CHARACTERIZATION AND FORMULATION OF GELATIN BASED SOFT CANDIES

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

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ABSTRACT

CHARACTERIZATION AND FORMULATION OF GELATIN BASED SOFT CANDIES

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High caloric value of the candies is the major concern of the consumers. Soft candies are produced by using different gelling agents and sugar constitutes almost 60% of the formulation. In this study, it is aimed to produce low calorie soft candies by using gelatin as the main gelling agent. Different low-calorie sweeteners; isomalt, maltitol and stevia were substituted with sucrose at different ratios and it was explored to find the best formulation similar to the candies prepared with the control formulation. To investigate physical properties, pH, total soluble solid content, water activity, moisture content, color, firmness, springiness, differential scanning calorimeter (DSC) and Low and High Field Nuclear Resonance (LF/HF-NMR Relaxometry) Relaxometry (T_1 and T_2 relaxation times) experiments were performed.

Results confirmed that, moisture content, water activity, firmness, and springiness values all depend on the sweetener type. Maillard browning reactions did not occur due to using non-reducing sugars thus brown color formation was not observed at significant rates.

DSC results showed that T_g values were quite low which could be related with the stability of the samples.

NMR Relaxometry experiments performed at both high and low field systems were consistent and complemented each other. T_1 and T_2 relaxation times were measured and T_2 times showed the presence of two proton pools which were related with water proton compartmentalization in the candies. As expected longer T_1 values were obtained at high field system. T_2 results showed no significant difference between two systems ($p>0.05$) for both components except RA_1. At high field system, temperature dependent experiments were also conducted and increase in temperature resulted in an increase on both T_1 and T_2 times.

Keywords: Low caloric soft candy; isomalt; maltitol; stevia; NMR Relaxometry

ÖZ

JELATIN IÇERIKLI YUMUŞAK ŞEKERLEMELERIN KARAKTERIZASYONU VE FORMÜLASYONU

Efe, Nilgün

Yüksek Lisans, Gıda Mühendisliği Bölümü

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Ağustos 2017, 114 sayfa

Yüksek kalorili şekerlemeler tüketiciler için kaygı konusudur. Yumuşak şekerlemelerde değişik jelleşme ajanları kullanılabilir ve seker oranı yaklaşık olarak formülasyonun %60 idir. Bu çalışmada, jelatin kullanılarak düşük kalorili yumuşak şekerlemelerin üretilmesi amaçlanmıştır. Farklı tatlandırıcılar, izomalt, maltitol ve stevia, sofra şekerine en yakın formülasyonun bulunması için farklı şekerlerle birlikte farklı oranlar kullanılmıştır. Hazırlanan şekerlemelerin fiziksel karakterizasyonu için pH, toplam çözünmüş kati madde miktarı, su aktivitesi, nem içeriği, renk, sertlik, yumuşaklık, diferansiyel taramalı kalorimetre (DTK), düşük ve yüksek rezolüsyonlu Nükleer Manyetik Rezonans Relaksometre (NMR) (T_1 ve T_2 relaksasyon) deneyleri yapılmıştır.

Sonuçlara göre nem içeriği, su aktivitesi, sertlik, yumuşaklık değerleri seker tipine göre farklılık göstermiştir. Şekerlemelerde indirgen seker olmadığı için Maillard reaksiyonu gözlemlenmemiştir.

Bununla beraber, kalorimetrik sonuçlarda, düşük T_g'nin örneklerin stabilitesinde etkili olduğu görülmektedir.

Düşük ve yüksek rezolüsyonlu NMR Relaksometre uygulandığında tutarlı ve tümler sonuçlar elde edilmiştir. T_1 ve T_2 relaksasyon zamanları ölçülmüş ve T_2 zamanları 2 proton havuzu olduğunu göstermiş ve bu havuzlar şekerlemelerde bölümleşme olduğunu göstermiştir. Beklenildiği gibi daha uzun T_1 zamanları yüksek rezolüsyonlu NMR Relaksometre sonuçlarında gözlemlenmiştir. T_2 sonuçlarında ise birinci sinyalden gelen alanın dışında 2 sistem arasında belirgin bir fark görülmemiştir ($p>0.05$). Buna ek olarak, yüksek rezolüsyonlu NMR tekniği kullanılarak sıcaklığa bağlı deneyler yapılmış ve sıcaklık arttıkça T_1 ve T_2 sürelerinde artış gözlemlenmiştir.

Anahtar kelimeler: dusuk kalorili yumusak sekerleme, izomalt, maltitol, stevia, NMR Relaksometri

To my beloved family,

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ABBREVIATION

a_w	Water Activity
T_g	Glass Transition Temperature
DE	Dextrose Equivalence
QS	*quantum satis*
FDA	Food and Drug Administration
GRAS	Generally Recognized as Safe
MW	Molecular Weight
TSSC	Total Soluable Solid Content
DSC	Differential Scanning Calorimeter
T_m	Melting Temperature
NMR	Nuclear Magnetic Resonance
MRI	Magnetic Resonance Imaging
IR	Inversion Recovery
RF	Radio Frequency
CPMG	Carr-Purcell-Meiboom-Gill
TD	Time Domain
CS	Corn Syrup
LF-NMR	Low Field Nuclear Magnetic Resonance
HF-NMR	High Field Nuclear Magnetic Resonance

CHAPTER 1

INTRODUCTION

1. CONFECTIONARY

Sugar based confectionery products include, marshmallows, starch, pectin, gelatin based soft candies, hard candies, chocolate, Turkish delight and many others (Lees & Jackson, E., 1973). The most important and main ingredients of these products are sugar and corn syrup. However, they differ with respect to the cooking method, moisture content, and additional ingredients (Alikonis, 1979). As an example, an additional ingredient, egg albumen, provides gelation in marshmallow production while starch and high temperature (T>100°C) are important steps on the production of starch-based jellies (Alikonis, 1979).

1.1. Candy

Candies are generally made of cane and beet sugars, corn syrup and additional flavor and colorings (“Semad,” n.d.). Some of the gelling agents used in candies include starch, pectin, gum arabic, and gelatin and these are the most important ingredients that affect the final textural properties of the products (soft, firm or solid). These polymers can be used by themselves or as in the

form of mixtures for producing candies with different textures. (Edwards, W., 2000; Lees & Jackson, E., 1973).

In sugar confectionery, significant quality parameters for the candies formulations are relative sweetness of the sweetening agent (compared to sucrose), solubility of

the sweetener, added flavor, and moisture content of the final product (Alikonis, 1979). The moisture content of final product is very important as it determines the textural properties and shelf life of the product (Ergun, Lietha, & Hartel, 2015).

Confectionery products can be classified according to the ingredients, process they exposed to and the water content (Alikonis, 1979) such as, gums, nougats, marshmallows and candies (hard candies and soft candies) (Lees & Jackson, E., 1973).

1.1.1. Hard Candy

Hard candies are cooked at high temperatures by mixing refined sugar and water (Alikonis, 1979). Alternatively, they can be defined as supersaturated mixtures with respect to sucrose (Ergun et al., 2015).

Production steps of hard candy are given in Figure 1.1 (Labropoulos & Anestis, 2012).

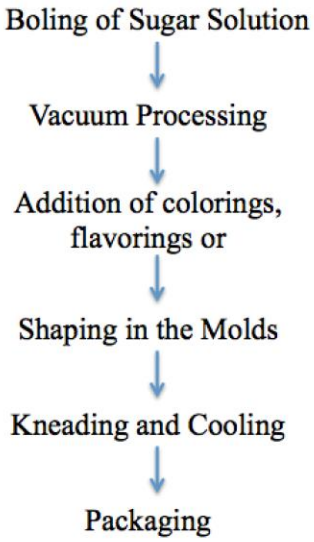


Figure 1.1 Hard candy production flow chart

The main differences between hard and soft candy are moisture content and textural properties. Hard candies are boiled to high temperatures and due to that they have very low moisture content changing in the range of 2-5% when compared to soft candies. Soft candies have higher moisture content, 8-22%, and softer texture.

1.1.2. Soft Candy

Starch, pectin and gelatin based candies are called as soft candies which have higher demand in Europe (Lees & Jackson, E., 1973). These products are softer than hard candies due to the gelling agent type and high moisture content (Lees & Jackson, E., 1973).

The general production line of soft candies is dissolving, concentrating, depositing or molding, drying or stoving as shown in Figure 1.2 (Lees & Jackson, E., 1973).

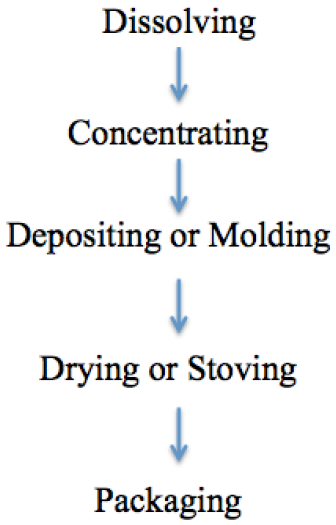


Figure 1.2 Soft candy production process

In soft candy production, molding can be done either by using starch molds or pouring the solution into rubbery molds. Starch molds have an advantage since starch absorbs the moist from the jellies, form a skin around them and makes it

easier to remove the jellies from molds without damaging the shape (Edwards, W., 2000). Therefore, in order to obtain the desirable water content, jellies should rest for 24-72 hours in starch molds (Ergun et al., 2015).

In gelatin based candies and gummies, the moisture content is between 8-22% and the water activity is between 0.50-0.75 (Ergun et al., 2015). In Table 1.1, the gelling agents used in hard and soft candy production, and their specific properties while making candies are given in detail (Lees & Jackson, E., 1973).

Table 1.1 Gelling agent properties in hard and soft candy production (Lees & Jackson, E., 1973)

	Gum Arabic	Starch	Gelatin	Pectin
Percent Use in Confectionery (%)	35-45	9-12	5-12	1-11
Temperature of Solution (°C)	25	71-82	60-65	93-100
Sweetener Ratio Sucrose/Glucose Syrup Ratio	66/33-50/50	66/33-50/50	66/33-50/50	60/40-50/50
Setting Temperature (°C)	20-37	20-37	20-37	71-82
Time in Molds (hours)	36-72	12-36	12-24	6-12
Total Solids (%)	68-70	72-78	72-78	76-78
	85+	78+	78+	78+
Texture	Smooth, Hard Bite	Short	Tough-Long	Clean Bite
pH during Cooking (Recommended)	5.0-6.0	5.0-6.0	5.0-6.0	4.0-5.0
Percent Acid for Flavoring (%)	0.3-0.45	0.2-0.4	0.2-0.3	0.4-0.7
Final pH of Product	4.2-5.0	4.2-5.0	4.4-5.0	3.2-3.5
Ease of Manufacture	Good	Excellent especially continuous production	Good	Fair

Since in this study soft candy was studied its proprties and production will be described in more detail.

1.2. Ingredients of Soft Candy

The main ingredients of soft candy are,

- Sucrose,
- Corn syrup,
- Gelling agent,
- Water,
- Aroma,
- Colorings,
- Acid (optional).

In this section, role of each ingredient will be discussed in detail.

1.2.1. Sugar Types / Sweeteners

1.2.1.1. Sucrose

Sucrose also known as saccharose is commonly known as table sugar, found in plants and generally sugar cane and sugar beet are used for its production (Labropoulos & Anestis, 2012). Sucrose is a non-reducing sugar (Figure 1.3). The sweetness rating (sweetness value) of sucrose is considered as 100 and other sweeteners have sweetness rating according to sucrose and the caloric value of sucrose is 4 cal/gram (IFIC Foundation, 2018). Solubility of sucrose is 2.0047 g sucrose/g water at 20 °C and the molecular weight (MW) is 342.297 g/mol.

Moreover, there is a relation between water activity (a_w) and sucrose concentration. a_w of water is represented as 1.0 and increasing sucrose concentration decreases water activity. Thus, sucrose may be called as water activity depressor and it may decrease the microbial spoilage in foods. In addition to the relation between water activity and sucrose concentration, glass transition

temperature (T_g) of sucrose and water activity of sucrose has a correlation. T_g is the temperature that the sample changes the physical property from glassy state to rubbery state and a small addition of water decreases T_g more (Ergun et al., 2015). When water activity of sucrose is 0.00, T_g is 56.6$\pm$3.4 °C and when a_w is 0.33, T_g is 12.6$\pm$ 0.9. As a result, if water activity increases, T_g decreases (Mathlouthi & Reiser, 1995). This relates important as the sucrose and moisture content relation directly affect the physical properties of candies (mostly texture).

