Thus, this recovery can be formulated as:

$$M_z(t) = M_0\,(1 - e^{-\frac{t}{T_1}}).$$

T_1 time depends on the magnetic field strength so that as magnetic field strength increases, T_1 time also increases. In addition, intensity of the proton in the sample determines how high or low the value of T_1. For instance, while the pure water has T_1 value around 2.7 seconds, solids (*if they are not in the crystal form*) can have shorter T_1 values because of their low proton intensity that is coming from the less water inside the solids (Hu & Nayak, 2010). Indeed, it was shown that the range of T_1 relaxation time is changing from milliseconds to several seconds according to the proton intensity of a substance. It is also important to point out that low water content does not always indicate shorter T_1 times. Crystals, due to their perfect ordering, give off the energy back very slowly, resulting in longer T_1 times.

1.3.2. Spin-spin relaxation (T_2) time

Apart from T_1 relaxation time, the other important time is known as Spin-spin relaxation (T_2) or transverse relaxation time. T_2 relaxation time is defined as the time that determines the rate at which the x-y component of the magnetization (M_{xy}) decays (Figure 1.7) (Kirtil & Oztop, 2016).

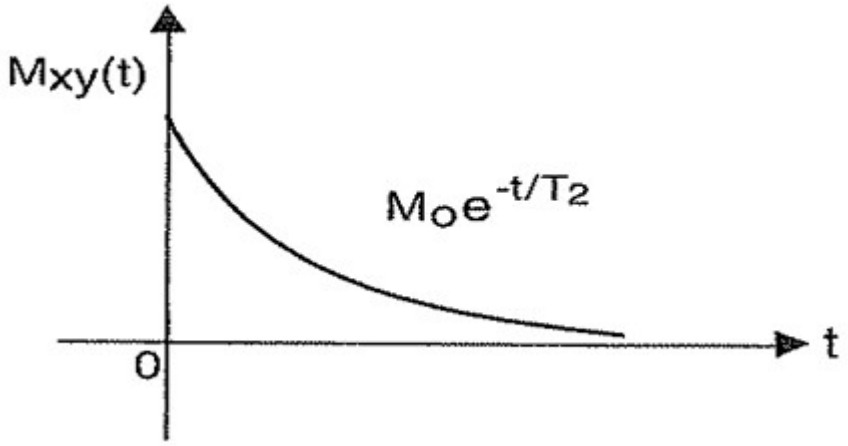

Figure 1.7. Decay of the transverse magnetization (Hashemi, 2010).

Therefore, this decaying of M_{xy} component is formulated as:

$$M_{xy}(t) = M_0\,(e^{-t/T_2}).$$

When the relaxation times T_1 and T_2 were compared it was seen that T_1 relaxation time is 5 to 10 times longer than T_2 relaxation time. This difference is explained by dephasing. Dephasing occurs due to spin-spin interactions and inhomogeneity of the external magnetic field. Hence, while T_1 can go up to several minutes, T_2 values might be hundreds of milliseconds (Hoffmann et al., 2012).

1.3.3. Repetition Time (TR) and Echo Delay Time (TE)

Before an NMR experiment was conducted, there are some parameters to be defined and controlled by the operator. They are repetition time (TR) and echo delay time or time to echo (TE). TR is the interval of time between RF pulses. Thus, to get accurate signal from the sequence, TR should be set to a sufficient value. If this is not achieved, some portion of the recovery along the x-axis would not recover fully, hence, the taken data would be biased. On the other hand, TE is the waiting time between the RF pulses. It should be chosen enough so that RF pulse could be sent without any fault (Boston University, 2012).

1.3.4. Pulse Sequences Used in NMR

A pulse sequence can be described as the set of RF pulses which are applied repeatedly in a certain NMR experiment. The amount and the frequency of these RF pulses mostly depend on the experiment set-up. Although the most basic pulse sequence consists of a single pulse (e.g. free induction decay (FID)), most of the pulse sequences contains more than one pulse such as solid echo (SE), magic sandwich echo (MSE). These pulse sequences will be explained in detail in following sections.

1.3.4.1. Free Induction Decay

Free induction decay (FID) is a type of NMR signal, produced by a rotating magnetic field, that induces an electric current in a stationary coil. FID is caused by dephasing which resulting weaker signals than expected. As a result, the signal will spiral to

the center of the x-y plane with time(Hashemi, 2010). This decaying oscillating signal can be seen in the Figure 1.8.

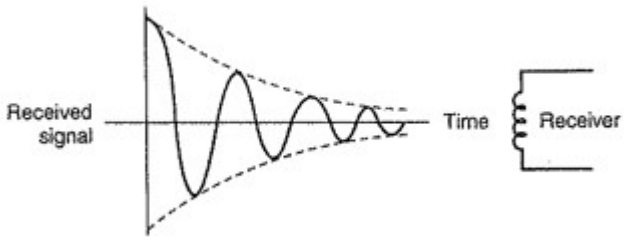

Figure 1.8. The decaying sinusodial waveform of the received signal (FID) (Hashemi, 2010)

An important application of the FID in food industry is the official analysis method of Solid Fat Content determination. The Solid Fat Content (SFC) is an important property of foods that needs to be measured since it directly affects their appearance, spreadibility and organoleptic properties (Teles dos Santos et al., 2014). To determine the solid and fat content in food samples, FID sequence has been used. There is in fact an official method of analysis that is used for that purpose (AOCS Official Method Cd 16b-93, 2009).

However, there is a 5-10 μs dead time (could extend up to 20 μs) which passes before the first recording is taken, and thus the signal coming from the solid fraction of the sample is often missed. In order to overcome this, a correction factor (F) is usually applied (Dejong & Hartel, 2016). Combining FID sequence with a Carr-Purcell-Meiboom-Gill sequence has also enabled the measurement of SFC and crystal polymorphism with one measurement to avoid the issue of dead time (van Duynhoven et al., 2002).

1.3.4.2. Solid Echo (SE) and Magic Sandwich Echo (MSE)

As dead time in pulse sequences is an issue, other sequences have been developed to determine crystalline regions in a solid sample as well as obtain knowledge about the amorphous fraction (L. Grunin et al., 2019). Solid Echo is one such pulse sequence which allows partial refocusing of the solid fraction and obtain crystallinity of the sample by back extrapolation over a series of experiments with carrying echo delays. As shown in Figure 1.9, it includes two 90° pulses with a delay. As the second pulse is applied out of phase, it allows for signal to be obtained from the initial part of the FID, thus resolving the dead time issue. (Maus et al., 2006).

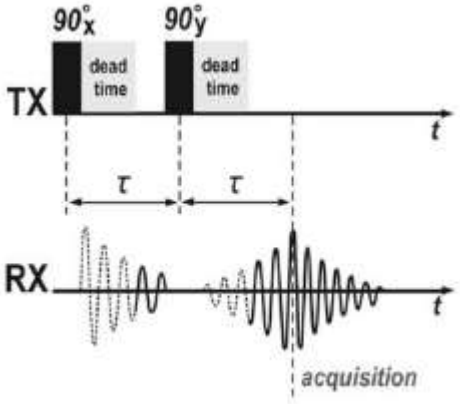

Figure 1.9. Pulse diagram of SE (L. Grunin et al., 2019) pulse sequence

While SE mainly refocuses dipole interactions within pair of spins, the initial part of FID can be refocused by using Magic Sandwich Echo (MSE) sequence with correct parameters and a phase cycling routine (L. Grunin et al., 2019). MSE was developed by Rhim et al (1970) almost 50 years ago (Rhim W-K, Pines A., 1970). It is a modified form of SE which prevents the dead time problem by refocusing the initial part of the free induction decay (FID) as well. MSE has proven to be a more robust method to investigate polymer mobility, and compared to SE, it allows to for a better refocusing of multi spin dipolar interactions. (Papon et al., 2011). However, SE sequence is a much shorter and faster sequence compared to MSE which requires at

least four phase cycling steps as shown in Figure 1.10. In this study, MSE has been used for characterizing soft candies for the 1st time.

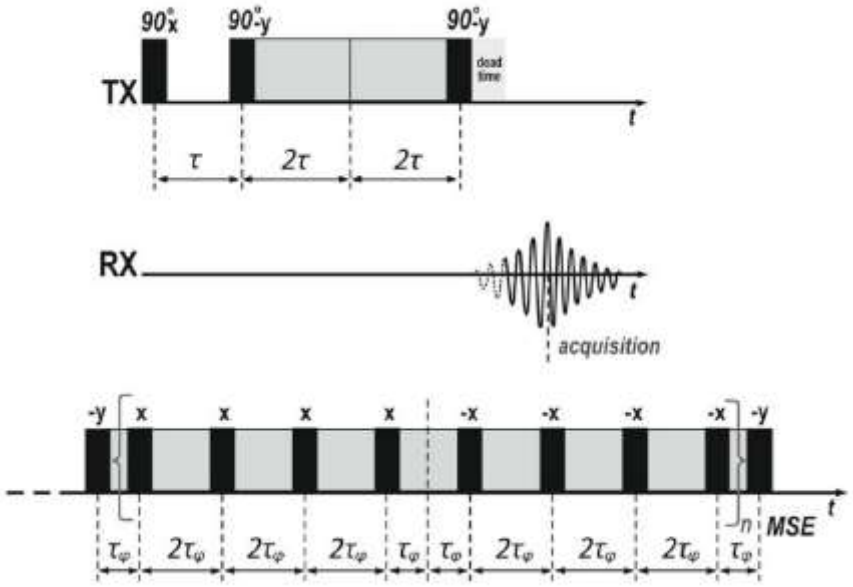

Figure 1.10. Pulse diagram of MSE (L. Grunin et al., 2019) pulse sequence

1.3.4.3. Spin Diffusion

Spin diffusion is observed when there is a gradient of magnetization between phases with different mobility. The polarization transfer is the leakage of magnetization from ^{1}H nuclei of soft protons to crystalline regions. The leakage itself is observed by the reduction of the "long" component of FID and simultaneous increase of the "short" component contribution into overall signal during the increase of the spin diffusion time (L. Y. Grunin et al., 2017).

Spin diffusion can be used for characterization of the interface between amorphous and rigid crystalline phases. The crystalline size can be investigated as a function of

crystallization time with spin diffusion in addition to the total crystalline content (Leisen et al., 2004). Spin diffusion was also conducted for evaluation of the linear sizes of the surface of cellulose crystallites (L. Y. Grunin et al., 2017). Goldman-Shen sequence was first introduced in 1966 to study the cross relaxation in LaF_3 (Goldman & Shen, 1966). The schematic of the sequence is given in Fig 1.11.