Water behaves a plasticizer in amorphous sugars and as water content increases T_g decreases (Mathlouthi & Reiser, 1995).

In food industry, sucrose is important as it helps to improve physical and chemical properties, such as, solubility, water activity, flavor enhancement, provides good sensorial properties mainly sweetness (Mathlouthi & Reiser, 1995). The advantage of using sucrose is its accessibility. However it should not be forgotten that compare to corn syrup it is quiet expensive.

In confectionery, sweetness and flavor are crucial issues, which are usually adjusted through modifying sugar content, and the microbial growth can also be prevented by the help of sugar addition. In addition its sweetness and humectancy properties, sucrose is also a supporter on the gelation of pectin and starch based jellies (Mathlouthi & Reiser, 1995).

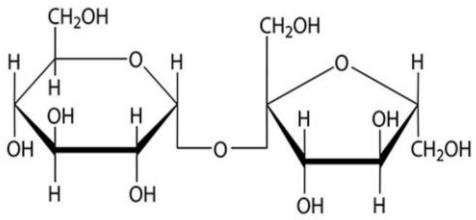


Figure 1. 3 Chemical structure of sucrose (“Sucrose,” 2016)

1.2.1.2. Corn Syrup

Corn syrup is made from cornstarch, and also called as glucose syrup. The caloric value of glucose syrup on dry basis is 4 cal/g (Labropoulos & Anestis, 2012) and the molecular weight of corn syrup is around 198.171 g/mol and it is a weak reducing agent (PubChem, n.d.).

Glucose syrup can be obtained from different sources such as, wheat, rice and potatoes starch (Pomeranz, 1985). It can be produced by acid hydrolysis or enzymatic hydrolysis of starch slurry and the end products of different hydrolyses are different including glucose, maltose etc. The enzyme, which is used in the first step of production, is α-amylase and the products are oligosaccharides. γ-amylase is used afterwards to break down oligosaccharides to glucose. Finally, D-xylose isomerase is added to convert glucose to fructose (Labropoulos & Anestis, 2012) if high fructose corn syrup (HFCS) is desired to be produced. . This hydrolysis is significant since the level of hydrolysis determines the sweetness and viscosity of the syrup. The efficiency of the hydrolysis is usually quantified by Dextrose Equivalence (DE) (Pomeranz, 1985). The main DE levels present in the market for glucose syrup are 42 DE and 63 DE. Functional properties (viscosity, nutritive value, etc.) and also sweetness values depend on DE. As an example; sweetness value of 42 DE glucose syrup is 50, whereas; sweetness of 63 DE glucose syrup is 70 (Labropoulos & Anestis, 2012). Viscosities of low DE syrups are also higher, which affects the texture and taste of the product (bland taste). Glucose syrup is commonly used in food industry (confections, soft drinks) not because it's functional properties but also the high production yield, being cheaper compared to sucrose and also the sweetness (Labropoulos & Anestis, 2012).

1.2.1.3. Maltitol

Maltitol is a non-reducing sugar alcohol and is not commonly found in the nature. Sweetness of maltitol syrup is 75 compared to sucrose which is 100 and the caloric value of maltitol syrup is 3 cal/g compared to sucrose which is 4 cal/g (Labropoulos & Anestis, 2012). The molecular weight of maltitol is 344.313 g/mol (Schouten et al., 1999).

Maltitol could be obtained throughout enzymatic hydrolysis of starch to obtain high amount of maltose, which is reduced with the help of a catalyst to produce maltitol (Fig. 1.4). After hydrolysis, refining takes place and the product is concentrated. The yield of the hydrolysis is between 50 and 90 %. Maltitol syrup is categorized into three types in general; 50-55%, 72-77%, and 80-90%. Pure maltitol has a powder like white crystalline structure. It is perfectly heat stable up to 200 °C, and as being non-reduced is not involved Maillard reactions. Solubility of maltitol is 160 g /100 g water at 20 °C. With increasing temperature, the solubility of maltitol increases.

Maltitol can also lower the water activity (a_w) of a food product. The products containing maltitol syrup has longer shelf life (due to microbial activity point

of view) than the other sugar alcohols, such as sorbitol or glycerol. This is an advantage of maltitol syrup to be used in confectionary gums and jellies. Furthermore, the gums and jellies, consisting of 73-77% maltitol syrup, were found to have equal sensorial characteristics as the ones that are made by sucrose or glucose (Wilson, 2007).

Maltitol may be used in the formulation of gelatin gums, hard-boiled candies, pectin jellies. In gelatin based gums additional maltitol syrup improves the power of gelatin. For hard-boiled candies maltitol may be used up to 80%, and improves transparency and makes product favorable (Wilson, 2007). The other advantages of maltitol in confectionery products are that it is not cariogenic and since not

metabolized as does glucose it is suitable for diabetics (Wilson, 2007). According to Turkish Food Codex the usage limitation is *quantum satis* (QS) for low caloric candies ("Turkish Food Codex Legislation," n.d.). Maltitol has GRAS status accepted by Food and Drug Administration (FDA) as filling nutritive sweetener, stabilizer, and thickener. In soft candies, the level up to 85% maltitol may be used ("Calorie Control Council," n.d.).

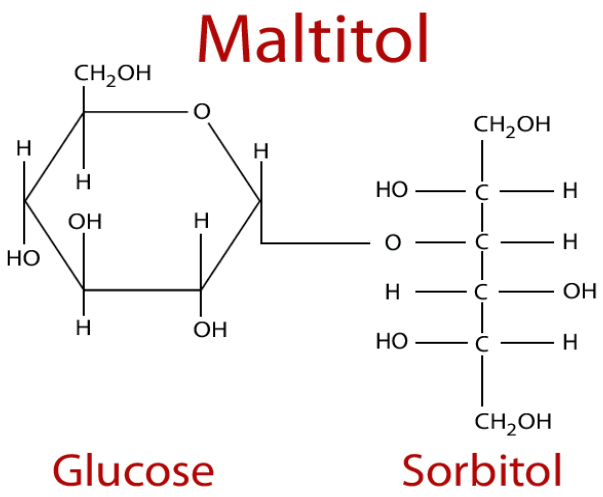


Figure 1. 4 Chemical structure of maltitol

1.2.1.4. Isomalt

Isomalt is a sugar alcohol and it has a white, odorless, crystalline structure. Isomalt is composed of two molecules are namely, 6-O- -D-glucopyranosyl-D-sorbitol (1,6-GPS) and 1-O- -D-glucopyranosyl-D-mannitol (1,1-GPM) and it is obtained from sucrose by enzymatic alteration of sucrose and then hydrogenated with hydrogen atoms (Figure 1.5).

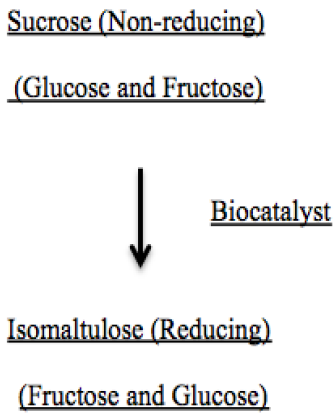


Figure 1. 5 The enzymatic alteration of sucrose to isomaltulose

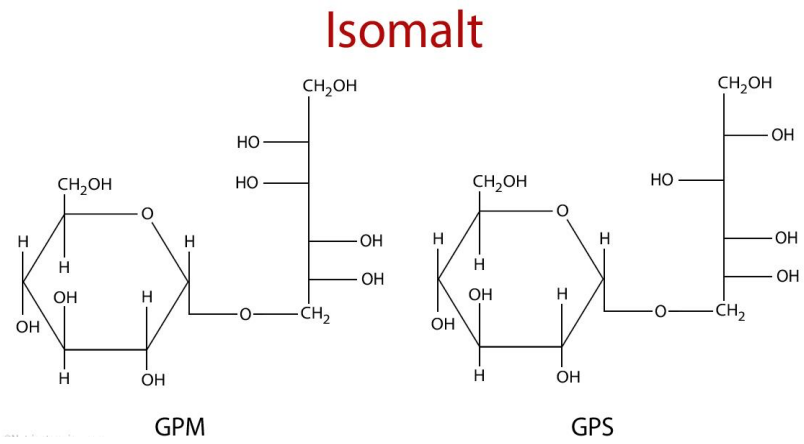


Figure 1. 6 Hydrogenation step from isomaltulose to isomalt structure

The MW of isomalt is 344.313 g/mol ("Isomalt | - PubChem," n.d.). Isomalt gives 2.1 cal/g calorie the sweetness of isomalt is 50 when sucrose is called 100 (Labropoulos & Anestis, 2012). The solubility of isomalt is 24 g in 100 g solution at 20 °C and it increases when the solution temperature increases (Wilson, 2007). Isomalt has high heat stability, and that is the reason to select this sweetener for

use in heat treatment containing processes such as baking and/or boiling. In addition, isomalt is non-reducing sugar thus; it does not participate in any browning reaction (Wilson, 2007).

Isomalt is very stable for the absorption of water and for isomalt-based products; texture is not sticky, due to the low moisture absorption. Also, it can be used widely in food industry and it is accepted by the consumers since it has no different taste profile than sucrose (Wilson, 2007). However, isomalt should be combined with the other sweeteners for the desired sweetness (Labropoulos & Anestis, 2012). Furthermore, isomalt has low glycemic index, it is not cariogenic, and it is suitable for the diabetics (Wilson, 2007). In Turkish Food Codex, the limitation is *quantum satis* for confectionery ("Turkish Food Codex Legislation," n.d.). In US Food and Drug Administration (FDA) regulation, isomalt has been accepted for filling in U.S. and called GRAS (Generally Recognized as Safe) ("Calorie Control Council," n.d.).

1.2.1.5. Stevia

Stevia is a natural sweetener and it is extracted from the leaves of a plant named as; *Stevia rebaudiana*. The major components are stevioside and rebaudioside A (Labropoulos & Anestis, 2012). 'The mixture of purified Stevia sweeteners is called steviol glycosides' (Wilson, 2007). The pure stevioside have sweetness of 300 times greater than sucrose (Labropoulos & Anestis, 2012). Steviol glycosides are called as non-nutritive sweeteners ("US Food and Drug Administration (FDA)," n.d.). The chemical structure of stevia is shown in the Figure 1.6 ("IPCS INCHEM," n.d.).

Even if, stevia has a sweet taste, it has a little bitter and astringent aftertaste (Labropoulos & Anestis, 2012). The use in combination with the other sweeteners is a good solution to overcome this aftertaste. Stevia has white crystalline

structure, like isomalt and pure maltitol. Depending on the temperature, solubility of stevia could change between 30 g/100g and 80 g /100 g water (Wilson, 2007). The MW of steviol is 318.457 g/mol and rebaudioside A is 967.021 g/mol ("Rebaudioside A - PubChem," n.d., "Steviol - PubChem," n.d.).

Pure stevioside is heat stable up to 100 °C for 1 hour in alkali foods and 60 °C in acidic foods. Pure stevioside is also very stable in the pH range of 3- 9. Owing to these characteristics, stevia is an appropriate sweetener for confectionery and ice cream production. Stevia is non-carcinogenic, non-caloric material which supports good dental health and is also good for diabetics (Wilson, 2007).

According to Turkish Food Regulations only 200 mg/kg steviol glycosides can be used in low calorie foods ("Turkish Food Codex Legislation," n.d.). Steviol glucosides with high rebaudioside are stated as GRAS in U.S. as a tabletop sweetener; moreover, rebaudioside A is also called as GRAS in U.S. with the purpose of sweetener and may be used in foods and beverages ("Calorie Control Council," n.d.)

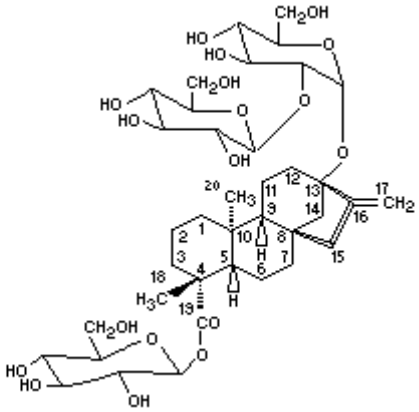


Figure 1. 7 Chemical structure of stevia

1.2.2. Gelling Agents

1.2.2.1. Pectin

Pectin is a polysaccharide from plant sources and it is commercially obtained from citrus (orange peel and lemons) and pomaceous (apple) fruits by using acid (hydrochloric) at pH 2.0 and is mainly used in jam, confectionery and candy production (Labropoulos & Anestis, 2012; Lees & Jackson, E., 1973). The difference between citrus pectin and apple pectin is the color of the product. Citrus pectin has a more brown color than apple pectin and this limits its use in confectionery (Lees & Jackson, E., 1973).

In gel formation, the amount of pectin, the solution temperature, the pH of the solution and the sugar concentration are significant. Low pH (up to 3.2) provides firm gels however, below this pH the desired firmness cannot be formed since carboxylic acids groups on the pectin back bone needs to be protonated to provide crosslinking through H-bonding. The gel strength of pectin is described as the sugar amount (gram) that produces a standard gel texture with one-gram pectin. In candy production, the gel strength of pectin is 150 (Lees & Jackson, E., 1973).

1.2.2.2. Starch

Starch is the reserve tissue of the many types of plants. In human diet, starch provides 70-80% of carbohydrates (Labropoulos & Anestis, 2012). Starch has white, odorless, and powder structure and it is produced from many sources such as, potato, rice and corn whose physical and chemical properties differs from each other (Lees & Jackson, E., 1973).

In sugar confectionery, mainly corn starch is used as a gelation agent, and coating agent due to its reliability, better textural properties and production efficiency (Lees & Jackson, E., 1973).

Starch has two components, namely, amylose (linear-chain) and amylopectin (branched-chain) (Labropoulos & Anestis, 2012). The concentrations of these vary according to the plant source (Lees & Jackson, E., 1973). Yet, starch includes 20-30% amylose and 70-80% amylopectin, in general (Labropoulos & Anestis, 2012).

When a starch solution is heated and temperature reaches to a certain point, starch gelatinization, which is specific for the type of the starch, occurs.

The gelatinization temperature differs according to the source of the starch and this gelatinization establishes enhancement of viscosity and solubilization (Labropoulos & Anestis, 2012). Starch is insoluble in cold water yet the viscosity increases with the increasing temperature (Labropoulos & Anestis, 2012).

The native starch has some limitations in food industry, such as, retrogradation (Ashogbon & Akintayo, 2014). In order overcome these drawbacks, the starch is modified in diverse forms such as, thin boiling starch and oxidized starch which are mostly used in confectionery (Labropoulos & Anestis, 2012; Lees & Jackson, E., 1973). A thin boiling starch has a better firmness than native starch and oxidized starch has a better stability than unmodified starch (Lees & Jackson, E., 1973). As an example, for Turkish delight production modified starch is used mostly.

1.2.2.3. Gelatin (Bovine)

Gelatin is composed of 85-92% proteins and the remaining are minerals, salts, and water. Collagen, in animals (from cattle hide, pigskin) and human tissues, is partially hydrolyzed to obtain gelatin (Schrieber & Gareis, 2007). Moreover,

gelatin includes all essential amino acids except tryptophan. It is known that, 10 g of gelatin has the same amount of glycine as 160 g meat (Schrieber & Gareis, 2007).

There are two types of gelatin with respect to the different isoelectric points: acid-conditioned type A and alkaline-conditioned high-Bloom type B (Schrieber & Gareis, 2007).

Gel strength (gelling power) is the most crucial parameter to describe gelatin, which is determined by Bloom test. Bloom test measures gel firmness of 6.67% gelatin which is aged for 17h at 10 °C as a function of time and the absolute value is shown as grams (Schrieber & Gareis, 2007). Bloom values have the range of 50-300 and owing to this, three types of gelatin arise that are namely, high-Bloom (200-300), medium-Bloom (100-200) and low-Bloom (50-100). High-Bloom gelatin has higher melting point, shorter time of gelation, more neutral taste, lighter color, and stronger gel strength. Schematic of the gelation for gelatin mechanism is given in Figure 1.8. In gelatin-based gels the gelation is reversible (thermoreversible) (Schrieber & Gareis, 2007).

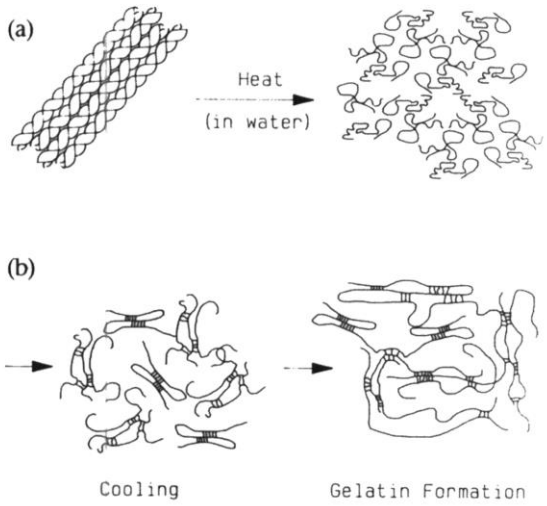


Figure 1. 8 Gelatin formation

Gelatin has many different characteristics such as, texturizing, foaming, stabilization, thickening, water binding and most importantly an excellent gelling agent. This why, gelatin is so popular and used in different various industries including pharmaceutical, paper processing and food. In confectionery, gelatin is mainly used in fruit jellies since due to melting point at the body temperature by absorbing water quickly it results in the release of flavor. The physical properties of gelling agents are shown in Table 1.2 (Schrieber & Gareis, 2007).

Table 1.2 Physical Properties of Gelling Agents Used in Confectionery Products

Gelling Agent	Gel Formation	Thickening Effect	Transparency	pH Stability
Gelatin	High	High	High	Middle
Modified Starch	High	High	Low	Middle
Native Starch	High	High	Low	Low
Pectin	High	Middle	High	Low

1.2.3. Physical Properties of Soft Candies

1.2.3.1. Moisture Content Determination

In food industry, water content should be investigated and determined accurately as it affects many chemical and physical properties of the food materials such as,

the growth of microorganisms (molds, yeasts), stability, texture, organoleptic characteristics and shelf life (Yetim & Kesmen, 2009).

In order to measure the water content of a food product, many techniques can be used such as, drying, refractometer, spectroscopy and chromatography. Drying is the commonly used method in food industry and it can be achieved through many ways such as oven drying (the most common) and vacuum-oven drying. For oven-drying, relatively high temperatures are used (100 °C - 135 °C) (Ergun et al., 2015). Vacuum-oven drying is based on the vacuum that is applied to the sample in a vacuum chamber at relatively lower temperature (∼70 °C) than the traditional oven for a longer time period (4-6 hours) (Yetim & Kesmen, 2009). Vacuum-oven drying has an advantage on the heat sensible food samples but it takes longer time to get the equilibrium moisture content. The calculation for moisture content determination of a sample is shown in the Equation 1.1 (FAO, n.d.).

$$\textbf{Moisture Content } (\textbf{MC})_{\textbf{wet basis}} = \frac{\textbf{Wet Weight} - \textbf{Dry Weight}}{\textbf{Wet Weight}} \times \textbf{100} \quad \text{Equation 1. 1}$$

Furthermore, water content has a relation of the other characteristics of the jellies such as glass transition temperature (T_g) since soft candies are semi-solid food matrixes. T_g is the temperature showing the physicochemical change of the amorphous material from glassy state to the rubbery state and the water content has a direct effect on the glass transition temperature (Gustavo V., Anthony J., Shelly J., & Labuza, 2008). They both have impacts on the stability of the foods (Ergun et al., 2015).

Table 1. 3 Moisture contents of some confectionery products.

Product	Moisture content (%)
Hard Candy	2-5
Marshmallow	12-20
Gummies and Jellies	8-22

1.2.3.2. Water Activity Measurement

The main description of the water activity (a_w) is the ratio of the partial vapor pressure of the food over the partial vapor pressure of the pure water at a given temperature, which is described below;

$$\mathbf{a_w} = \left(\frac{\mathbf{p_w}}{\mathbf{p_w^\circ}}\right)_{\mathbf{T}} \qquad \text{Equation 1. 2}$$

Since water activity is mostly related to the microorganism growth in the food systems, the control of a_w is very significant for the growth of microorganisms resulted as end of the shelf life of the product (Ergun et al., 2015). Water activities of some confectionery products are shown in Table 1.4 (Ergun et al., 2015).

Table 1. 4 Water activities of some confectionery products.

Product	**a_w**
Hard Candy	0.25-0.40
Marshmallow	0.60-0.75
Gummies and Jellies	0.50-0.75

1.2.3.3. PH Measurements

pH is the negative logarithm of the H^+ ions concentration of the aqueous solution. Since fruit flavored confectionery products require to maintain the similar acidity of the fruits to have a closer taste to its original taste, some acidic components should be added to the solution. Moreover, when there is a hydrocolloid in the product, pH also affects the stability and gelling ability of the polymer (Edwards, W., 2000). This is usually provided through citric acid addition. Especially for crosslinking in the gel matrices, pH has a significant effect. For gelatin based soft candy production, pH is usually set around 4.4 (Lees & Jackson, E., 1973).

1.2.3.4. Total Soluble Solid Determination

Total soluble solid content (TSSC) determines the soluble ingredients of a solution. A sufficient and fast determination method during production of jellies is to use refractometer (Lees & Jackson, E., 1973). A refractometer uses different refractive index of the materials and it helps to understand the concentration and the total soluble solid amount of the solution or as a reverse approach, it gives information about the water content (Ergun et al., 2015). In sugar confectionary, refractive index is a significant parameter and in most instruments the result is

obtained as °Brix value, which is represented as the percent sugar, by weight in aqueous solution as seen in Equation 1.3 (Wrolstad, Ronald, 2012).

$$\mathbf{°Brix} = \frac{(\mathbf{weight\ sugar})}{(\mathbf{weight\ sugar + weight\ water})} \times \mathbf{100} \qquad \text{Equation 1. 3}$$

1.2.3.5. Firmness and Springiness Analysis

Firmness and springiness are the significant parameters for consumers' perception since these affects the quality while eating (McKenna, 2003). Firmness is defined as, the force requires to break/bite the product in the mouth. The food material recovers itself after the first bite in the mouth is called as springiness (McKenna, 2003). In confectionery, these features are significant to determine the quality of the products (DeMars and Ziegler, 2001). For instance, when there is a decrease in gelatin or starch concentration in the formulation of candies, the gel strength decreases and due to less interaction between the molecules (Zayas, 1997).

1.2.3.6. Color of Candies

As color of a product is closely associated with consumer perception, color measurement is very significant for food industry. There are different methods to determine the color of a sample such as spectrophotometers and colorimeters. Spectrophotometer quantifies the light, which is absorbed or reflected from the sample with respect to a standard material ("Colorimeter vs. Spectrophotometer - HunterLab," n.d.). A colorimeter is a very rapid measurement, which is designed like human-eye by using the sensation of psycho-physical point. The spectrophotometric and colorimetric methods are both accurate. However, a colorimeter is cheaper than a spectrophotometer and spectrophotometer is suitable

more for liquid samples (Ege University, n.d.). CIE L^* a^* b^* color spaces or Hunter lab color system are the ones used commonly for quantifying the color of a product (Sahin & Sumnu, 2006). In CIE L^* a^* b^* color spaces system, there are three axis. These axis give the information about the color coordinates and is represented by the letters L^*, a^*, b^*. L^* indicates the lightness and has the range between 0-100 and L^* is always positive. a^* and b^* letters indicate the main colors, red-green and yellow-blue, respectively. A positive a^* value indicates redness ($+a^*$), the negative a^* indicates greenness ($-a^*$); Positive and negative b^* values are indicators of blue ($-b^*$) and yellow ($+b^*$), respectively (Sahin & Sumnu, 2006). For the Hunter lab color system, L, a, b is equivalent to CIE L^* a^* b^* color spaces system. CIE L^* a^* b^* color spaces system is more widely used than Hunter L^* a^* b^* color system (Hunter Lab, 1976).

1.2.3.7. Differential Scanning Calorimeter (DSC) Analysis

Differential Scanning Calorimeter (DSC) is used to understand the thermal transitions of a material by analyzing the melting point (T_m), glass transition (T_g), the crystallization temperatures, and the degree of crystallinity. Glass transition temperature (T_g) is the physical change of the material from glassy state (hard, brittle) to rubbery state (soft, rubbery). The degree of crystallinity represents the fractional amount of polymer that is crystalline.

A DSC experiment results in a curve that relates heat flow rate versus temperature and is known as a thermogram. For sampling, hermetically sealed aluminum pans are used. In confectionery, there is a relation between T_g, moisture content, and molecular weight of the sweetener. T_g decreases when moisture content increases. If the molecular weight of sugar is high, T_g is usually higher (Ergun et al., 2015). T_gs of some confectionery materials is given in Table 1.5. A representative DSC thermogram is shown in figure 1.9 (Tau & Gunasekaran, 2016).