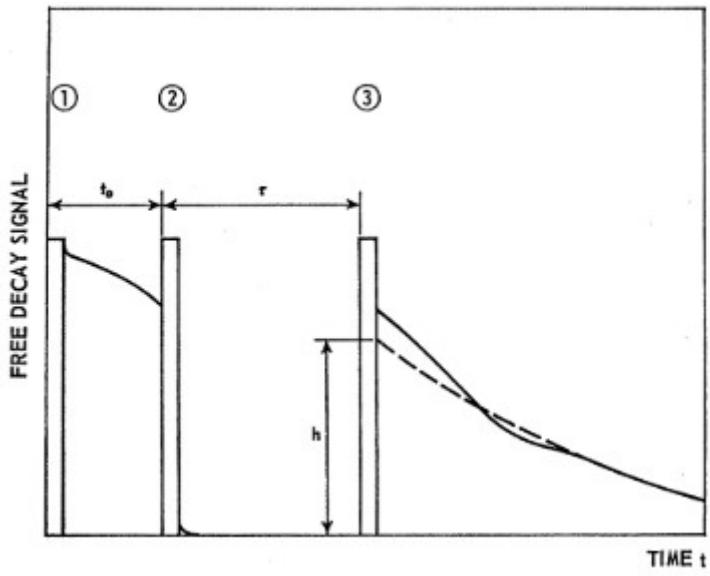

Figure 1.11. Goldman-Shen pulse sequence to study spin diffusion (Goldman & Shen, 1966)

As seen in the figure, the sequence is constituted by three π/2 pulses. The first two pulses are separated by a time τ_0, after which the magnetization from hard segments has decayed to zero due to short effective transverse relaxation time T_2*. The second pulse rotates the remaining magnetization onto the z-axis. Then, during a diffusion time τ, the magnetization diffuses from the soft to the hard component, allowing the magnetization arising from the hard fraction to increase; the third pulse tips the magnetization in the transverse plane, where it can be detected for the spin diffusion measurements (Besghini et al., 2019).

Spin diffusion can be used to explore sample homogeneity and calculate the domain sizes (Buda et al., 2003; Schäler et al., 2015). Spin diffusion is observed as a result of the magnetization transfer under dipolar interactions due to the exchange of magnetization state between neighboring spins through a flip-flop process (Adams, 2016; Schäler, 2012) . The magnetization diffuses following the Fick's law, and the equilibrium state is affected from the material, the density of the source and sink and on the domain sizes. Its detection is based upon mobility filtering. A phase with specific mobility is selected, by using a double quantum (DQ) filter and the magnetization is allowed to transfer from the selected phase to the other, letting the signal from that phase detectable (Mauri et al., 2008). The sequence is composed of periods in which the phase generating the magnetization is selected (also called as *filter period*), a *spin diffusion period* with a variable diffusion time in which the magnetization flows to the other phases present in the material, and a *phase-resolved detection*, that can be preceded by a magic sandwich echo sequence (MSE) to completely recover the rigid-phase signal that was lost during the dead time. From the fitting of all data, the domain sizes can be estimated, down to tens of nanometers (Schäler et al., 2015).

1.3.5. Use of NMR in Food Systems

In most of the industrial areas, high field NMR (HF-NMR) is used since it gives high signal to noise ratio, sensitivity and resolution. However, HF-NMR is not useful for lab scale as it requires large space and high maintenance cost. On the other hand, low field NMR (LF-NMR) has been used for many purposes that are relevant to food industry. Also, it does not require large spaces, can easily be used at bench scale and offers affordable cost (Barbosa et al., 2013).

NMR relaxometry is a crucial analytical method that can be used in materials in the state of solid or liquid. There are many food related applications in which NMR relaxometry can be used. Lipids, seeds, plant cells, meat, vegetables, beverages and sugar containing systems are just some examples (Hatzakis, 2019).

NMR relaxometry has an important place in foods in the case of quality control (Hamed et al., 2018). It has been used for understanding adulteration in honey (Ribeiro et al., 2014); olive oil (Ok, 2017), to characterize cheese systems (Scano et al., 2019); to characterize gel systems (Ozel, Uguz, et al., 2017). NMR relaxometry can be used to understand the formulations of food by considering the interaction with water of the compounds in that formulations. It is a fact that different substances in food show different behavior with water. Thus, with the help of NMR relaxometry, those differences can be detected. In that regard, NMR relaxometry can be considered as an important quality control tool for the food industry (Li et al., 2016).

1.4. Objectives

TD-NMR has proven to be a cost-effective and efficient method for quality control analysis, and thus its use can be promoted in the food industry as it provides easier solutions to experiments which otherwise require long experiment times and extensive knowledge. This study focuses on the use of non-conventional TD-NMR approaches rather than the relaxation times which are known as the conventional TD-NMR parameters.

The rather ‘non-conventional’ TD-NMR approaches of Solid echo, Magic Sandwich Echo and Spin diffusion techniques have been used for the first time for food gels.

Gelatin-based candies have been selected as a model food since they have a standard way of preparation which can enable to make generalized statements.

The study has been structured as two parts. The first part focused on the use of corn syrups having different compositions, and thus the objective of this part was to see how the syrup interaction with water and gelatin affect the properties of candies. Five different corn syrups with different glucose/fructose ratios have been used. The amounts of these syrups were varied and replaced by sucrose alternatively. T_1 and T_2 relaxation time measurements were also conducted to support the information obtained from SE/MSE and Spin Diffusion experiments. To complement the information obtained from NMR experiments, water activity, total soluble solids (Brix°) values and hardness values of the gels were also measured since these parameters of the candies were also affected by water-syrup interactions

The objective of the second part of the study was to see if different types of gelatin (bovine and porcine) could have significant effects on properties of candies and whether these differences can be analyzed using different TD-NMR approaches and also whether non-conventional NMR approaches were capable of identifying the gelatin type in a candy.

The results of this study could provide input to develop new quality methods to detect adulteration in the food industry, specifically in food products containing different types of gelatin as well as corn syrups.

CHAPTER 2

MATERIAL AND METHODS

2.1. Materials

Commercial sucrose (Bal Kupu, Turkey) was purchased from a local market. Bovine (250 bloom value) and porcine (300 bloom value) gelatins were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Corn syrups were purchased from Sunar Misir Entegre Tesisleri Sanayi ve Ticaret A.S. (Adana, Turkey). The specific details of the syrups are provided in Table 2.1.

Table 2.1. Specifications of Corn Syrups

Product Name	Brix (20°C)	Glucose (%)	Fructose (%)
SBF 10 Syrup	79 ± 1.00	36 ± 2.00	10.5 ± 2.50
SHFSLF20 Syrup	79 ± 1.00	27.5 ± 2.50	20 ± 3.00
SMF 42 Syrup	70 ± 1.00	51 ± 3.00	42.5 ± 2.50
SCG 40 Syrup	83 ± 1.00	40.5 ± 3.50	-
SCG 60 Syrup	81.75 ± 1.25	60.5 ± 3.50	-

Sodium azide (≥99.99% trace metals basis) (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) was used at a final concentration of 0.01% (w/w) in all formulations to prevent microbial growth.

2.2. Methods

2.2.1. Preparation of Soft Candies

Experiments were divided according to sample types. One part included soft candy preparation using bovine gelatin along with five different syrup types and sucrose. The second part included comparison of soft candy prepared with bovine and porcine gelatin while using only one type of corn syrup.

The sample preparation was similar for both parts. It is also given in Figure 2.1. First, 8 g of gelatin was mixed with 15 ml distilled water while the magnetic stirrer was set at 100°C. At the same time, corn syrup, sucrose (exact amounts are given in Table 2.2. and 2.3.) and 17 ml distilled water were mixed in a separate container. The total amount of syrup and sucrose equaled 60 g. Mixing was stopped when the mixture temperature reached 100°C. After this, these two mixtures were added to another beaker and were stirred at 85°C and 350 rpm. Mixing was stopped when brix value of mixture reached 70°. Then, the mixture was poured into molds and allowed to cool. Analysis were carried out on the samples after being stored overnight in the mold.

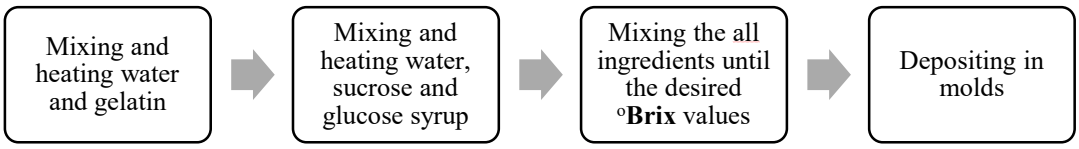

Figure 2.1. Gelatin-based Candy Production Process

Table 2.2. Candy formulations prepared for the 1st part of the study

Sample	Sucrose (g/100 g mix)	Glucose (g/100 g mix)	Fructose (g/100 g mix)
SCG40,30%	30	11	-
SCG60,30%	30	17.1	-
SMF42,30%	30	14.4	12
SBF10,30%	30	10.2	2.4
SHFSLF20,30%	30	6.9	5.1
SCG40,60%	-	22	-
SCG60,60%	-	34.2	-
SMF42,60%	-	28.8	24
SBF10,60%	-	20.4	48
SHFSLF20,60%	-	13.8	10.2
Sucrose	60	-	-

Table 2.3. Candy formulations prepared for the 2nd part of the study

Sample	Sucrose (g/100 g mix)	Glucose (g/100 g mix)	Fructose (g/100 g mix)
Bovine	30	12.6	-
Porcine	30	12.6	-

2.2.2. Water Activity Measurement

Water activity was measured using Aqualab 4TE (Meter Group, Pullman, USA). The samples were cut into thin layers and the water activity was directly measured at 25°C.

2.2.3. Texture Profile Analysis

Hardness of the soft candies was measured using Texture Analyzer (Brookfield Ametek CT3, TA18 probe, Middleboro, MA, USA). Samples were cut in dimensions of 3*3*3. 0.05 N load was used, and test speed was set to 1 mm/s. Moreover, chewiness data were also measured but since data were not useful, only hardness results were recorded in this study.

2.2.4. ^{o}Brix Measurement

^{o}Brix values of the soft candies were measured by using HANNA HI 96801 Refractometer (HANNA Instruments, USA). Samples were melted before measurement.

2.2.5. Time Domain Nuclear Magnetic Resonance (TD-NMR) Relaxometry Experiments

Time domain NMR Relaxometry measurements were conducted by using 0.5 T NMR instrument operating at a ^{1}H Larmor frequency of 20.34 MHz (Spin Track, Resonance Systems GmbH, Kirchheim/Teck, Germany).

2.2.5.1. Measurement of Spin-lattice (T_1) Relaxation Times

A saturation recovery pulse sequence was used to obtain T_1 relaxation times. Parameters were set as given in table 2.4. Sample type indicates the samples from the first and the second part of the experiment.

Table 2.4. Parameters for Saturation Recovery Pulse Sequence

Sample Type	Relaxation Period (μs)	Time of Observation (μs)	Number of Scans
1	300	300000	8
2	300	400000	8

2.2.5.2. Measurement of Spin-spin (T_2) Relaxation Time

A CPMG (Carr-Purrcell-Meiboom-Gill) pulse sequence was used to measure T_2 relaxation times. Parameters were set as given in table 2.5. Sample type indicates the samples from the first and the second part of the experiment.

Table 2.5. Parameters for CPMG Pulse Sequence

Sample Type	Relaxation Period (μs)	Echo Time (μs)	Number of Echoes	Number of Scans
1	300	500-700	200	8
2	300	40	2500	8

2.2.5.3. Solid Echo and Magic Sandwich Echo sequence experiments

For both SE and MSE sequences, relaxation period was set as 10000 ms and number of scans were set as 16. Probe ringing time was 9 μs.

2.2.5.4. Spin Diffusion Experiments

Goldman-Shen sequence was used for the Spin Diffusion experiment. Time of observation was set as 1 s and relaxation period was set as 1000 ms. The number of scans was kept at 64.