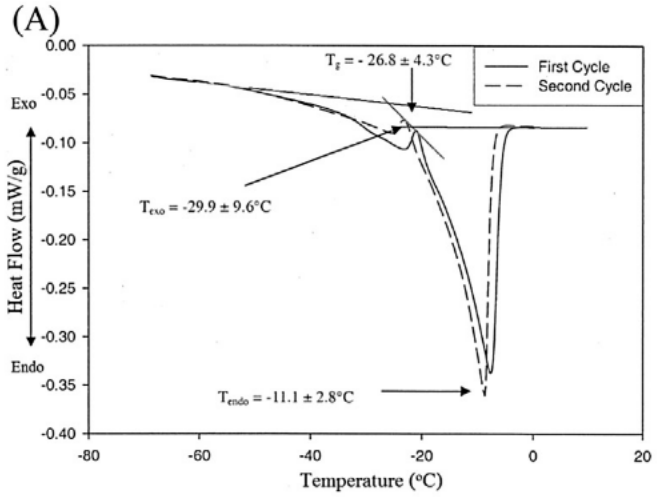


Figure 1. 9 A representative DSC thermogram (Tau & Gunasekaran, 2016)

Table 1. 5 Tg of the some confectionery materials (Hartel, Ergun, & Vogel, n.d.; Lemus-Mondaca, Ah-Hen, & Vega-Galvez, 2015; Raudonus, Bernard, Janûen, Kowalczyk, & Carle, n.d.; Siniti, Jabrane, & Le Âtoffe Â, n.d.)

Material	**Tg (ºC)**
Sucrose	62 to 70
Corn Syrup (42 DE)	79
Citric Acid	6
Water	-139.2
Isomalt	63.6
Maltitol	39
Stevia*	73.86

*Data belongs to freeze-dried stevia

1.2.3.8. Nuclear Magnetic Resonance (NMR) Analysis

Nuclear magnetic resonance (NMR) is a nondestructive analytical method that can be used for characterizing food materials. This technique has a similar working principle to magnetic resonance imaging (MRI). MRI is a three dimensional technique, which is usually used in medical science and uses gradients to create an image (Hashemi, William, & Christopher, 2011). The uniformity of a sample could also be assessed using MRI. On the other hand, in NMR Relaxometry spatial encoding is not required to acquire a signal and the signal comes from the whole sample.

In food industry, low field NMR Relaxometry can be applicable in dairy products, emulsions, baking products etc. in order to understand water content, water distribution and structural changes (Kirtil & Oztop, 2015b). Nuclear Magnetic Resonance (NMR) Relaxometry has been a great tool to understand water distribution and the mobility of protons in gel systems. NMR relaxation time distributions give information about the proton pools present in the samples and these are usually related with the microstructure of the products (Emrah Kirtil, Cikrikci, McCarthy, & Oztop, 2017; Emrah Kirtil & Oztop, 2015a; Baris Ozel, Cikrikci, Aydin, & Oztop, 2017; Baris Ozel, Dag, Kilercioglu, Sumnu, & Oztop, 2017a). With the advancements on benchtop NMR instruments, conducting relaxation measurements is now affordable and very easy to implement (E. Kirtil, Cikrikci, McCarthy, & Oztop, 2017). In this study, T_1 and T_2 relaxation times obtained at different magnetic field strengths were measured to characterize soft candies prepared with different sweeteners.

The logic behind NMR is the magnetization transfer. The magnetization depends on the magnets and there are many types of magnets namely, ultra-high field (4.0-7.0 Tesla (T)), high field (1.5-3 T), low field NMR (< 1.5 T) (Hashemi et al., 2011). There is a magnetic field generated from the magnet and an a radio frequency pulse (RF pulse) at proper frequency that is used to excite the protons in

the samples precessing at the frequency of the magnetic field strength (Kirtil & Oztop, 2015b). After the RF pulse is turned off, protons in the samples relaxes back to their original state and NMR signal is acquired (Hashemi et al., 2011).

Generated signals from the disturbance are depended on how the signal is applied. Thus, different signal types can be obtained by using this technique such as, T_1 and T_2, relaxation times.

1.2.3.8.1. T_1 and T_2 Relaxation Times

T_1 is called as longitudinal relaxation time or spin-lattice relaxation time. T_1 relaxation time denotes the growth of magnetization in z-axis. Inversion/Saturation recovery sequences are used to measure T_1 relaxation times.

In addition to T_1, T_2 relaxation is called spin-spin relaxation and represents the connatural interactions between protons. Carr-Purcell-Meiboom-Gill (T_2 CPMG) is a popular pulse sequence, which is used for the measurement of T_2. Spin-spin relaxation time is a decaying curve of magnetization in x-y plane. While longitudinal magnetization vector recovers itself, the transverse vector will decay at an independent rate (Hashemi et al., 2011). The typical T_1 and T_2 relaxation plots and their functions are shown in Figure 1.10 and 1.11 (Kirtil & Oztop, 2015b).

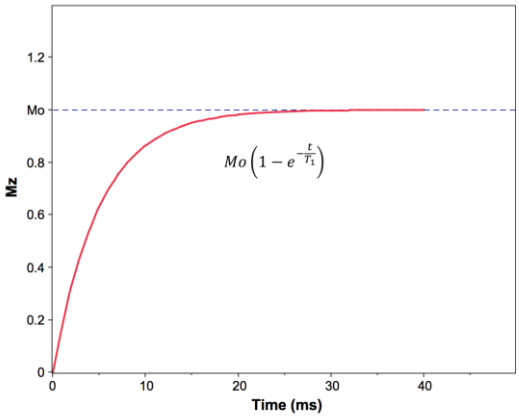


Figure 1. 10 A representative T_1 curve obtained through Saturation Recovery Sequence

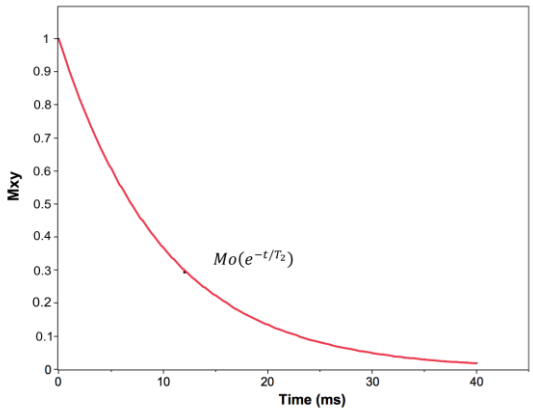


Figure 1. 11 A representative T_2 curve obtained through CPMG sequence

1.3. Aim of the Study

The aim of this study is to formulate and characterize the physical chemical properties of low calorie gelatin based soft candy by using different sweetener types. Maltitol, isomalt and stevia are the sweeteners substituted with sucrose at different ratios. Physical properties of the candies through; water activity, color, texture, moisture content and differential scanning calorimeter analysis. Moreover, high and low-resolution time domain (TD) NMR are the new unique other tools used for characterization and to understand the influence of the contribution of different ingredients on the final texture of the candies. Use of Time Domain High and Low Resolution NMR made this study unique, as there is not such a study in the literature that complemented physical characterization of soft sugar based candies using these techniques.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

In this study for soft candy production, bovine gelatin, sucrose, corn syrup, maltitol (^{o}Brix: 77) (Yılmaz Kimya Inş. San. Tic. A.Ş., Istanbul, Turkey), isomalt (Smart Kimya Tic. And Dan. Ltd. Şti, Izmir, Turkey) and stevia (Fibrelle, Turkey) were used. Bovine gelatin (Blooming Index: 250) and corn syrup (DE 43-47, ^{o}Brix: 82-84) were kindly provided by Kervan Gıda Sanayi ve Ticaret A.Ş. (Istanbul, Turkey). Sucrose (Bal Kupu, Turkey) was purchased from a local market.

2.2 Methods

2.2.1 Production of Soft Candy

For lab scale candy production, bovine gelatin concentration was chosen as 8%. Lower concentrations were also tested and after preliminary examinations, 8% which was also the concentration used by Marfill et al. (Marfil, Anhe, & Telis, 2012) was decided to be used for all formulations. The total solid sugar concentration was set to 40%. Keeping this fixed concentration substitution with different sweeteners were done at different ratios 30:70; 50:50; 70:30 while the first ratio denotes sucrose concentration and the other being the sweetener. Since, using maltitol, isomalt and stevia as the sole sugar did not not give the desired texture of the sugar candies as does 100% sucrose these formulations were not

tested. Another sugar ingredient in the formulation was corn syrup (CS). As the name implies since it was in liquid form, it was not considered under the total solid sugar concentration. (CS) concentration was chosen as 20% and overall sugar content was fixed to 60% considering the formulations of the commercial products. Trace amounts of citric acid solution were added to obtain the desired pH (4.4±0.1) of the soft candies. The rest of the solution was distilled water. An experimental design table that shows the formulations is given at the end of the section in Table 2.1.

To prepare candies, first gelatin-water (1:2) and sugar-water solutions were prepared separately. It was crucial to keep the initial temperature of the gelatin-water solution between 80-90 °C (Schrieber & Gareis, 2007). Then, manual stirring and magnetic stirring were applied for both solutions, respectively. Magnetic stirrer was set to 75°C at 220 rpm. Solution temperature was kept between 53-59 °C. Afterwards, the two solutions were mixed; they were kept for 30 minutes at 65°C in a water bath. Additional stirring was done for sugar-water solution, if mixture had no sugar crystals inside. Then, they were mixed to obtain the main gelling solution. The main solution was mixed further at the magnetic stirrer at 75 °C for 5-10 minutes at 220 rpm. Finally, the main solution was poured into rectangular silicon molds of 3.7*3.7*1.8 (w*d*h) cm dimensions and stored at 25 °C for 24 hours until gelatin sets. Flow chart for candy production and initial photos of the candies are given in Figure 2.1 and 2.2

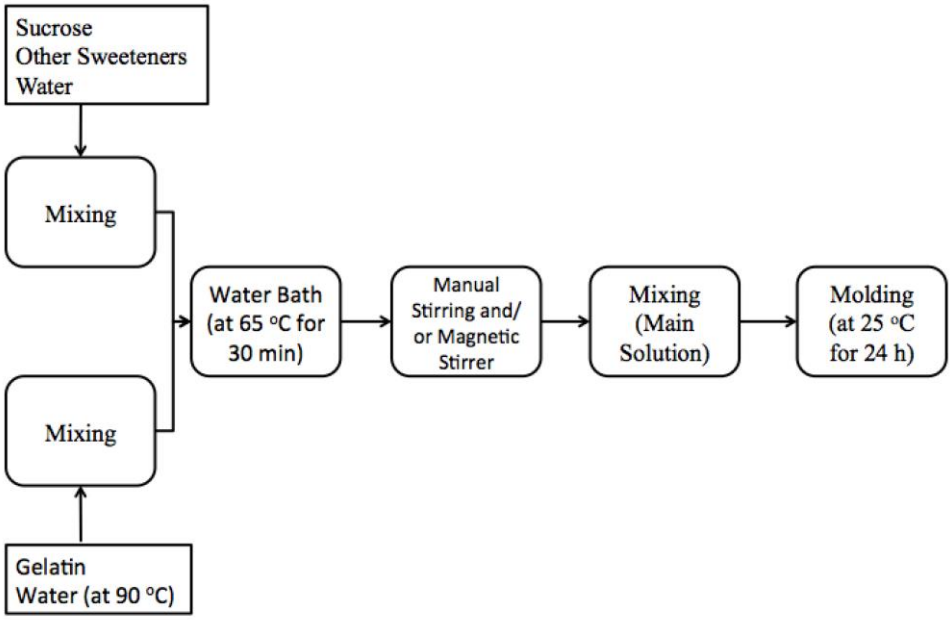


Figure 2. 1 Flow chart of soft candy production

Figure 2. 2 Soft candies right after molding

2.2.2 Moisture Content Determination

In this experiment, vacuum oven drying, which is a less destructive method was used (Mathlouthi, 2001). Initially, the jellies were weighted and a desiccator was used to transfer the samples after vacuum oven. The vacuum oven was set to 70^{o}C and the pressure was dropped down to -0. 1MPa. The jellies were kept at these conditions for 8h (Sessler, Weiss, & Vodovotz, 2013). 8h was confirmed with preliminary trials also. The final masses of the jellies were recorded at the end of 8 hours. Experiments were conducted as triplicates.

2.2.3 Water Activity Analysis

Water activity (a_w) of the soft candies was measured by a water activity meter (AquaLab, Dew Point Water Activity Meter 4TE, Pullman, USA). Small pieces of jellies were put in a sample chamber and waited until the equilibrium is attained. Experiments were conducted at 25^{o}C as triplicates.

2.2.4 pH Analysis

In confectionery products, pH is important for the stability of the gel during cooking and before molding (Lees & Jackson, E., 1973). According to Lees and Jackson (1973), the pH of the solution should be between 4.4 and 5.0 for gelatin-based products so in this study. pHs lower than 6 can contribute to an promote an increase in reducing sugar which could result in Maillard reaction (Ergun et al., 2015) and consequently flavor change.

pH of the solutions before molding were measured using HANNA FC with the 2022/HALO^{TM} solid pH probe (HANNA Instruments, Romania).This probe is the one that is commonly used for semi solid food materials.

pH value of all the solutions were kept between 4.3 and 4.5 and adjusted through citric acid. Experiments were conducted as triplicates.

2.2.5 Total Soluble Solid Determination

To analyze the total soluble solid content of the gummy jelly solutions HANNA HI 96801 Refractometer (HANNA Instruments, Romania) was used. The working range of the instrument is 0-85 ^{o}Brix. A small aliquot of warm main solution was poured drop by drop to the instrument reservoir. Then, ^{o}Brix values were recorded. To calibrate the instrument distilled water at room temperature was used.

2.2.6 Firmness and Springiness Analysis

TA-XT plus Texture Analyser, (Stable Microsystems, Godalming, UK) was used and the specified classifications for the types of the materials the gummy confectionary class was chosen for conducting the analysis. Firmness and springiness values (which is called elasticity) were obtained while using this section. Firmness value was given as the peak force and springiness value was calculated as the Ratio F 1:2 in the software which was shown in Figure 2.3.

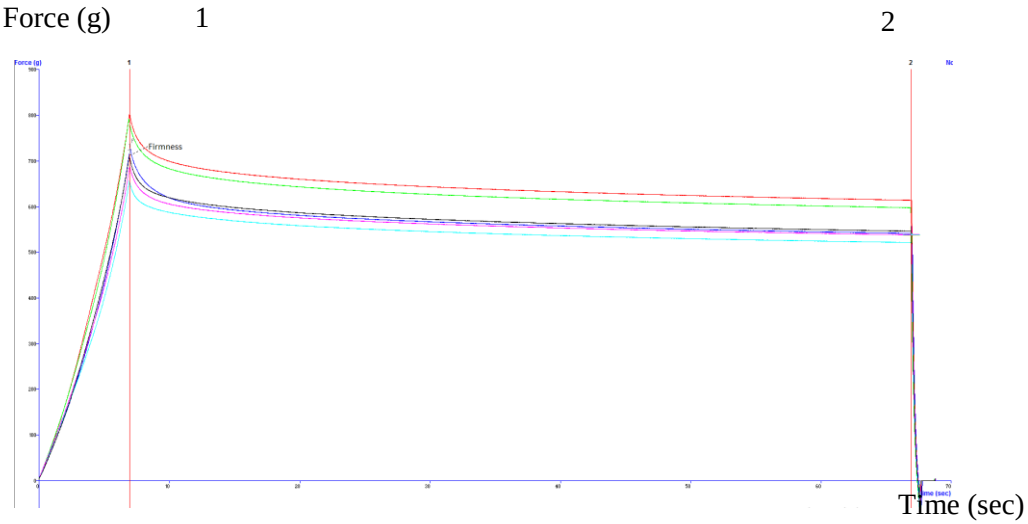


Figure 2. 3 A representative TPA result from the instrument

The detailed test settings were:

- Test mode: Compression. The pre-test, test and post-test speeds were set at 1.0 mm/s.
- Target mode: Strain; Strain: 50% Holding time: 60 sec; Time: 10.0 s;
- Trigger type: Auto (force); Trigger force: 5.0 g; Advanced Options: Off;

The load cell (2 kg) was calibrated with a 2 kg weight. Probe height calibration was done by setting the return distance as 10mm, return speed as 10mm/sec and contact force as 1g. The probe diameter was 1.2 cm. The experiments were conducted as triplicates.

2.2.7 Differential Scanning Calorimeter (DSC) Experiments for Glass Transition Measurements

A single furnace DSC (DSC 4000, Perkin Elmer, MA, USA) was used to obtain the T_g values of the different formulated jellies. The working temperature range of the instrument is between -100 °C and +450 °C ("PerkinElmer," 2017). The sample (8-20 mg) was placed into aluminum pans and hermetically sealed immediately before the analysis to avoid moisture loss and an empty pan was used as the reference. The DSC cycle contained 5 steps, heating, holding, cooling, holding and heating step. Holding time was 2 min for all scans. The temperature range was -50 °C – +20 °C and the heating rate were set as 10 °C /min determined wrt to the preliminary experiments and methods followed by other researchers (Tau & Gunasekaran, 2016). This experiment was done in triplicates.

2.2.8 Color Measurements

The instrument Color Reader CR-10 (Konica Minolta Optics, Japan) was used for color measurements and L^*, a^*, b^* data were recorded for this experiment and CIE L^* a^* b^* color spaces system were chosen. For the reference, compact white chalk powder was used. This experiment was done in triplicates.

2.2.9 NMR Relaxation Measurements

NMR Relaxometry experiments were performed at 2 different field strengths (0.52 Tesla/22.34 MHz and 11.7 Tesla/500 MHz). Low field experiments were conducted at METU, Food Engineering Department whereas high field experiments were performed at *Institute of Molecular Physics, Poznan/Poland* using a high resolution NMR instrument.

2.2.9.1 Low Field NMR Relaxometry Experiments

Low field 0.5 T NMR spectrometer operating at a *Larmor* frequency of 22.35 MHz equipped with a 10-mm diameter radio frequency coil (SpinCore Inc., Gainsville, FL, USA) was used for the experiments. Inversion recovery and Carr-Purcell-Meiboom-Gill (CPMG) sequences were used to measure relaxation times of T_1 and T_2 respectively. For T_2 CPMG sequence, 2 ms echo time, 150 echoes, 128 scans and 0.5 s repetition delay were used. For the T_1 measurements, the number of points was 512, repetition delay was 1 s, and number of scans was 16. MATLAB was used to analyze T_1 data. Discrete component analysis was conducted using XPfit software to explore the components/proton pools in CPMG decay.

2.2.9.2 High Field NMR Relaxometry Experiments

Ultra-high field Bruker AVANCE spectrometer, 500 MHz (^{1}H) was used in this experiment. T_1 inversion recovery experiment, T_2 CPMG experiments were conducted for 100_S, 30S_70M, 30S_70I, 30S_70St samples at 25, 35, 45, 55 and 65^{o}C to explore the effect of temperature on the soft candies. Origin, MATLAB and XPFit software were used to analyze the data.

2.2.10 Statistical Analysis

All experiments were conducted in at least triplicates. Data were analyzed by using Minitab 16 (Minitab Inc., Penn State, USA). Analysis of Variance (ANOVA) was conducted at 95% confidence interval. All statistical analysis results were given in Appendix A.

2.3 Experimental Design

In this study, maltitol, isomalt, and stevia were combined with sucrose at different ratios. The sugar types were mainly chosen according to their caloric value to decrease the sugar content of the soft candies. The caloric value of the formulations are calculated according to their contribution and their caloric value/gram which can be seen in Table 2.1 at the end of the section and the oil – fat % which is coming from gelatin is assumed as zero while doing the calculations.

In order to determine the best gelatin concentration different concentrations were also tested. Preliminary results showed that 8% was gelling concentration for the selected production method and that was also consistent with the literature. Candies containing only one sweetener ware not formulated as the preliminary trials showed that the candies with 100% sweetener experienced significant problems in terms texture. In the light of these results, Table 2.2 shows the experimental design of the study and sample numbering.

Table 2. 1 The caloric values of the soft candy formulations

Sweetener Type	Gelatin (cal)	Corn Syrup (cal)	Sucrose (cal)	Sweeteners (cal)	Total (cal)
100S	32	80	160	0	272
30S_70I	32	80	48	58.8	218.8
30S_70M	32	80	48	84	244
30S_70ST	32	80	48	0	160
50S_50I	32	80	80	42	234
50S_50M	32	80	80	60	252
50S_50ST	32	80	80	0	192
70S_30I	32	80	112	25.2	249.2
70S_30M	32	80	112	36	260
70S_30ST	32	80	112	0	224

Table 2. 2 Experimental design of the study

Sample Name	Sucrose (%)	Isomalt (%)	Maltitol (%)	Stevia (%)
100S	100	-	-	-
30S_70I	30	70	-	-
30S_70M	30	-	70	-
30S_70ST	30	-	-	70
50S_50I	50	50	-	-
50S_50M	50	-	50	-
50S_50ST	50	-	-	50
70S_30I	70	30	-	-
70S_30M	70	-	30	-
70S_30ST	70	-	-	30

S: Sucrose, M: Maltitol, I: Isomalt, ST: Stevia; 100, 70,50,30 represents percentages ratios of overall sugar concentration which is 40%.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Total Soluble Solid Content (TSSC)

Brix value is the representative value of the total soluble solids in the solution. ^{o}Brix value is a fast and sufficient parameter that is used to understand total soluble solid content in confectionery products (Lees & Jackson, E., 1973).

In Table A.1, it is seen that sweetener type and concentration have significant effects on the results separately ($p<0.05$). Samples containing maltitol were significantly different from the other samples wrt total soluble solid content regardless of the sweetener concentration; this might be because of the ingredient of maltitol, which is used in syrup form (^{o}Brix value of maltitol syrup is 77.4) in the experiments ($p<0.05$) (Table A.1).

According to Table A.1, 0%, 30%, 50% are similar whereas 70% had higher values than these samples. In Figure 3.1 it can be seen that 30S_70I and 30S_70St samples were similar to 100S samples. Thus, despite the lower solubility of these sugars wrt to sucrose, they can substitute sucrose even at higher concentrations with the help of relatively high temperatures (65 C^{o}).

As seen in Table A.1 maltitol concentration difference might be due to the form of the sweetener used ($p<0.05$). Since, other sweeteners were used as powdered while maltitol was used as sugar. Syrup form contains some water inside and TSSC of maltitol might be scaled down compared to the others. Concentration results also promote this hypothesis since an increase of the maltitol concentration decreases

°Brix values of the soft candies mostly. Moreover, there was a significant interaction between sweetener type and concentration ($p<0.05$) (Table A.1).

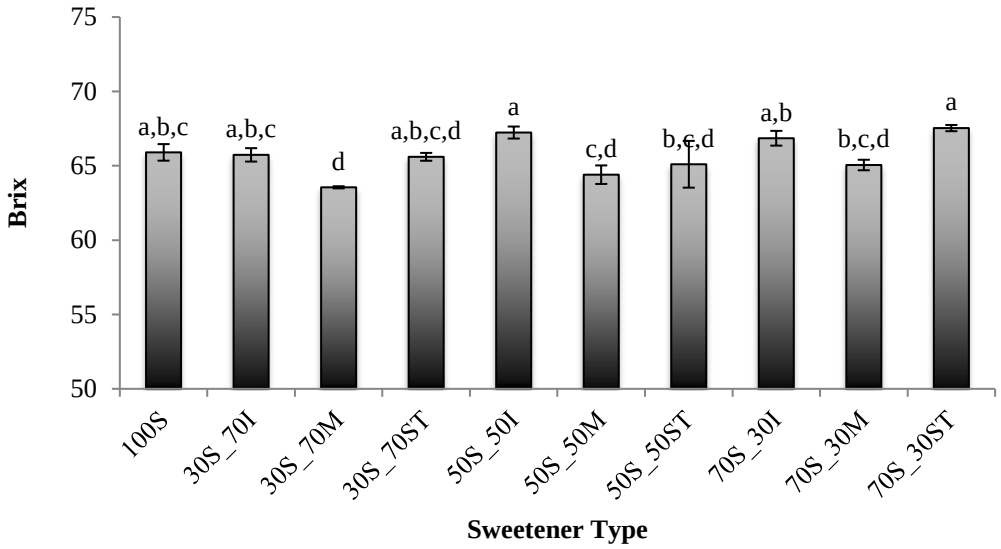


Figure 3. 1 Brix values of the main solutions before molding

Briefly, a typical jelly product should have approximately 65% total soluble solid content (Tau & Gunasekaran, 2016). Thus, Figure 3.1 shows that, 30S_70I, 30S_70ST and 70S_30M samples were similar to 100S samples.