2.2.5.5. Data Analysis

T_1 and T_2 relaxation data were calculated using MATLAB (version R2019b). SE/MSE data were analyzed using the special module in RELAX 8 (Resonance Systems, Kirchheim/Teck Germany). Spin diffusion data was analyzed by using Origin 7. The detailed approached followed will be discussed in the next section

2.2.6. Statistical Analysis

Experiments were carried out in replicates of three and data was represented as mean ± standard deviation. Statistical analysis was carried out on all experimental data using Minitab (Minitab Inc., Coventry, UK). In order to understand the effect of different variables, analysis of variance (ANOVA) was done. The assumptions of ANOVA (Normality and Test of Equal Variances) were confirmed and outlier data was excluded before further analysis of the data. Results were compared using Tukey's comparison test at a 95% confidence interval.

2.2.7. Experimental Design

Table 2.6. Experimental Design for Part I

Factors	Levels	Responses
Syrup Type	SCG40, SCG60, SMF42, SBF10, SHFSLF20	1. Water Activity 2. Texture
Syrup Amount (g/100 g mix)	0, 30, 60,	3. Brix 4. Spin-lattice (T_1) Relaxation Time 5. Spin-spin (T_2) Relaxation Time 6. Solid Echo and Magic Sandwich Echo 7. Spin Diffusion

Table 2.7. Experimental Design for Part II

Factors	Levels	Responses
Gelatin Type	Bovine, Porcine	1. Water Activity 2. Texture 3. Brix 4. Spin-lattice (T_1) Relaxation Time 5. Spin-spin (T_2) Relaxation Time 6. Solid Echo and Magic Sandwich Echo 7. Spin Diffusion

CHAPTER 3

RESULTS AND DISCUSSION

As explained in the last part of Chapter 2, this study is structured in 2 sections. In the 1st part, effect of different corn syrups on gelatin soft candies will be evaluated using non-conventional TD-NMR approaches such as SE/MSE and Spin Diffusion. In addition, conventional TD-NMR experiment results such as T_1 and T_2 relaxation times will also be discussed. Physical measurements such as water activity; hardness; ^{o}Brix measurements were also performed to complement the results obtained from NMR.

In the 2nd part of the study, use of non-conventional TD-NMR approaches will be explored to see if these methods have any potential on identifying the type of the gelatin used in a candy formulation. For that purpose, 2 different candies were prepared, one with bovine and the other with porcine gelatin. In addition to TD-NMR, physical measurements as described in the 1st part were also conducted.

3.1 Effect of various corns syrups on the characteristics of gelatin candies

In this section, firstly physical characterization experiments will be discussed and then the results of the TD-NMR will be explained.

3.1.1 °Brix

°Brix values of the corn syrups used in the study changed in a range of 79-83. SCG 60 and SCG 40 syrups had the highest °Brix values whereas SBF10 and SHFSLF20 had the lowest. °Brix values of the candies are given in Fig 3.1.

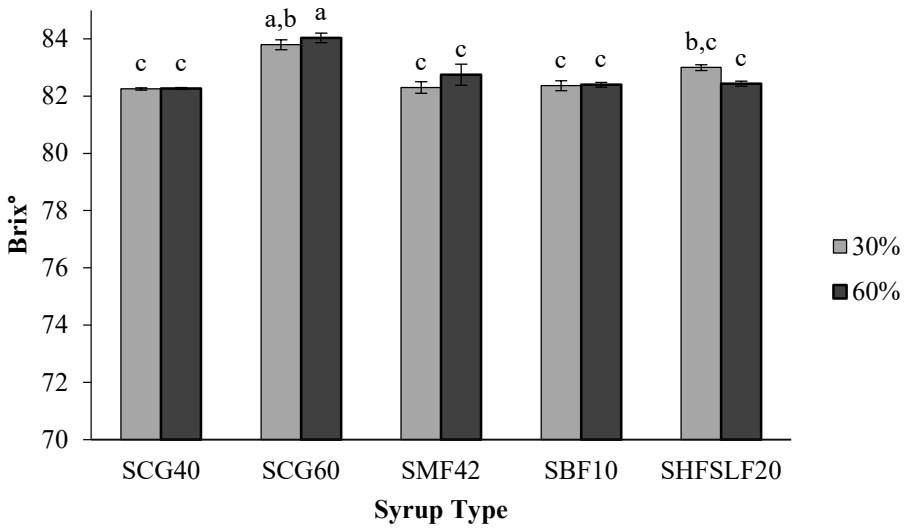

Figure 3.1. Effect of corn syrup type on the °Brix values of the formulated candies

When a refractometer reading is used for measuring the concentration of a confectionery, the scale reading is more correctly termed as °Brix to represent the equivalent sucrose concentration of a syrup with that refractive index (Ergun et al., 2010). °Brix values are not exactly the total solids (or water) content, with the errors increasing when less sucrose is present in the mixture. However, for many confectionery products, a correction factor is not used and ◦Brix is assumed to be sufficiently close to the true *total solids concentration* (and water content by difference). Kaya (2019) also showed that °Brix values had significant and high correlation with the moisture content values for gelatin based soft candies and

suggested the use of °Brix values as a direct parameter for moisture content comparison (*Determination of the Best Drying Conditions for Gelatin*, 2019).

For our samples, effect of corn syrup type was found significant on the SS ($p<0.05$) whereas the % corn syrup used did not have a significant effect ($p>0.05$). Samples prepared with SCG60 had the highest SS values which was parallel to the high °Brix values of the syrup itself. So, in overall, results showed that sugar composition of the syrup used in the formulations did not have a significant effect on the moisture content, and thus is not a good indicator to identify the type of the corn syrup used in candy formulation as long as the °Brix values of the syrups are not too high or low.

3.1.2 Water activity (a_w)

Water activity is an important thermodynamic property that affects the shelf life of foods. It depends on the food composition. Particularly sugars have a significant effect on lowering the water activity since their water binding ability is quite high. Mostly, jelly candies' water activity changes between 0.5 and 0.7 and the final product should achieve at least 75% total soluble solids to prevent mold growth (*Determination of the Best Drying Conditions for Gelatin*, 2019; Ergun et al., 2010). For the prepared candies, water activity experiments were also performed and results are given in Fig 3.2. Except the SCG40 samples prepared with 60% syrup, a_w of all samples were in the expected range (≤ 0.70).

It is important to mention that the 60% syrup containing formulations prepared in this study are not preferred in the industry. As stated in Chapter 1, confectionery gels

usually contain sucrose to balance the sweetness. 60% formulations in this study were just prepared to see the effect of the syrups clearly.

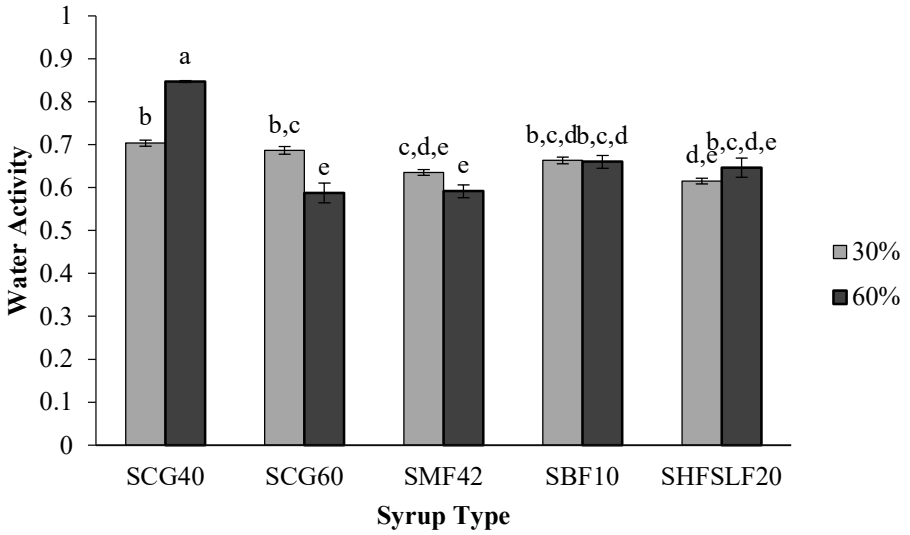

Figure 3.2. Effect of corn syrup type on the aw of the formulated candies

According to ANOVA results, syrup type was found significant ($p<0.05$) whereas syrup content did not have an effect. On the other hand, different than °Brix values, interaction between syrup type and syrup concentration were found significant as well. SCG40 samples had the highest a_w values. This was direct result of the higher °Brix values of the syrup used. This finding was also important since it showed that the lowest water content syrup resulted in higher a_w values. SCG40 had the highest °Brix values of 83 and resulted in highest a_w samples (SCG40, 60%). SCG40 syrup includes 40% glucose and the rest is maltose. Although SCG60 syrups had a similar °Brix value with SCG40, in a_w results the SCG40 samples were followed by SBF10 and SCG60 with lower a_w values. SCG60 syrups had higher glucose content than SCG40. Since concentration of glucose is higher in SCG60, it is reasonable to have lower water activities compared to SCG40. More glucose molecules result in more H-bonding, and thus decrease water activity. The rest of the syrup in SCG40 and SCG 60 contain maltose and glucose is known to decrease water activity more

compared to maltose as being a higher molecular weight compound. SBF10 samples had 36% glucose and 10% fructose whereas SCG40 samples had 40% glucose. The slightly lower activity of SBF10 samples compared to SCG40 could be explained by the presence of fructose in the syrup. Effect of fructose is more obvious in SMF42 samples. Despite the fact that SMF42 had the highest water content, samples prepared with SMF42 had the lowest a_w values. SMF42 contains 51% glucose and 42% fructose. Fructose is always known to be a better humectant than glucose and decreases water activity more wrt to glucose (Bussiere & Serpelloni, 1985). Water activity values of the ~0.61 was a direct reflection of this fact. These results also showed how the composition of a syrup had a significant impact on the a_w values of different formulations.

3.1.3 Hardness

Texture is a very important attribute of a confectionary product. Firmness and springiness are the significant parameters for consumers' perception since these affects the quality while eating (McKenna, 2003). Chewiness is also important for a candy product. Since the goal in this study was to see the effect of corn syrup type on candies rather than having a candy with a good sensory profile, only hardness values were measured. Hardness results of the candies are given in Fig. 3.3.

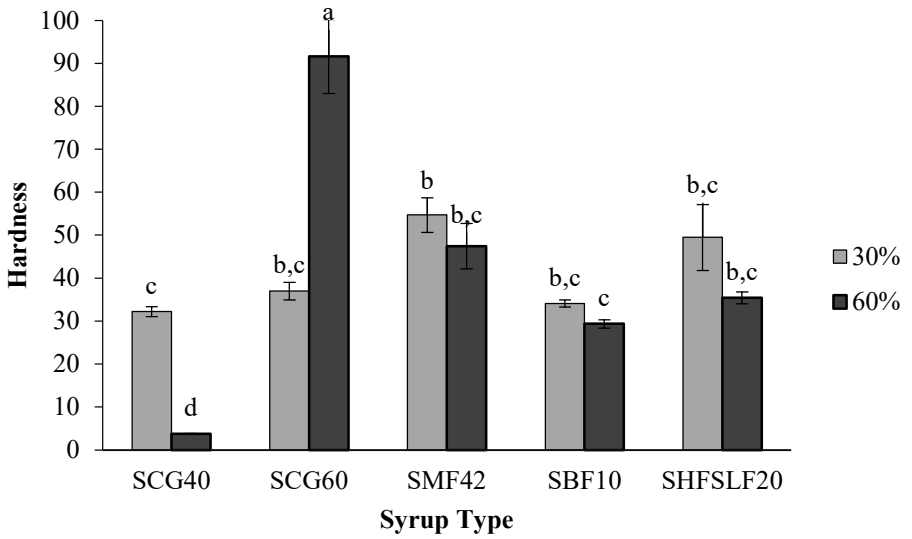

Figure 3.3. Effect of corn syrup type on the hardness of the formulated candies

Syrup type was again significant on the hardness of the candies ($p<0.05$) whereas syrup concentration was insignificant ($p>0.05$). Interaction between syrup type and concentration was also significant ($p<0.05$). SCG60 samples had the highest hardness values whereas SCG40 samples were the softest samples. Higher a_w values for SCG40 samples was in parallel with the soft gels obtained with this syrup. SCG60 (60%) samples were the hardest. Higher ^{o}Brix values of those samples could be a possible explanation for this behavior. Other samples had almost similar hardness values. Pearson correlations results also showed that there is a significant positive correlation between hardness and ^{o}Brix values.