3.2 Moisture Content

Water affects the stability of textural properties and shelf life of the food products, and also the growth of the microorganisms (Yetim & Kesmen, 2009). In this study, vacuum oven drying method was used to understand moisture content of jellies. In soft candy production, moisture content is usually between 8-22 %. In this study, moisture content of the final products before molding was found to be around ~32 %.

In Table A.2, it is clear that that sweetener type has a significant impact on the results ($p<0.05$). The samples including maltitol have the highest moisture content, which is 35.1, and stevia samples have the lowest mean values around 31.9. In this result maltitol had the highest moisture content results (mean). This can be related to that, for maltitol samples, sugar rich and polymer rich phases with fewer interconnections in the structure (Altay & Gunasekaran, 2013). Maltitol samples might have bigger water molecules that are entrapped in the gel matrix thus; moisture content results might be higher too.

Sweetener concentration was also found to be significant ($p<0.05$) (Table A.2). Increasing sweetener concentration resulted an increase on the moisture content results. If sweetener concentration increases, water holding capacity decreases. The water holding capacity of sucrose might be higher than other sweetener types. Besides, this result may also be related to the solubility of the sweeteners. The solubilities of sweeteners were indicated in the introduction part. Thus, sucrose is very soluble in the solution. (200g/100g water at 20 ^{o}C) which is much higher than other sweeteners.

Moreover, as seen in Table A.2, there was a significant interaction between sweetener type and concentration ($p<0.05$).

In the Figure 3.2, 30S_70M and 50S_50M samples were significantly different than the other formulations, which showed that up to 30 % concentration of maltitol used in the formulation moisture content was similar to the control sample of 100S and after 30% there is a significant increase of the maltitol jellies ($p<0.05$) (Table A.2). The reason for the different moisture content results at different concentration was thought that maltitol used as a syrup form while sucrose, isomalt and stevia were as a powder. Since, in high concentration like 70% there was a sharp increase in MC results (Figure 3.2).

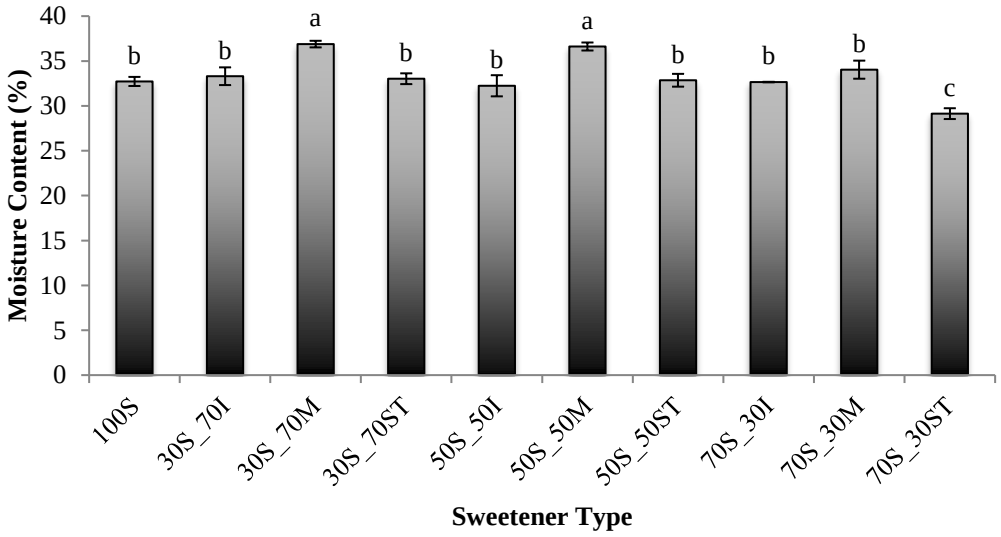


Figure 3. 2 Moisture Content results of the samples after molding 24 hours by using vacuum oven technique

3.3 Water Activity

It is reported that water activity of soft candies should be between 0.5-0.75 (Ergun et al., 2015). Ingredients with high molecular weight and solubility such as proteins, gums, have little effect on decreasing a_w and there is a relation between solubility, molecular weight and a_w (Ergun et al., 2015). Gelatin, as being a protein, had the little effect on a_w and the relation between sugar types between a_w was observed.

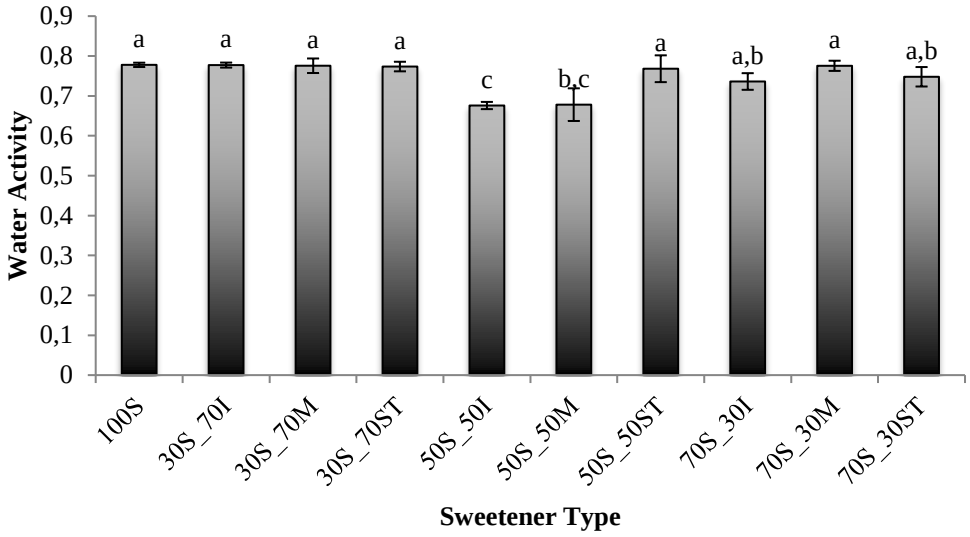


Figure 3. 3 Water Activity Results of the Low Calorie Soft Candies

As seen in Table A.3, sweetener type has a significant impact on the water activity of differently formulated jellies ($p<0.05$). The samples containing stevia have the highest mean values than others whereas; isomalt has the lowest values. Stevia has the highest molecular weight due to the rebaudioside A part compared with other. When the molecular weight is low with high solubility, a decrease in a_w is expected thus, in this study stevia samples had the highest a_w (Ergun et al., 2015).

Moreover, maltitol, isomalt and corn syrup are commonly used humectants (Ergun et al., 2015). Isomalt as including two sugar alcohol moiety could bind more than other sweeteners therefore, a_w of isomalt samples were found to be higher. This also confirms that humectant characteristic of the isomalt could have been higher than maltitol.

In addition sweetener concentration was found to be significant (Table A.3) ($p<0.05$). The water activities of 50% concentrations were significantly different than the ones of 0%, 30 % and 70% concentrations ($p<0.05$). The water activity values of 0%, 30% and 70% concentrations were higher than others, which might

be explained by the interaction between sucrose and sweeteners when the replacement ratio is 1:1.

Despite of 50S_50I and 50S_50M samples, the water activities of all jellies were statistically similar ($p>0.05$) (Figure 3.3). However, moisture content results had statistically different results for all formulations compared to a_w results (Figure 3.2). a_w and moisture content may not be directly related since a_w is thermodynamical phenomenon whereas moisture content is the complete water inside the sample.

Furthermore, sweetener type and concentration had an impact on water activity together according to the Table A.3 significantly ($p<0.05$).

3.4 Color

In confectionery, color is important for consumer perspective. In this study, lightness and yellowness, which are mainly affected by gelatin content, were taken into consideration since there was no coloring agent included to the solutions. Gelatin and sugar types (depending on whether it is reducing or not) gave the color base to products and color results were discussed below. The a^* value of the samples changed between 0.2 and 1.4 which was very low and close to zero thus was not taken into account and in most of the studies in the literature b value was used (Oliver, Blakeney, & Allen, 2009).

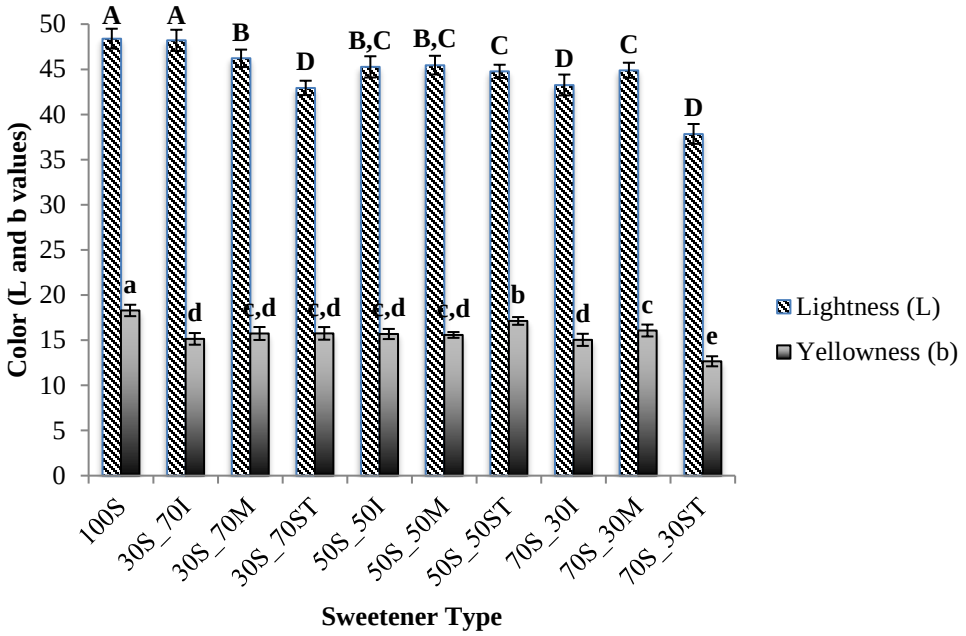


Figure 3. 4 L^* and b^* values of the low calorie soft candies

To begin with, the lightness statistical results showed that sweetener type and concentrations both affect final form of the gelatin-based soft candies significantly ($p<0.05$). There was also a synergistic effect between sweetener type and sweetener concentrations ($p<0.05$) (Table A.4).

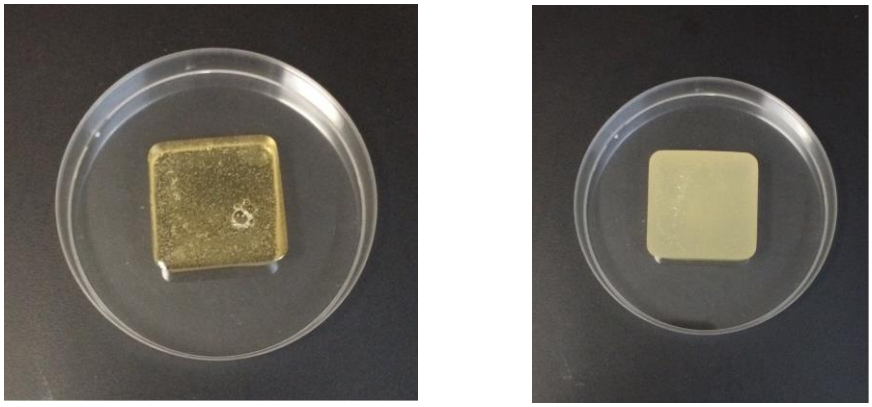
In Table A.4 isomalt and maltitol containing samples had the highest value according to the lightness results. On the contrary, stevia containing samples had the lowest value and these were significantly different ($p<0.05$).

Sweetener concentration showed that 100S samples had the highest L^* value and all concentrations were significantly different ($p<0.05$).

100S and 30S_70I samples behaved similar and they had the highest value of L^* which was shown in Figure 3.4. Results of the statistical analysis showed that, isomalt might be used as a sucrose replacement in that concentration according to the lightness results.

Stevia containing samples had lower lightness value and while doing the experiment they have opaque appearance, too.

Since none of the sweeteners including sucrose were all non-reducing their contribution to Maillard browning was insignificant thus color is not affected from that reaction. The only contribution to Maillard could have resulted from sucrose hydrolysis due to citric acid which would result in reactant formation for Maillard Reaction. However, lightness results did not show such an effect.



a) 100S b) 30S_70St

Figure 3. 5 a) represents 100S sample b) represents 30S_70St sample

Another color parameter was the b* (yellowness) values. In this study, all results were obtained in positive scale. In the Table A.5, it was seen that as; the yellowness of the samples was significantly different with regard to the sweetener type and sweetener concentration and there is also a significant interaction between them ($p<0.05$).