3.1.4 Conventional TD-NMR Approaches: NMR Relaxation Times

TD NMR Relaxometry enables to obtain microstructural information based on relaxation times (Nilgün Efe, 2018). Water distribution in hydrogels and in confectionery gels has been studied extensively by NMR relaxation times (Cikrikci

et al., 2018; Efe et al., 2019; Ilhan et al., 2020; Ozel et al., 2018, 2020; Ozel, Cikrikci, et al., 2017; Ozel, Uguz, et al., 2017; Oztop et al., 2010; Pocan et al., 2019).

In this study rather than relaxation times some other approaches have also been utilized but still the changes on the relaxation times with different syrup types and syrup concentrations were also investigated.

3.1.4.1 Spin-lattice relaxation times (T_1)

T_1, known also as spin-lattice relaxation time, gives information about the energy transfer between protons and the lattice of the sample (Kirtil & Oztop, 2016). T_1 of the pure water is around ~2.5 s and solids (except crystalline ones) have mostly shorter T_1 relaxation times. In this study T_1 times were also measured through a saturation recovery sequence and results are given in Fig. 3.4.

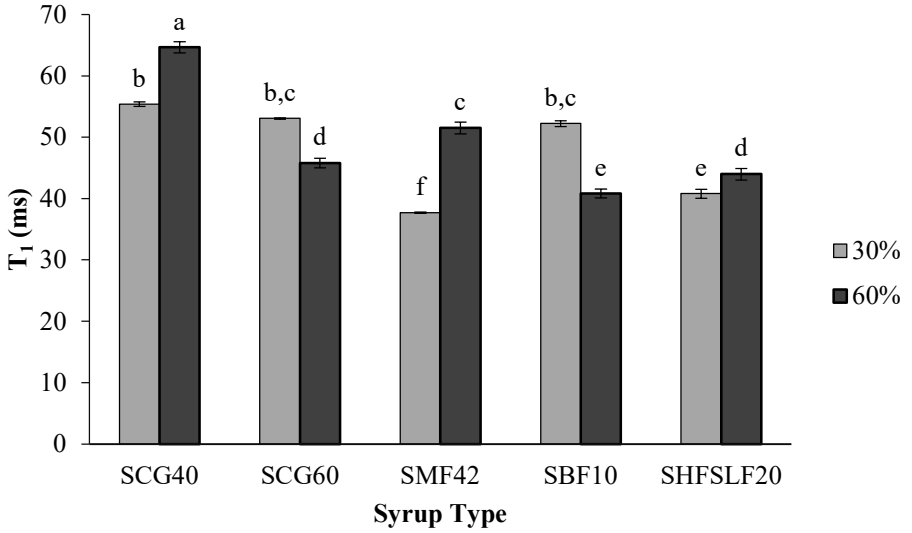

Figure 3.4. Effect of corn syrup type on the T1 times of the formulated candies

T_1 data were fitted to a monoexponential model. As gelatin is capable of forming a nice rigid gel network, observing a monoexponential behavior was not unreasonable at that field strength. Both syrup type and syrup concentration and also their interaction were found to be significant on the T_1 relaxation times ($p<0.05$). T_1 values changed in the range of 35-65 ms and the longest T_1 values were observed for the SCG40 (60%) samples whereas the shortest T_1 were observed on SMF42 (30%) samples. Effect of syrup concentration on T_1 was not monotonic but rather depended on the type of the syrup used. When the syrup type was considered as the main factor; it was observed that SMF42 and SHFSLF20 samples had the shortest mean relaxation times and they were not significantly different from each other ($p>0.05$). It is interesting to note that when the composition of these syrups is considered; they had higher fructose levels. Thus, it is possible to conclude that fructose resulted in shorter T_1 relaxation times.

T_1 is the shortest when the molecular tumbling rate (also known as the correlation time τ_c), is approximately equal to the Larmor frequency and it depends significantly on the viscosity of the sample. ºBrix values of the samples were between 82-84 and this small change probably did not reflect itself on the relaxation times since the higher water content syrup containing samples (SMF42) had the shortest relaxation times. This was an obvious result that shows how the composition could have dramatic effects on relaxation times regardless of the water content. Molecules tumbling faster or slower are less efficient at spin-lattice relaxation and have longer T_1s. Free water or crystals (Le Botlan et al., 1998) have longer relaxation times due to the wide range of tumbling rates and so most molecules in this state are inefficient at T_1 relaxation. The change in T_1 of the gels could also be attributed to the change in the correlation times. With increasing fructose amounts, correlation times could have decreased and decreased the T_1 relaxation. Fructose syrup samples also had lower water activity and lower T_1 times. Pearson correlation between a_w and T_1 were found to have a significant and positive correlation ($R=0.67$, $p<0.05$).

3.1.4.2 Spin-spin relaxation times (T_2)

T_1 experiments usually take longer time due to the nature of the process (*recovery of the longitudinal magnetization*). Especially for high water content; dynamic system's T_1 is usually not preferred as a monitoring parameter. T_2, which is the spin-spin relaxation times and that is more related with the microstructure of the samples, is usually conducted by a Hahn Echo or CPMG pulse sequence and takes shorter time for measurement. T_2 relaxation occurs due to the spin-spin interactions which causes dephasing of the magnetization and also is affected from the inhomogeneity of the magnetic field. However, CPMG pulse sequence considers the problem of magnetic field inhomogeneity and thus relaxation is observed just due to the dephasing of spins. T_2 results of the samples used in this study are given in Fig. 3.5.

Relaxations times changed in the range of 3-47 ms. Syrup concentration had a significant effect on the relaxation times ($p<0.05$). Syrup containing samples also included sucrose as the other sugar. It was obvious from the results that presence of sucrose decreased the T_2 times except SMF42 and SHFSLF20 samples. T_2 times are affected from the mobility of the protons in the system. Presence of sucrose hindered the mobility, and thus decreased the relaxation times. Effect of water content was very prominent on the T_2 results. As stated in Chapter 2, SMF42 samples had the lowest ^{o}Brix values (~71). The longest T_2 times of **47 ms** observed on SMF42 (60%) samples was a direct reflection of this fact. No correlation was detected between the physical parameters and T_2 relaxation times ($R<0.2$). It was also not possible to explain the dependence of T_2 relaxation times wrt glucose or fructose concentration. Interactions between sugars and water created a complicated picture for the T_2 relaxation thus the hypothesis that T_2 relaxations time could be an indicator for differentiating different corn syrup containing samples could not be made.

T_1 and T_2 relaxations times have been used in many gel systems to understand polymer water interactions; water mobility or to understand the effect of a certain ingredient on the characteristics of the gels. Pocan et al (2019) used TD-NMR to explore the behavior of D-Allulose substitution on gelatin gels and results were quite interesting. These gels were characterized with 3 different proton populations each having a different T_2 time. Efe et al (2019) examined the effect of sugar alcohols on gelatin candies and gels were shown to have a multicompartmental nature and 2 proton pools were used to explain the changes on the formulations.

In this study, gels were also shown to fit a 2 compartment multiexponential model. However, results were not sufficient enough to explain the changes on the system with the changes in syrup concentration or type. Thus, a monoexponential model was preferred to see the changes.

In overall, it was observed that the conventional TD-NMR approaches; relaxation times would not be the best method to differentiate gels formulated with different composition corn syrups. Therefore, new TD-NMR approaches have been tested in this study. In the next section these 2 new techniques will be discussed.

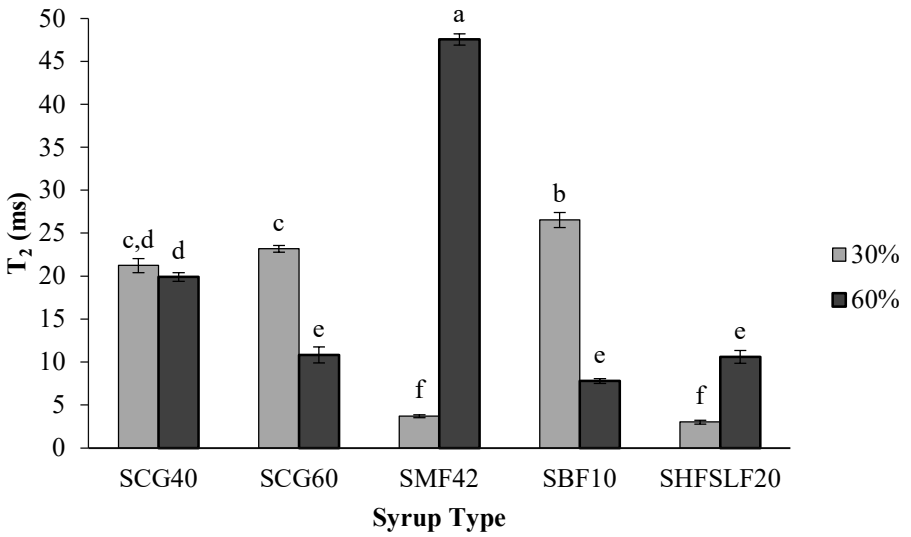

Figure 3.5. Effect of corn syrup type on the T2 times of the formulated candies

3.1.5 Non-Conventional TD-NMR Approaches

3.1.5.1 Solid Echo / Magic Sandwich Echo

Free **I**nduction **D**ecay also known as FID in NMR literature is the simplest signal obtained by a 90º rf pulse. FID has been used in food industry to develop the official method for measuring solid fat content (SFC) in fats and confectionery products (AOCS Official Method Cd 16b-93, 2009; Cobo et al., 2017). It based on the signal intensity difference between the initial and final parts of the signal. Since solid part (solid fat) usually has a short T_2 relaxation time, it decays quickly thus the information about solid part is usually hidden in the first points of the signal. On the other hand, liquid part (oils) decays slowly. A representative FID and how SFC is calculated is given in Fig. 3.6. Point **S** is also known as the 'Short Component' and point **L** is known as the long component. FID sequence has also been used quantify sucrose crystal content in fondants (Lenz & Hartel, 2005; Porter & Hartel, 2013) and monitor crystallization of sorbitol (Dejong & Hartel, 2016).

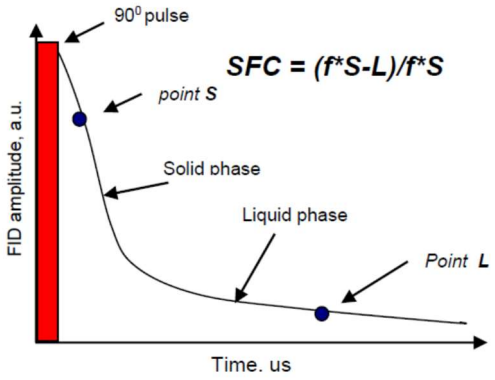

Figure 3.6. A representative FID signal and SFC

An important point about the FID sequence is the need for the correction factor '**f**' which is required due to the dead time. Dead time is known as the time that is lost in the rf probe until the first signal point is acquired. The larger the coil the longer the dead time. And as long as a special application is not included in the pulse sequence, dead time always exist. And the presence of deadtime cause a loss in the 'solid signal', thus the solid content could have some bias.

To overcome the problem of dead time, several alternatives exist. In order to determine the crystalline regions in the solid polymers and to monitor kinetics of the crystallization process as well as to obtain quantitative measurement on more mobile amorphous fractions, different approaches have been followed (L. Y. Grunin et al., 2017; Maus et al., 2006).

In 1971 , Rhim and his co-workers designed Magic Sandwich Echo (MSE) sequence which did not require any special instrumentation and works on the principle of refocusing the signal in the initial part of the FID and did not require any deadtime, and thus enabled the acquisition of the signal of the solid part (Matsui, 1991; Maus et al., 2006; Rhim et al., 1971). Maus et al (2006) showed that this approach would

let to quantify the crystalline and amorph regions in polymer systems (Maus et al., 2006).

Later this technique was utilized for different polymer studies (Papon et al., 2011; Pieruccini et al., 2015; S Sturniolo et al., 2014; Simone Sturniolo & Saalwächter, 2011; Vaca Chávez & Saalwächter, 2011). MSE Magic Sandwich Echo is in fact a solid echo (SE) sequence. By using a SE sequence it is also possible to partially refocus the solid fraction (Maus et al., 2006). However, SE sequence cannot fully refocus multiple dipolar interactions. SE mainly refocuses dipole interactions within pair of spins but for the MSE with correct parameters and the phase cycling refocusing nearly the entire Hamiltonian becomes possible. Pulse sequences and representative signals from FID, SE and MSE are shown in Fig. 3.7 and 3.8. As can be seen, the signal coming from MSE is higher compared to a regular FID and Solid Echo sequence.

In this study, SE and MSE sequences were used to calculate the 2^{nd} moment which has been shown to be an important parameter for estimating the crystallinity of different samples. The approach described by Grunin et al (2019) was used by using the module in Relax 8 software (Resonance Systems GmBH, Kirchheim, Germany) and 2^{nd} moment (M_2) values were calculated. M_2 is a well-known NMR parameter characterizing the structure and dynamics of the studied material. In fact, it is the second moment of the NMR absorption line which in scientific literature is often called the NMR second moment (Goc, 1998). The followed approach is a much simpler approach than the conventional way of fitting the Abrahamian based Time-Domain free induction signal and is rather based on the direct integration of the frequency-domain NMR spectrum. M_2 results of the samples for SE and MSE are given in are given in Table 3.1. The information obtained from SE are actually same but MSE provides data with a better Signal to Noise Ratio (SNR). A comparison plot for the MSE and SE for SCG40 (30%) samples is given in Figure. 3.9.

As expected, the 2nd moment values calculated from MSE and SE were highly correlated ($R=0.932$, $p<0.05$). This also confirmed the accuracy of the analysis. Higher M_2 values indicated higher crystallinity. It should be noted that the values obtained from M_2 are just relative values. For exact quantification of the crystallinity, a calibration curve should have been prepared as in the study of Grunin et. al (2019). Crystallinity values should also be calculated from another method like X-Ray diffraction (XRD) and later quantification should be performed. However, practically it is not very feasible to conduct this for different samples. Thus, in this study rather than exact crystallinity values, a comparison was made between the samples to show whether samples prepared from different corn syrup samples had different crystallinities.

ANOVA results showed that syrup type and its interaction with syrup concentration was significant on the crystallinity of the samples ($p<0.05$) and SCG40 samples had the highest crystallinity whereas SMF42 samples had the lowest. Glucose is known to have lower solubility than fructose and its crystallization tendency is quite high. For instance, in honey, which is a syrup composed of fructose and glucose; the crystals are formed due to the glucose (Berk et al., 2021). In that regard, the samples prepared with the syrups having high glucose content are expected to have higher amount of crystals and this was reflected in both SE and MSE results as seen in Table 3.1. It is also interesting to note the power of MSE based on the obtained results. As seen in the Table, for SE, SMF42 samples had also similarity with SBF10 which was also similar to rest of the samples. However, in MSE results, SMF42 isolated itself and crystallinity values were significantly different from the others ($p<0.05$). Another point that needs attention is that the candies visually did not suffer from sugar crystals, but it is the power of TD-NMR that has the ability to detect even low amount of crystals.

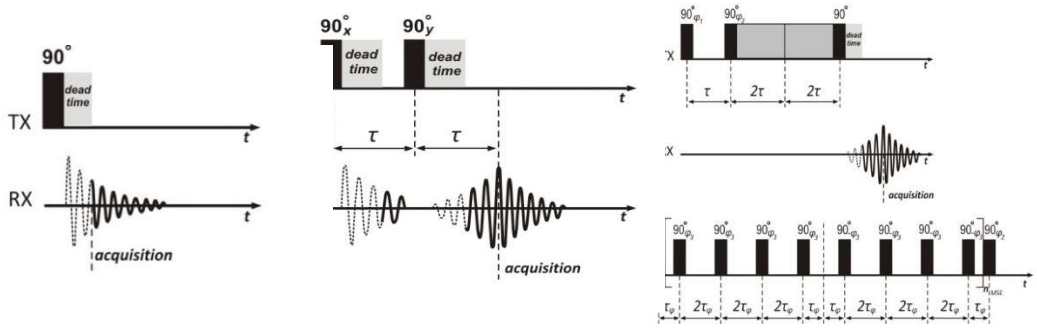

Figure 3.7. Pulse diagrams for FID, SE and MSE Sequences (Grunin, Oztop, Guner, & Baltaci, 2019)

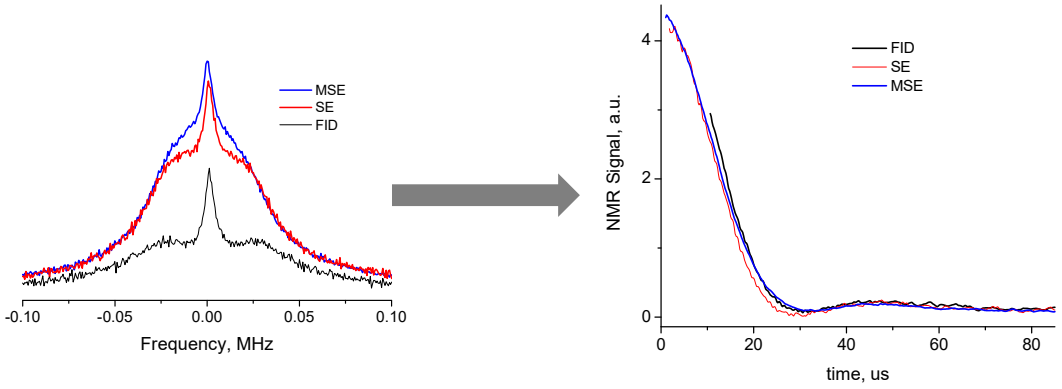

Figure 3.8. Representative signals of FID, SE and MSE (Grunin, Oztop, Guner, & Baltaci, 2019)

Table 3.1. 2nd moment (M_2) of the candy samples

	M_2 (SE)		M_2 (MSE)	
Syrup Amount (%) / *Syrup Type*	30	60	30	60
SCG40	7.00±0.10^{a,b}	7.46±0.07^{a}	11.76±0.08^{a,b}	11.99±0.07^{a}
SCG60	6.86±0.06^{a,b}	7.25±0.05^{a,b}	11.21±0.14^{a,b}	12.02±0.15^{a}
SMF42	6.14±0.27^{c}	5.31±0.08^{d}	9.21±0.23^{c}	8.55±0.35^{c}
SBF10	6.77±0.15^{b,c}	6.75±0.13^{b,c}	10.92±0.24^{b}	11.00±0.12^{b}
SHFSLF20	7.19±0.10^{a,b}	6.69±0.09^{b,c}	11.60±0.08^{a,b}	11.14±0.09^{a,b}

As described before, the crystallinity calculations were based on the direct integration of the frequency-domain NMR spectrum. This calculation helps to differentiate the crystalline and amorphous regions in the systems. On the other hand, in SFC experiments, a simple ratio is calculated and % crystal content is reported. When FID is used a correction factor '*f*' is needed. But for SE and MSE such a correction is not needed due to the refocusing of the solid part in the pulse sequence. It should also be pointed out that, if the researcher is not interested in amorphous vs crystalline fractions and there is a liquid system in which crystals are forming, that approach could also be helpful. Dejong et al (2016) and Berk et al (2021) used this approach to monitor the crystallization kinetics of sorbitol and honey respectively (Berk et al., 2021; Dejong & Hartel, 2016). In this study, we also used to follow this approach to calculate the 'possible' crystal content despite the fact that samples are in solid state. The values are not exactly the crystal contents but rather denote the solid portion in the samples that contributed to the 'solid signal'.

The following ratio was calculated from the SE/MSE data (Berk et al., 2021):

$$NMR\ value = \frac{Magn_{Short} - Magn_{Long}}{Magn_{Short}} \times 100 \quad \textbf{(1)}$$

$Magn_{short}$ (*point 's' in Fig. 3.5)* denoted the average signal coming from the solid and liquid portion, whereas $Magn_{long}$ denoted the liquid signal only (*point 'L' in Fig. 3.5*). $Magn_{short}$ covered the average of the data between **2.5-11** μs, whereas for $Magn_{long}$ data, between **100-150** μs was used. Results are given in Table 3.1.

Table 3.2. MSE/SE signal outputs as calculated in SFC experiments

	SE (% crystallinity)		*MSE (% crystallinity)*	
Syrup Amount (%) / *Syrup Type*	30	60	30	60
SCG40	88.25±0.12^{c}	87.61±0.08^{c}	84.21±0.18^{a,b}	84.51±0.15^{a,b}
SCG60	88.34±0.08^{c}	90±0.21^{a,b}	83.34±0.36^{a,b,c}	86.05±0.23^{a,b}
SMF42	87.93±0.43^{c}	88.39±0.51^{c}	80.91±1.39^{c,d}	79.29±0.48^{d}
SBF10	88.97±0.47^{b,c}	90.52±0.34^{a}	83.17±0.92^{b,c}	85.12±0.63^{a,b}
SHFSLF20	90.95±0.27^{a}	90.41±0.19^{a,b}	86.4±0.46^{a}	84.95±0.44^{a,b}

Results were quite interesting. Since the ratio is related with the % solid content it was expected that we would see a correlation between the ^{o}Brix values and the NMR outputs. However, no significant correlation was detected. ANOVA results showed that, both syrup type and syrup concentration were found to be significant ($p<0.05$) on the SE results whereas syrup % was insignificant ($p>0.05$) on MSE. Pearson correlation analysis between MSE and SE showed a smaller positive correlation ($R=0.67$, $p<0.05$) compared to the previous analysis. ANOVA results were also different for SE and MSE. According to SE experiments the highest values were obtained for SHFSLF20 samples and the lowest values were observed for SMF42

and SCG60 samples. On the other hand, MSE results showed the smallest values for SMF42 samples and the rest of the samples were same. MSE results were slightly parallel with the previous approach but still the other syrup types were not differentiated. The higher SNR for the samples was also confirmed for this approach. A signal plot for one of the candy formulations is given in Fig 3.8. As seen in the figure MSE had higher signal than SE for the same sample.