According to the type of the sweetener used, maltitol had the highest yellowness value (mean) than the others ($p<0.05$) (Table A.5). Concentration results indicated that all concentrations were varied from each other, which is shown in Table A.5 ($p<0.05$). The control sample (100S) had the highest mean value was 18.3.

According to the previous study (Martinez-Cervera, Sanz, Salvador, & Fiszman, 2012), polydextrose-sucralose substitution cause an increase in the $a^*$and b^* values of the crumb. From this result they concluded that the reduced sugar muffins had a more orangey color than 50% polydextrose-sucralose sample, which was the

control sample. In this study, the jellies do not attend in Maillard browning reactions due to their chemistry thus an increase in a^* and b^* value was not observed.

3.5 Firmness and Springiness

Firmness and springiness are the two analytic parameters to understand textural properties of the samples. For candy samples the recommended parameters by the instrument calculated these two crucial parameters.

According to the ANOVA results there is a significant difference on these textural parameters between the sweeteners used in the formulations ($p<0.05$). Maltitol samples had the lowest firmness value (mean) and stevia samples had the highest mean value. Maltitol samples did not have the similar firmness value with the control sample (100S) since, the moisture content of the jellies were higher than others. There was also a significant difference according to the sweetener concentrations ($p<0.05$). Moreover, there is a synergistic effect between sweetener type and concentration according to the firmness results ($p<0.05$) (Table A.6).

In this study, firmness value of the 30S_70M and 30S_70I sample was significantly different from 100S samples ($p<0.05$). This might be due to sugar alcohol type, which was used in the study. In literature, to determine the effect of the sugar alcohols type on muffins texture were studied. In that study, sorbitol, maltitol, and isomalt were used. The results of study show that muffins texture strongly depend on the type of sugar alcohol. It was seen that hardness was decreased by replacing sucrose with sorbitol and maltitol and isomalt has no significant impact on hardness value when comparing with sucrose (Martínez-cervera, Salvador, & Sanz, 2014). Thus, Table 3.1 shows that, maltitol containing sample (30S_70M) was significantly decrease the firmness value like in the Martínez- cervera et al (2014)'s study ($p<0.05$). However, isomalt did not show

the similar characteristic to the sucrose sample in fact, there was a significant increase ($p<0.05$). Gelatin provides a gel matrix according to its Bloom value. However, the solubility of isomalt is very low and the firmness value was very high. This can be related to that, isomalt could not dissolve in the solution and could not attain the bond interaction with water. This might be due to the gelatin-isomalt strong network interaction than water isomalt dissolving network.

According to Table A.7, sweetener type resulted in significant difference ($p<0.05$). And all types showed the different springiness value. Maltitol had the highest springiness mean value, which was 89.5, and stevia samples had the lowest springiness value of 72.5. According to the sweetener concentration, there was a significantly decreasing trend while adding ascendantly the different types of sweetener to the sucrose-gelatin-water solution ($p<0.05$). 70% had the springiness value near to the 0% sample. This might be due to the fact that, when the concentration getting higher, the sweetener might have shown their own characteristic in the gel matrix.

According to the study from (Martínez-cervera et al., 2014), they found there was no difference between sucrose and maltitol samples. However in this study, these samples were significantly different this might have been due to the gelatin matrix - sucrose - maltitol interaction difference.

Moreover, in Table 3.1 there was a decrease in springiness value of stevia samples while increase the stevia concentration in the formulation. According to a previous study, Zahn et al (2013) performed an experiment about replacing stevia with sucrose. They add stevia instead of sucrose about the ratio is 1/250. They also found their reference muffin springiness value as 0.74 and they found a decrease in springiness value. According to their study, this was related to the decrease of the strength of bonds in three dimensional crumb network (Zahn, Forker, Krügel, & Rohm, 2013). Thus, in this experiment, the control sample had the springiness

value of 75.55 % and the decrease of the springiness value of stevia might have strong bond interaction in gelatin network.

Table 3. 1 Firmness and springiness results of the low calorie soft candies

Sweetener Type	Firmness (g)*	Springiness (%)*
100S	666.62^{b}±7.84	75.55^{e}±0.85
30S_70I	761.57^{a}±8.07	83.79^{d}±1.38
30S_70M	638.29^{c}±6.00	92.35^{c}±0.62
30S_70ST	621.86^{c}±7.85	68.57^{f}±1.07
50S_50I	489.57^{e}±6.25	$93.80^{b,c}$±0.86
50S_50M	505.19^{e}±2.80	$94.01^{b,c}$±0.80
50S_50ST	674.15^{b}±7.53	69.10^{f}±0.74
70S_30I	576.31^{d}±3.92	$95.29^{a,b}$±1.01
70S_30M	508.48^{e}±8.83	96.25^{a}±0.70
70S_30ST	686.62^{b}±27.10	76.78^{e}±1.78

*Values are mean ± standard deviations for triplicate measurement.

3.6 DSC

DSC is a calorimetric method in order to understand the thermal transition of a sample. Melting point, crystallization temperature, and glass transition temperature determinations can be done by using this method. In this study T_g experiments were done. The results of T_g were generally very low (Table3.2) since a little amount of water resulted as a large decrease in confection (Ergun et al., 2015). The higher the T_g, the sharper and more brittle structure the sample have (Ergun et al., 2015) thus, the lower T_g showed the softness of the jelly samples.

Furthermore, lower T_g can be related with the stability of the sample (Tau & Gunasekaran, 2016). Moreover, according to Tau et al (2016), they found their T_g values for sucrose gel -26.8± 4.3, for sucralose gel -32.4±0.1, for aspartame gel -29.0±3.7. They were concluded these decrease in T_g values with increase in stability of the sample. For this study also, stable jellies were obtained according to T_g results. Furthermore, this result can be related with the gelatin water interaction (the solubility of gelatin) due to the amino acid structure inside the gelatin (Gekko, Li, & Makino, 1992).

In Table A.8 sweetener type did not have an impact on the jellies and the mean values were very close. However, according to Ergun et al (2010). If the molecular weight of sugar is high, T_g is usually higher and in this study, stevia was found to have the highest molecular weight and T_g was also higher than others. Moreover, according to Table 1.5 and Table A.8 and the formulations of the maltitol containing samples had the lowest T_g values, which could be also related to the moisture content. It is known that T_g decreases when moisture content increases (Ergun et al., 2015).

On the other hand, sweetener concentration had an impact on the formulations ($p<0.05$). In overall, 100S, which was the control sample, had the highest T_g values. Thus, sucrose is known as hygroscopic material and moisture content results were also lower than others like T_g results.

As seen in Table 3.2, all sweetener types and concentration resulted in a decrease on the T_g values wrt the 100S sample. (Martínez-cervera et al., 2014) showed that, according to the calorimetric results, maltitol samples were similar to sucrose. In this study, ANOVA results also showed that there was no significant interaction between sweetener type and concentration ($p>0.05$).

Table 3. 2 Tg results of the low calorie soft candies

Sweetener Type	Tg (C)*
100S	-42.15[a]±2.22
30S_70I	-44.63[b]±0.49
30S_70M	-43.81[b]±0.70
30S_70ST	-43.50[b]±1.12
50S_50I	-44.38[b]±0.20
50S_50M	-44.21[b]±0.64
50S_50ST	-44.21[b]±0.88
70S_30I	-43.76[b]±0.20
70S_30M	-44.32[b]±0.75
70S_30ST	-43.57[b]±0.20

*Values are mean ± standard deviations for triplicate measurement.

In Figure 3.2, the moisture content results were also very similar respectively. This might be because of the crystallization of sucrose other than water content of the jellies. The sugar inside the sample limited the crystallization and water might be more mobile inside the samples. Moreover, glucose syrups were known as non-crystalline or amorphous sugars, however; crystals can be formed at elevated temperature in low temperature range (Tau & Gunasekaran, 2016).

3.7 NMR Relaxometery

As stated in Materials and Methods section, NMR Relaxometry experiments were performed at 2 different field strengths (0.52 Tesla/22.34 MHz and 11.7 Tesla/500 MHz). Low field experiments were conducted at METU, Food Engineering Department whereas high field experiments were performed at Institute of Molecular Physics, Poznan/Poland using a high resolution NMR instrument.

For all samples described before low field experiments were conducted and as will be discussed afterwards, only for selected samples high field experiments were performed.

3.7.2 Low Field System Experiments

3.7.2.2 Spin Lattice (T_1) Relaxation Times

LF-NMR Relaxometry is becoming very popular in food researches since it is a non-destructive method. It is possible to obtain microstructural information based on relaxation times. Distribution of water in gels (Baris Ozel, Uguz, Kilercioglu, Grunin, & Oztop, 2016; Oztop, McCarthy, McCarthy, & Rosenberg, 2014; Oztop, Rosenberg, Rosenberg, McCarthy, & McCarthy, 2010; Williams, Oztop, Mccarthy, Mccarthy, & Lo, 2011), polymer water interactions (B. Ozel, Dag, Kilercioglu, Sumnu, & Oztop, 2017; Baris Ozel, Cikrikci, Aydin, & Oztop, 2017) could be explored using relaxation time distributions.

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, 2015b). T_1 of the pure water is very long (~2.5 s) and solids (except crystalline ones) has shorter T_1 relaxation times (Kirtil & Oztop, 2015b).

T_1 experiments conducted at the low field system through inversion recovery (IR) sequence showed that a mono exponential model was sufficient to describe the

relaxation times (Fig 3.5 and, Fig. 3.6). As gelatin is capable of forming a nice rigid gel network, observing a monoexponential behavior was not unreasonable. Results of T_1 relaxation showed that sweetener type and sucrose concentration were significant ($p<0.05$) (Table A.9). Maltitol containing samples had the highest mean values (73.9 ms) and isomalt and stevia samples had similar relaxation times ($p>0.05$). Substitution of sucrose with sweeteners definitely affected the T_1 values. Control samples that contained only sucrose had the lowest relaxation times which might be attributed to hygroscopicity of sucrose (Ergun, Lietha, & Hartel, 2010; Hartel & Shastry, 1991). As sucrose concentration decreased T_1 values increased which indicated the presence of a synergistic interaction between sucrose and the sweeteners and that resulted in a less hygroscopic (moisture binding) environment resulting in longer T_1 values. 30 % and % 50 sucrose concentration samples were not significantly different than each other ($p>0.05$) but significantly higher than 70 % ($p<0.05$) and control (100% sucrose) samples. Control and 70% sucrose content samples were also statistically significantly different from each other ($p<0.05$).

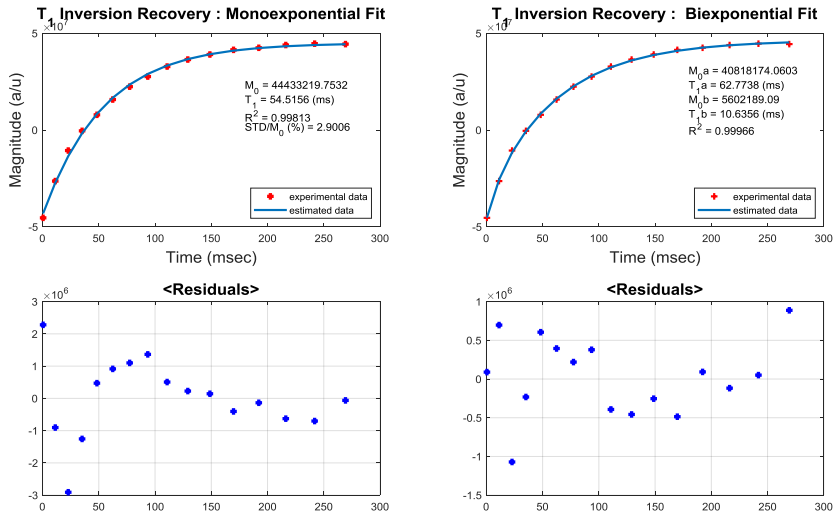


Figure 3. 6 Representative Inversion Recovery (IR) curve for T1 relaxation time measurements conducted at METU

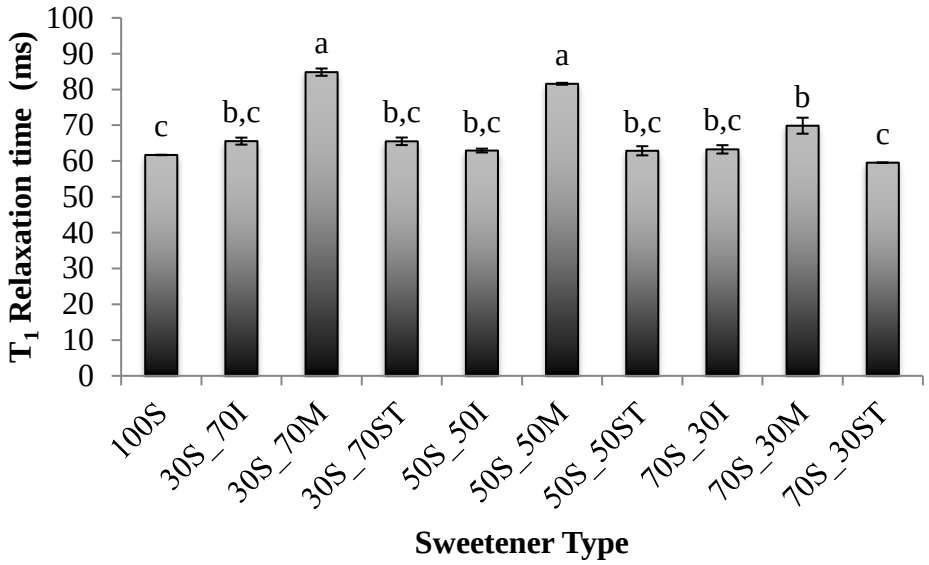


Figure 3. 7 T_1 relaxation time of samples obtained at the low field system at METU.