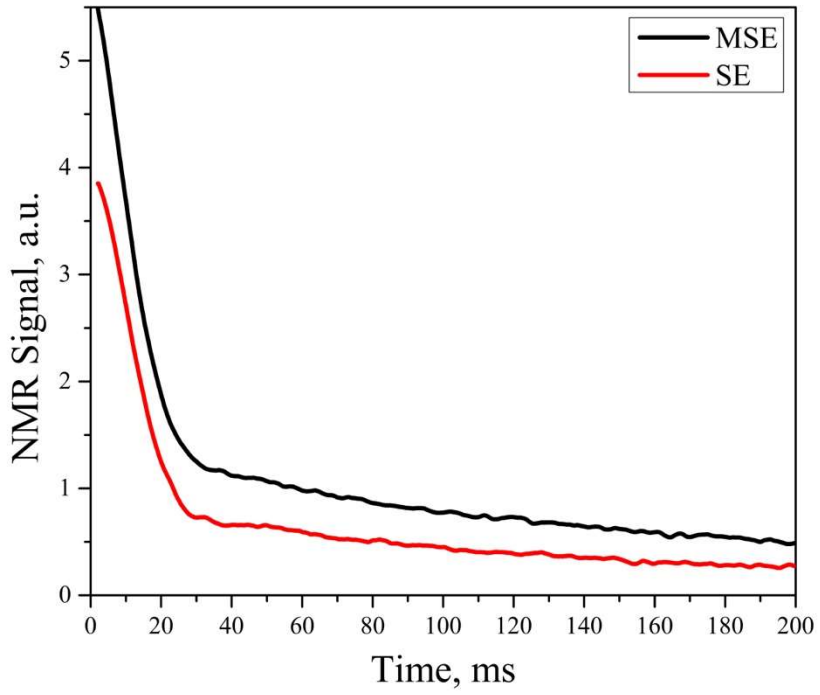

Fig. 3.9. Representative signals of SE and MSE sequences for the SCG40 (30%) samples

This SFC like analysis showed that differentiation between different formulations could not be detected sufficient enough. Results also showed that MSE results were more similar to the previous approach. Thus, MSE has really a good potential to be used for differentiating different formulations. But still with needs more experimentation to have generalized statements.

3.1.5.2 Spin Diffusion

As stated in Chapter 1, spin diffusion occurs when there is a gradient of magnetization between phases with different mobility. The polarization transfer is the leakage of magnetization from ^{1}H nuclei of soft protons to the crystalline regions. The leakage itself is observed by the reduction of the "*long*" component of FID and simultaneous increase of the "*short*" component contribution into overall signal during increase of the spin diffusion time (L. Y. Grunin et al., 2017). In our case, long component refers to the water that is trapped in the gel whereas short component is the solid portion and in the case of a soft candy it is the crystal sugars that could potentially form.

In other words, spin diffusion leads to communication among spins at various sites in a solid. It has been shown that such communication could provide useful information about the spatial inhomogeneity in the resonance frequencies and relaxation times. The rate of the spin- flip communication is directly proportional to the local dipolar field (Cheung, 1981). Thus, this behavior could help us to understand the differences in different candy formulations. The challenge of spin diffusion experiments is the data analysis conducted afterwards. *Spin Diffusion* experiments are usually conducted through the famous Goldman Shen (Goldman & Shen, 1966) pulse sequence as described in Chapter 1. What we get from the spin diffusion is a parameter that is related with the **domain sizes** which can be used as a characterization parameter for different candies. We need to have a SE or MSE signal and do some fitting as will be described below.

The 1st parameter that needs to be calculated is the 2nd moment as it will be required for the further calculations. Abrahamian-based Time-Domain Free Induction Decay turns to be as follows (L. Grunin et al., 2019);

$$s(t) = A_{cr} \exp\left(-\tfrac{1}{2}a^2t^2\right) \cdot \sin(bt)/bt + A_{am} \exp(-(t/T_2^{am})^2) + A_w \exp(-(t/T_2^{*w})^2)$$

(2)

where indexes *cr*, *am* and *w* relate to crystalline, amorphous phases and water respectively, T_2 denotes the spin-spin relaxation time and the second moments are:

$$M_2^{cr} = a^2 + b^2/3 \text{ and } M_2^{am} = 2/(T_2^{\text{am}})^2 \quad \textbf{(3)}$$

For the fitting of the Equation 2, MSE data was used. And since with the water fraction, fitting becomes very hard, it was first subtracted from the signal with a special module in **Relax 8** software. So, the fitting was performed to the 1st and 2nd exponents.

M_2^{am} was used for calculation of the effective spin diffusion coefficient (D_{sd}). r^2 (*mean square distance between spins*) value was estimated within a range of 0.22-0.25 nm similar to cellulose since average distance was nearly similar in all saccharides (Cheung, 1981).

$$D_{sd} = \frac{\sqrt{\pi}}{6}\langle r^2\rangle\sqrt{M_2} \quad \textbf{(4)}$$

The transfer thickness (d) of the interface layer which is between bounded water and crystalline was found by using

$$d = \frac{2\beta t^{0.5}}{\sqrt{\pi}}\sqrt{M_2} \quad \textbf{(5)}$$

assuming that the magnetization transfer was carried out in one direction (β = 1).

$t^{0.5}$ was calculated by conducting Goldman-Shen experiment (Fig. 3.10). Linear fitting was performed by using Origin software. Results of the 'd' values are given in Table 3.3

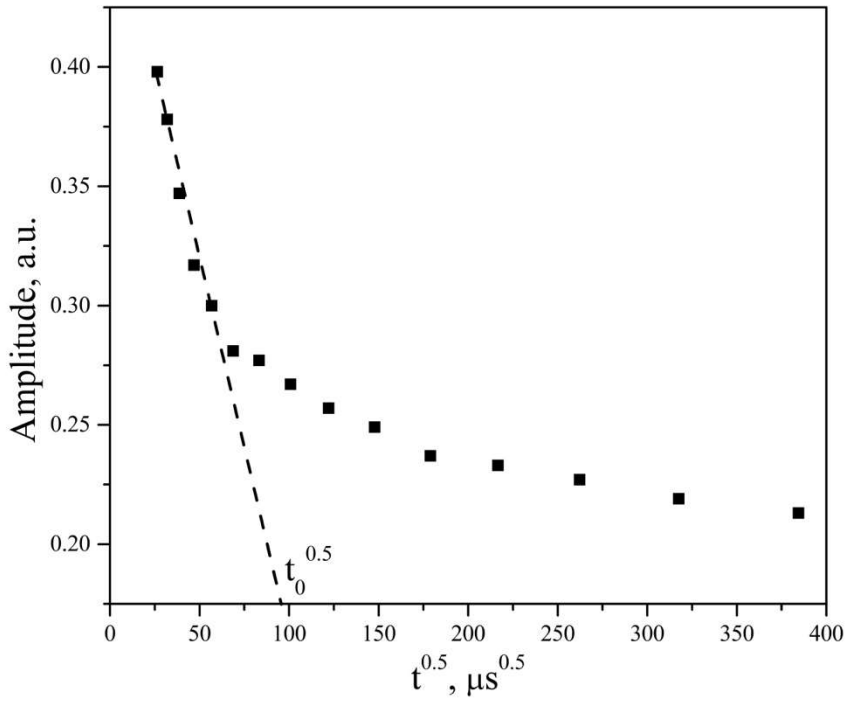

Fig. 3.10. An example of the determination of the parameter $t^{0.5}$ in Goldman-Shen Experiment

Table 3.3. Interface layer thickness of the soft candies calculated by *Goldman-Shen* pulse sequence

Interface layer thickness (Å)		
Syrup Amount (%) / *Syrup Type*	30	60
SCG40	47.5 ± 1.8^{g}	$62.2\pm1.4^{d,e}$
SCG60	$50.2\pm1.5^{f,g}$	69.4 ± 2.4^{d}
SMF42	83.6 ± 1.8^{c}	641 ± 3.8^{a}
SBF10	$57.7\pm2.4^{e,f}$	95.8 ± 1.7^{c}
SHFSLF20	60 ± 1^{e}	113 ± 3.5^{b}

Both syrup type, syrup concentration and also their interaction were found to be significant on the 'd' values ($p<0.05$). Higher syrup concentrations resulted in larger thickness values. Glucose syrups of SCG40 and SCG60 had similar values whereas the fructose syrup samples differed significantly from each other ($p<0.05$). It was quite interesting to see that as fructose content of the syrup increased; interface thickness increased. There was a monotonic increasing trend of the 'd' values wrt fructose concentration of the syrup. The transfer thickness (d) is defined as the thickness of the interface layer which is between bounded water and crystalline. Thus, the presence of fructose increased that thickness and could have resulted in lower crystallinity values which was also confirmed by the lower crystallinity values in SE/MSE experiments. This result also showed that if the water binding is high; interface layer thickness increases and thus crystallinity decreases. Interface thickness of being different for different syrups showed that this approach had the potential to differentiate the candies prepared from different syrups from each other.

Results of this section showed that, the non-conventional NMR approaches provided more differentiation among the candies compared to the relaxation times. So, these

methods have a potential to detect the presence of different ingredients in formulations.

3.2 Effect of gelatin source on the NMR characteristics of gelatin candies

Bovine, porcine and fish gelatins are the commercially available sources. However, the most preferred one is porcine as being cheaper and having greater gelation ability. As stated before, the difference between bovine and porcine gelatin comes from the differences on amino acid compositions (Hafidz et al., 2011).

In this study, conventional and nonconventional TD-NMR approaches have been used to see the differences in different gelatin-based candies. As stated, the candies were prepared from the same bloom index porcine (PC) and bovine (BC) based candies. Sucrose, corn syrup (SCG40) amounts were kept same in the formulations and they were used at a ratio of 50-50% as in the case of 30 % syrup formulations in the 1st part of the study. Only difference was the type of the gelatin. Table 3.4 shows the aw and °Brix values for the formulations. As seen on the results, gelatin type had a significant effect on the water activity ($p<0.05$) but not on the °Brix values. PC gels had higher a_w values than the BC gels. However, the difference in aw could not be used as a tool for differentiation since in industrial production, candies are usually conditioned in storage rooms to achieve the same a_w levels.

Table 3.4. Effect of gelatin source on the a_w and °Brix of candies

Gelatin Type	*a_w*	*°Brix*
Porcine	0.6709 ± 0.0018^{a}	13.7 ± 0.17^{a}
Bovine	0.6202 ± 0.0015^{b}	13.33 ± 0.09^{a}

Relaxation times of the gels were also measured. Results are given in Table 3.5. T_1 times were not significantly different from each other but despite the higher a_w of porcine gelatin samples they had shorter T_2 relaxation times. Porcine gelatin is known to have better gel forming ability (Sezer et al., 2019). Shorter T_2 times indicated the hindered mobility of the water protons in the gel network.

Table 3.5. Effect of gelatin source on the relaxation times of the candies

Gelatin Type	*T_1 (ms)*	*T_2 (ms)*
Porcine	45.56±0.92[a]	12.37±0.24[b]
Bovine	48.77±1.68[a]	14.67±0.41[a]

In addition to relaxation times, SE/MSE and Spin Diffusion experiments were also conducted for the 2 candies. Results are given Table 3.6. This time SE/MSE experiments were also performed for the candies who have lost most of their water during storage at 25 ºC for 3 months. This was intentionally done since SE/MSE are known to work best for really 'solid' samples. Candies had a moisture content around 16-20%. To see whether differentiation would be much better at lower water content SE/MSE data were analyzed, both before and after storage by the 2nd moment values, as described in the 1st part. However, samples did not have a significant difference before or after storage ($p>0.05$). Thus, this approach was not capable of detecting the differences between the samples.

Table 3.6. 2nd moment values of the bovine/porcine gelatin candies*

	Before Storage		*After Storage*	
Gelatin Type	*M_2 (SE)*	*M_2 (MSE)*	*M_2 (SE)*	*M_2 (MSE)*
Porcine	5.84±0.96[a]	8.67±0.82[a]	9.23±0.08[a]	14.37±0.16[a]
Bovine	6.14±0.27[a]	9.21±0.23[a]	8.97±0.50[a]	14.40±0.42[a]

**Lettering indicates the significant differences between rows ($p<0.05$)*

Spin diffusion experiments were also performed to calculate the interface thickness before and after storage (Table 3.7). *Before storage* thickness values did not differ between the gelatin types ($p>0.05$), however for '*after storage'* a huge significant difference was detected between the samples ($p<0.05$). Thickness increase was much more in the case of BC samples. This was an indication that PC had a more stable gel structure compared to BC. And the huge difference on the thickness values showed that SD approach have potential to identify different gelatin types.

Table 3.7. Effect of gelatin source on the interface layer thickness of the soft candies calculated by *Goldman-Shen* pulse sequence

Gelatin Type	*Interface layer thickness (Å) Before Storage*	*Interface layer thickness (Å) After storage*
Porcine	45.3 ± 2.1[a]	65.67±2.91[b]
Bovine	47.5 ± 1.8[a]	227±28.84[a]

CHAPTER 4

CONCLUSION

In this study, to our knowledge we have used the TD-NMR approaches of SE/MSE and Spin Diffusion for the 1st time on the characterization of confectionery gels. In fact, spin diffusion has not been studied for any food systems at all.

Since food systems are complex, we decided to work with a simple system of which we know the composition well and we can prepare at controlled conditions. In that regard, we decided to go with the soft confectionery gels. Then, we asked the question '*What are the problematic issues of the confectionery gels that the industry is facing*?' and the following problems come out:

1. Use of corn syrup in confectionery products is quite speculative nowadays. Its use in other confectionery products is even more controversial. And there is not an easy way to identify the source of the sugar used in a product with a simple method. But it is known that, corn syrup retards crystallization in soft candies. SE/MSE and SD approaches have been shown to be capable of providing information on the crystallinity of food samples. Then, *why not we use these approaches for candies made with different corn syrups and see what are the differences*?

   So, our research question became: '*Can we use these non-conventional NMR approaches for understanding the presence of corn syrup in candies?*

2. The 2nd question that we asked was related with the gelatin type. In Turkey, porcine gelatin as not being halal is a very important problem that many

industries suffer from. There are methods to understand the difference but still it is not that easy. So, we asked the question '*If we prepare candies having almost same physical properties; would it be possible to differentiate them through these non-conventional TD-NMR approaches*?

So, in the 1st part of the study, we investigated candies prepared by using 5 different corn syrups at different syrup concentrations. Physical properties of a_w, hardness and ^oBrix were measured and some differences were detected. The conventional TD-NMR approaches of T_1 and T_2 relaxation times were also measured but they were found insufficient indicators for differentiating the samples. Later, SE/MSE sequences were used on the samples. Crystallinity of the fructose containing samples were found to be the lowest which was in accordance with the basic '*food chemistry'* facts. Spin diffusion calculations enabled us to calculate the interface thickness and the results showed a good variation within the samples. Presence of fructose affected the thickness significantly and the thickness increased with increasing fructose concentration. That was an important finding, but still to develop a generalized method for identifying the nature of the syrup in unknown samples, further experiments are required. Physical parameters should be known and by using a large data set, multivariate analysis models can be constructed, and the thickness values could indicate the type of the syrup used in the formulations.

In the 2nd part of the study, we investigated 2 candies having exactly the same formulation but one prepared with porcine and the other prepared with bovine gelatin. T_2 times and SD results were able to differentiate the samples. Especially the interface layers calculated after 3 months of storage showed significant increase on the bovine gelatin samples indicating the high stability for the porcine ones. So, SD has also been shown to be an alternative approach for differentiation. But using this method for an unknown candy again requires a larger data set and further analysis.

In summary, in this study we just showed the fact that there are these ‘non-conventional NMR methods’ used in polymer science literature and they have significant potential to solve food quality related problems that the industry is facing.

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APPENDICES

A. Statistical analyses for the effect of Corn Syrup on the Candies

Table A.1. ANOVA and Tukey's Comparison Test for water activity of the samples

General Linear Model: aw versus Syrup Type, Syrup(%)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Syrup Type	Fixed	5	SBF10, SCG40, SCG60, SHFSLF20, SMF42
Syrup(%)	Fixed	2	30, 60

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Syrup Type	4	0.100684	0.025171	47.72	0.000
Syrup(%)	1	0.000253	0.000253	0.48	0.497
Sample*Syrup(%)	4	0.049682	0.012420	23.55	0.000
Error	20	0.010549	0.000527		
Total	29	0.161168			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0229663	93.45%	90.51%	85.27%

Comparisons for aw

Tukey Pairwise Comparisons: Syrup Type

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping		
SCG40	6	0.775283	A		
SBF10	6	0.661833		B	
SCG60	6	0.637033		B	C
SHFSLF20	6	0.630783		B	C
SMF42	6	0.613383			C

Tukey Pairwise Comparisons: Syrup(%)

Grouping Information Using the Tukey Method and 95% Confidence

Syrup(%)	N	Mean	Grouping
60	15	0.666567	A
30	15	0.660760	A

Tukey Pairwise Comparisons: Syrup type*Syrup(%)

Grouping Information Using the Tukey Method and 95% Confidence

Sample*Syrup(%)	N	Mean	Grouping			
SCG40 60	3	0.847000	A			
SCG40 30	3	0.703567		B		
SCG60 30	3	0.686500		B	C	
SBF10 30	3	0.663367		B	C	D
SBF10 60	3	0.660300		B	C	D
SHFSLF20 60	3	0.646533		B	C	D	E
SMF42 30	3	0.635333			C	D	E
SHFSLF20 30	3	0.615033				D	E
SMF42 60	3	0.591433					E
SCG60 60	3	0.587567					E

Table A.2. ANOVA and Tukey's Comparison Test for brix of the samples

General Linear Model: Brix versus Syrup Type, Syrup(%)

Method

Factor coding (-1, 0, +1)
Rows unused 3

Factor Information

Factor	Type	Levels	Values
Syrup Type	Fixed	5	SBF10, SCG40, SCG60, SHFSLF20, SMF42
Syrup(%)	Fixed	2	30, 60

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Syrup Type	4	10.1250	2.53126	32.68	0.000
Syrup(%)	1	0.0072	0.00725	0.09	0.763
Syrup Type*Syrup(%)	4	0.8065	0.20162	2.60	0.073
Error	17	1.3167	0.07745		

Total	26	12.4985

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.278300	89.47%	83.89%	70.28%

Comparisons for Brix

Tukey Pairwise Comparisons: Syrup Type

Grouping Information Using the Tukey Method and 95% Confidence

Syrup Type	N	Mean	Grouping		
SCG60	6	83.9167	A		
SHFSLF20	6	82.7167		B	
SMF42	5	82.5250		B	
SBF10	5	82.3833		B	
SCG40	5	82.2583		B	

Tukey Pairwise Comparisons: Syrup(%)

Grouping Information Using the Tukey Method and 95% Confidence

Syrup(%)	N	Mean	Grouping
60	13	82.7767	A
30	14	82.7433	A

Tukey Pairwise Comparisons: Syrup Type*Syrup(%)

Grouping Information Using the Tukey Method and 95% Confidence

Syrup Type*Syrup(%)	N	Mean	Grouping		
SCG60 60	3	84.0333	A		
SCG60 30	3	83.8000	A	B	
SHFSLF20 30	3	83.0000		B	C
SMF42 60	2	82.7500			C
SHFSLF20 60	3	82.4333			C
SBF10 60	2	82.4000			C
SBF10 30	3	82.3667			C
SMF42 30	3	82.3000			C
SCG40 60	3	82.2667			C
SCG40 30	2	82.2500			C

Table A.3. ANOVA and Tukey's Comparison Test for hardness of the samples

General Linear Model: Hardness versus Syrup Type, Syrup(%)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Syrup Type	Fixed	5	SBF10, SCG40, SCG60, SHFSLF20, SMF42
Syrup(%)	Fixed	2	30, 60

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Syrup Type	4	7568.4	1892.10	33.87	0.000
Syrup(%)	1	0.0	0.00	0.00	0.997
Syrup Type*Syrup(%)	4	6096.7	1524.19	27.28	0.000
Error	20	1117.4	55.87		
Total	29	14782.5			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
7.47461	92.44%	89.04%	82.99%

Comparisons for Hardness

Tukey Pairwise Comparisons: Syrup Type

Grouping Information Using the Tukey Method and 95% Confidence

Syrup Type	N	Mean	Grouping		
SCG60	6	64.300	A		
SMF42	6	51.075		B	
SHFSLF20	6	42.450		B	C
SBF10	6	31.725			C
SCG40	6	17.975			D

Tukey Pairwise Comparisons: Syrup(%)

Grouping Information Using the Tukey Method and 95% Confidence

Syrup(%)	N	Mean	Grouping
60	15	41.51	A
30	15	41.50	A

Tukey Pairwise Comparisons: Syrup Type*Syrup(%)

Grouping Information Using the Tukey Method and 95% Confidence

Syrup Type*Syrup(%)	N	Mean	Grouping		
SCG60 60	3	91.60	A		
SMF42 30	3	54.70		B	
SHFSLF20 30	3	49.50		B	C
SMF42 60	3	47.45		B	C
SCG60 30	3	37.00		B	C
SHFSLF20 60	3	35.40		B	C
SBF10 30	3	34.10		B	C
SCG40 30	3	32.20			C
SBF10 60	3	29.35			C
SCG40 60	3	3.75			D

Table A.4. ANOVA and Tukey's Comparison Test for T_1 of the samples

General Linear Model: logT_1 versus Syrup Type, Syrup(%)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Syrup Type	Fixed	5	SBF10, SCG40, SCG60, SHFSLF20, SMF42
Syrup(%)	Fixed	2	30, 60

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Syrup Type	4	0.083598	0.020899	179.10	0.000
Syrup(%)	1	0.001239	0.001239	10.62	0.004
Syrup Type*Syrup(%)	4	0.057913	0.014478	124.07	0.000
Error	20	0.002334	0.000117		
Total	29	0.145084			

Comparisons for logT$_1$

Tukey Pairwise Comparisons: Syrup Type

Grouping Information Using the Tukey Method and 95% Confidence

Syrup Type	N	Mean	Grouping
SCG40	6	1.77705	A
SCG60	6	1.69282	B
SBF10	6	1.66446	C
SMF42	6	1.64402	D
SHFSLF20	6	1.62689	D

Tukey Pairwise Comparisons: Syrup(%)

Grouping Information Using the Tukey Method and 95% Confidence

Syrup(%)	N	Mean	Grouping
60	15	1.68748	A
30	15	1.67462	B

Tukey Pairwise Comparisons: Syrup Type*Syrup(%)

Grouping Information Using the Tukey Method and 95% Confidence

Syrup Type*Syrup(%)	N	Mean	Grouping
SCG40 60	3	1.81066	A
SCG40 30	3	1.74343	B
SCG60 30	3	1.72483	B C
SBF10 30	3	1.71788	B C
SMF42 60	3	1.71176	C
SCG60 60	3	1.66080	D
SHFSLF20 60	3	1.64310	D
SBF10 60	3	1.61104	E
SHFSLF20 30	3	1.61069	E
SMF42 30	3	1.57629	F

Table A.5. ANOVA and Tukey's Comparison Test for T_2 of the samples

General Linear Model: T_2 versus Syrup Type, Syrup(%)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Syrup Type	Fixed	5	SBF10, SCG40, SCG60, SHFSLF20, SMF42
Syrup(%)	Fixed	2	30, 60

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Syrup Type	4	1140.90	285.224	246.93	0.000
Syrup(%)	1	108.22	108.220	93.69	0.000
Syrup Type*Syrup(%)	4	3620.92	905.229	783.68	0.000
Error	20	23.10	1.155		
Total	29	4893.14			

Comparisons for T_2

Tukey Pairwise Comparisons: Syrup Type

Grouping Information Using the Tukey Method and 95% Confidence

Syrup Type	N	Mean	Grouping		
SMF42	6	25.6298	A		
SCG40	6	20.5706		B	
SBF10	6	17.1628			C
SCG60	6	17.0110			C
SHFSLF20	6	6.8091				D

Tukey Pairwise Comparisons: Syrup(%)

Grouping Information Using the Tukey Method and 95% Confidence

Syrup(%)	N	Mean	Grouping	
60	15	19.3360	A	
30	15	15.5374		B

Tukey Pairwise Comparisons: Syrup Type*Syrup(%)

Grouping Information Using the Tukey Method and 95% Confidence

Syrup Type*Syrup(%)	N	Mean	Grouping				
SMF42 60	3	47.5507	A				
SBF10 30	3	26.5397		B			
SCG60 30	3	23.1936			C		
SCG40 30	3	21.2317			C	D	
SCG40 60	3	19.9095				D	
SCG60 60	3	10.8285					E
SHFSLF20 60	3	10.6052					E
SBF10 60	3	7.7860					E
SMF42 30	3	3.7089					F
SHFSLF20 30	3	3.0130					F

Table A.6. ANOVA and Tukey's Comparison Test for second moment (derived via SE sequence) of the samples

General Linear Model: Second Moment (SE) versus Syrup Type, Syrup(%)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Syrup Type	Fixed	5	SBF10, SCG40, SCG60, SHFSLF20, SMF42
Syrup(%)	Fixed	2	30, 60

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Syrup Type	4	8.4915	2.12288	44.12	0.000
Syrup(%)	1	0.0788	0.07881	1.64	0.215
Syrup Type*Syrup(%)	4	1.8696	0.46741	9.71	0.000
Error	20	0.9623	0.04811		
Total	29	11.4023			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.219351	91.56%	87.76%	81.01%

Comparisons for Second Moment (SE)

Tukey Pairwise Comparisons: Syrup Type

Grouping Information Using the Tukey Method and 95% Confidence

Syrup Type	N	Mean	Grouping		
SCG40	6	7.23018	A		
SCG60	6	7.05555	A	B	
SHFSLF20	6	6.94218	A	B	
SBF10	6	6.75680		B	
SMF42	6	5.72302			C

Tukey Pairwise Comparisons: Syrup(%)

Grouping Information Using the Tukey Method and 95% Confidence

Syrup(%)	N	Mean	Grouping	
30	15	6.79280	A	
60	15	6.69029	A	

Tukey Pairwise Comparisons: Syrup Type*Syrup(%)

Grouping Information Using the Tukey Method and 95% Confidence

Syrup Type*Syrup(%)	N	Mean	Grouping			
SCG40 60	3	7.45620	A			
SCG60 60	3	7.24963	A	B		
SHFSLF20 30	3	7.19440	A	B		
SCG40 30	3	7.00417	A	B		
SCG60 30	3	6.86147	A	B		
SBF10 30	3	6.76587		B	C	
SBF10 60	3	6.74773		B	C	
SHFSLF20 60	3	6.68997		B	C	
SMF42 30	3	6.13810			C	
SMF42 60	3	5.30793				D

Table A.7. ANOVA and Tukey's Comparison Test for second moment (derived via MSE sequence) of the samples

General Linear Model: Second Moment (MSE) versus Syrup Type, Syrup(%)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Syrup Type	Fixed	5	SBF10, SCG40, SCG60, SHFSLF20, SMF42
Syrup(%)	Fixed	2	30, 60

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Syrup Type	4	34.4506	8.61266	90.97	0.000
Syrup(%)	1	0.0000	0.00004	0.00	0.984
Syrup Type*Syrup(%)	4	2.0590	0.51474	5.44	0.004
Error	20	1.8936	0.09468		
Total	29	38.4033			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.307702	95.07%	92.85%	88.91%

Comparisons for Second Moment (MSE)

Tukey Pairwise Comparisons: Syrup Type

Grouping Information Using the Tukey Method and 95% Confidence

Syrup Type	N	Mean	Grouping		
SCG40	6	11.8731	A		
SCG60	6	11.6164	A		
SHFSLF20	6	11.3734	A	B	
SBF10	6	10.9611		B	
SMF42	6	8.8845			C

Tukey Pairwise Comparisons: Syrup(%)

Grouping Information Using the Tukey Method and 95% Confidence

Syrup(%)	N	Mean	Grouping
60	15	10.9429	A
30	15	10.9406	A

Tukey Pairwise Comparisons: Syrup Type*Syrup(%)

Grouping Information Using the Tukey Method and 95% Confidence

Syrup Type*Syrup(%)	N	Mean	Grouping		
SCG60 60	3	12.0237	A		
SCG40 60	3	11.9890	A		
SCG40 30	3	11.7572	A	B	
SHFSLF20 30	3	11.6038	A	B	
SCG60 30	3	11.2092	A	B	
SHFSLF20 60	3	11.1430	A	B	
SBF10 60	3	11.0042		B	
SBF10 30	3	10.9180		B	
SMF42 30	3	9.2145			C
SMF42 60	3	8.5544			C

Table A.8. ANOVA and Tukey's Comparison Test for interface layer thickness of the samples

General Linear Model: log(Interface layer thickness) versus Syrup Type, Syrup(%)

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Syrup Type	Fixed	5	SBF10, SCG40, SCG60, SHFSLF20, SMF42
Syrup(%)	Fixed	2	30, 60

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Syrup Type	4	1.53566	0.383914	863.15	0.000
Syrup(%)	1	0.80519	0.805189	1810.29	0.000

Syrup	4	0.60582	0.151454	340.51	0.000
Type*Syrup(%)					
Error	20	0.00890	0.000445		
Total	29	2.95556			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0210899	99.70%	99.56%	99.32%

Comparisons for log(Interface Layer Thickness)

Tukey Pairwise Comparisons: Syrup Type

Grouping Information Using the Tukey Method and 95% Confidence

Syrup Type	N	Mean	Grouping			
SMF42	6	2.36442	A			
SHFSLF20	6	1.91535		B		
SBF10	6	1.87083			C	
SCG60	6	1.77059				D
SCG40	6	1.73481				D

Tukey Pairwise Comparisons: Syrup(%)

Grouping Information Using the Tukey Method and 95% Confidence

Syrup(%)	N	Mean	Grouping	
60	15	2.09503	A	
30	15	1.76737		B

Tukey Pairwise Comparisons: Syrup Type*Syrup(%)

Grouping Information Using the Tukey Method and 95% Confidence

Syrup Type*Syrup(%)	N	Mean	Grouping					
SMF42 60	3	2.80684	A					
SHFSLF20 60	3	2.05267		B				
SBF10 60	3	1.98122			C			
SMF42 30	3	1.92200			C			
SCG60 60	3	1.84084				D		
SCG40 60	3	1.79356				D	E	
SHFSLF20 30	3	1.77803					E	
SBF10 30	3	1.76043					E	F
SCG60 30	3	1.70033						F G
SCG40 30	3	1.67605						G

B. Statistical analyses for the effect of Gelatin Types on the Candies

Table B.1. ANOVA for water activity of the samples

General Linear Model: aw versus Gelatin Type

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Gelatin Type	Fixed	2	Bovine, Porcine

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gelatin Type	1	0.003856	0.003856	469.35	0.000
Error	4	0.000033	0.000008		
Total	5	0.003889			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0028662	99.15%	98.94%	98.10%

Table B.2. ANOVA for brix of the samples

General Linear Model: Brix versus Gelatin Type

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Gelatin Type	Fixed	2	Bovine, Porcine

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gelatin Type	1	0.2017	0.20167	3.56	0.132
Error	4	0.2267	0.05667		
Total	5	0.4283			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.238048	47.08%	33.85%	0.00%

Table B.3. ANOVA for T_1 of the samples

General Linear Model: T_1 versus Gelatin Type

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Gelatin Type	Fixed	2	Bovine, Porcine

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gelatin Type	1	15.37	15.374	2.78	0.171
Error	4	22.11	5.527		
Total	5	37.48			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
2.35106	41.01%	26.27%	0.00%

Table B.4. ANOVA for T_2 of the samples

General Linear Model: T_2 versus Gelatin Type

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Gelatin Type	Fixed	2	Bovine, Porcine

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gelatin Type	1	7.906	7.9060	23.46	0.008
Error	4	1.348	0.3369		
Total	5	9.254			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.580473	85.44%	81.79%	67.23%

Table B.5. ANOVA for second moment (derived via SE sequence) of the samples before storage

General Linear Model: Second Moment (SE) BS versus Gelatin Type

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Gelatin Type	Fixed	2	Bovine, Porcine

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gelatin Type	1	0.9189	0.91885	27.38	0.006
Error	4	0.1343	0.03356		
Total	5	1.0531			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.183207	87.25%	84.06%	71.32%

Table B.6. ANOVA for second moment (derived via SE sequence) of the samples after storage

General Linear Model: Second Moment (SE) AS versus Gelatin Type

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Gelatin Type	Fixed	2	Bovine, Porcine

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gelatin Type	1	0.09670	0.09670	0.25	0.642
Error	4	1.53445	0.38361		
Total	5	1.63115			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.619365	5.93%	0.00%	0.00%

Table B.7. ANOVA for second moment (derived via MSE sequence) of the samples before storage

General Linear Model: Second Moment (MSE) BS versus Gelatin Type

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Gelatin Type	Fixed	2	Bovine, Porcine

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gelatin Type	1	0.003700	0.003700	0.02	0.887
Error	4	0.650667	0.162667		
Total	5	0.654367			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.403320	0.57%	0.00%	0.00%

Table B.8. ANOVA for second moment (derived via MSE sequence) of the samples after storage

General Linear Model: Second Moment (MSE) AS versus Gelatin Type

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Gelatin Type	Fixed	2	Bovine, Porcine

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gelatin Type	1	0.00200	0.002002	0.01	0.938
Error	4	1.18435	0.296088		
Total	5	1.18636			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.544140	0.17%	0.00%	0.00%

Table B.9. ANOVA for interface layer thickness of the samples

General Linear Model: Interface layer thickness (Å) versus Gelatin Type

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Gelatin Type	Fixed	2	Bovine, Porcine

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gelatin Type	1	39043	39043	30.97	0.005
Error	4	5043	1261		
Total	5	44085			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
35.5059	88.56%	85.70%	74.26%