3.7.2.2 Spin-Spin (T_2) Relaxation Times

Spin-spin relaxation times gives information about the neighboring spins and thus closely related with the microstructure of the sample rather than the lattice. It gives the energy transfer information between closer spins (Kirtil & Oztop, 2015b). For solid forms of the food T_2 is shorter than the liquid forms since, in solids molecules are tight and energy transfer is slow.

T_2 values were recorded using a CPGM sequence which is a very famous sequence known to be robust for the magnetic field inhomogeneities. As known, the most important drawbacks of low resolution systems are magnetic field inhomogeneities and CPGM sequence compensated these with the application of multiple 180^o pulses. A representative CPGM curve and its model fitting to different models are given in Fig. 3.7.

Results of the low field experiments showed that T_2 relaxation times are better described by a biexponential model rather than mono exponential. XPFit software with discrete analysis mode was used to find the T_2 values and contribution of the

proton populations present in the samples. Output of XPfit is given in Fig. 3.8. T_2 values of all samples are given in Table 3.3. T_{2_1} and T_{2_2} denote the relaxation times of the 2 components present in the samples and RA_1 and RA_2 are the corresponding contribution of the pools to the overall signal.

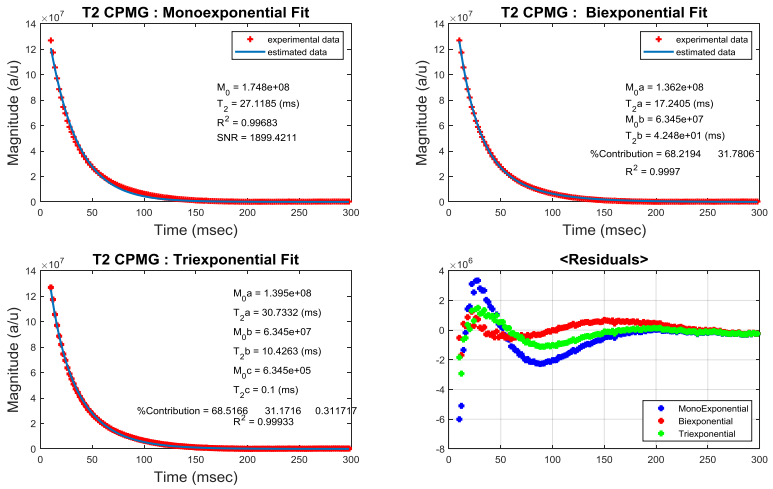
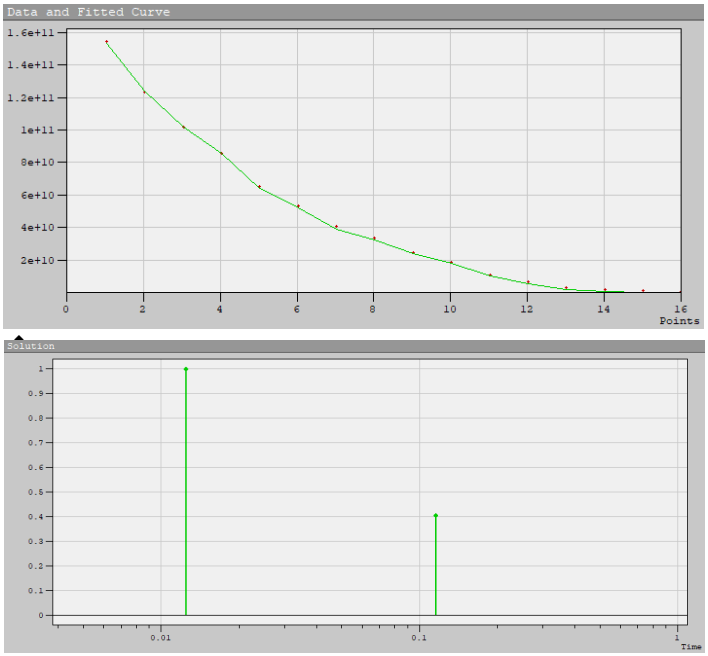


Figure 3. 8 Representative CPMG (IR) curve for T2 relaxation time measurements conducted at METU



❖ **Discrete Components Analysis**

Fitting range : [1; 507]

□□^{2} : 110.1

Exponential	**B (magnitude)**	**Bnorm**	**□□□□ (s)**
1	2.7e+7	0.923	0.037
2	2e+6	0.077	0.066

Figure 3. 9 Discrete Component Analysis mode of XPFit software for a representative T_2 data.

Table 3. 3 T_2 relaxation time of samples obtained at the low field system at METU

Sweetener Type	RA_1 (%)	RA_2 (%)	T_{21} (ms)	T_{22} (ms)
100S	61.51+2.90^{c}	38.49+2.90	4.67+0.58^{c}	21.00+3.00^{e}
30S_70I	50.91 ±1.28^{d}	49.10 ±1.28	7.00±1.41^{b,c}	29.50 ±2.12^{c,d,e}
30S_70M	71.50 ±2.12^{b}	28.50 ±2.12	12.50 ±0.71^{a,b,c}	35.00 ±0.00^{b,c,d}
30S_70ST	95.62 ±0.57^{a}	4.38 ±0.57	10.50 ±0.71^{a,b,c}	32.50 ±4.95^{c,d}
50S_50I	44.77 ±1.03^{d}	55.23 ±1.03	9.67 ±0.58^{b,c}	29.67 ±2.31^{c,d,e}
50S_50M	43.87 ±0.37^{d}	56.14 ±0.37	15.00 ±1.41^{a,b}	45.00 ±2.83^{b}
50S_50ST	67.64 ±2.08^{b,c}	32.36 ±2.08	9.50 ±0.71^{b,c}	26.00 ±1.41^{d,e}
70S_30I	49.77 ±5.02^{d}	50.23 ±5.02	9.67 ±8.39^{b,c}	37.00 ±4.36^{b,c}
70S_30M	51.53 ±1.10^{d}	48.47 ±1.10	20.00 ±1.00^{a}	55.67 ±2.08^{a}
70S_30ST	61.87 ±0.47^{c}	38.13 ±0.47	9.00 ±0.00^{b,c}	28.00 ±0.00^{c,d,e}

Statistical analysis results given in Table A.2 showed that sweetener type and sucrose concentration are also significant on both T_2 values and the relative area of the population 1 (RA_1). Since the relative areas should sum up to 100% ANOVA was conducted only for RA1. Similar to T_1 results, maltitol was found to have the longest T_2 times for both proton populations (T_{21} and T_{22}) followed by isomalt and stevia samples, which were not different from each other ($p>0.05$). Sucrose concentration also had a significantly impact on the T_2 relaxation results ($p<0.05$). It was interesting to note for the 1st proton pool that is the shortest T_2 component which could be associated with non-exchanging proton pools, substitution of sucrose changed the relaxation times with respect to control sample of 100% sucrose but the addition of sweetener did not create a difference at different concentrations. It was hypothesized that the contribution of non-exchanging proton pools at all substitutions had the same effect. On the other hand, the effect of sucrose concentration becomes significant for T_{22} values. 30% and 100% were the lowest and highest values respectively and different than each other ($p<0.05$) wherease the 50% and 70% were found to have the same relaxation times. The second proton pool that was thought to be associated with the water that was entrapped in the gel network. Similar results were also obtained in high resolution experiments and they will be explained afterwards.

In terms of the contribution of non-exchanging proton pools to the T_2 signal (RA_1); all sweeteners were found be different than each other, stevia ones having the highest contribution. This was also observed at higher field experiments. Effect of sucrose concentration was also significant and the highest contribution was found for 70% sucrose containing samples. 30% and 50% samples were not found to be statistically different than each other ($p>0.05$).

3.7.2 High Resolution NMR Experiments

T_1 (spin lattice) and T_2 (spin-spin) experiments were conducted at different temperature values for selected samples. Only sucrose and 70-30 sweetener/sucrose samples were examined at 25, 35, 45, 55 and 65 ^oC. Since the data quality of high resolution systems are very good and experiments take longer times there, only one measurement was conducted for each sample.

3.7.2.2 Spin Lattice (T1) Relaxation Times

T_1 Results obtained were not found to be correlated with the ones obtained at SpinCore system. There may be a couple of reasons for that including the high field of the system in the Poland; the inhomogeneity of the Spin Core system as being an open and low field system; the different parameters used in the sequences.

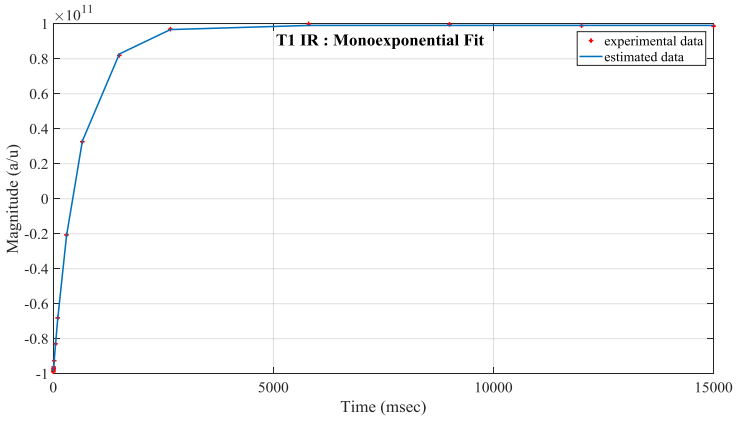


Figure 3. 10 Representative T_1 curve for sucrose based candies at 65 ^oC

T_1 values of samples showed a mono exponential behavior with R^2 values close to 0.9999. A representative T_1 plot is given in Fig 3.9. The most obvious result is the increase in T_1 with respect to temperature. For all samples T_1 increased with

increasing temperature and ANOVA results (Appendix A1) also showed that temperature was a significant factor ($p<0.05$). This was not an unexpected result as the soft candies were melting with the increase in temperature enabling entrapped the water to be more mobile and thus resulting in an increase in relaxation times (Kirtil & Oztop, 2015a; Baris Ozel, Cikrikci, et al., 2017; Baris Ozel, Dag, Kilercioglu, Sumnu, & Oztop, 2017; Baris Ozel et al., 2016). In terms of differentiating the samples based on T_1; it was obvious that sweetener containing samples had higher T_1s at all temperatures. Sucrose samples were found to have the shortest T1s ($p<0.05$). Maltitol and isomalt samples were similar to each other but stevia samples were definitely different ($p<0.05$). Substitution of sucrose with T_1 could have affected the mobility of water protons as sucrose is a better humectant. Due to its chemical nature and high crystalline purity, maltitol in its pure, crystalline form is less hygroscopic than sucrose which might also explain the different water binding behavior of sucrose with respect to maltitol (Rozzi, 2007).

T_1 as being the spin lattice relaxation time gives information about how the given RF pulse energy is released back in the system. The longer the T_1, the more difficult for the energy to be released. Stevia and maltitol samples had the longest T_1, which could indicate that in terms of microstructure the stevia samples could be more organized and the crystallinity could be higher. This could be further validated by other NMR experiments or X-Ray diffraction. Moreover, the presence of aglycone unit in stevia could have affected the water binding ability reversely and could have resulted in longer relaxation times. Maltitol as being added in syrup form could also have resulted in longer T_1s.

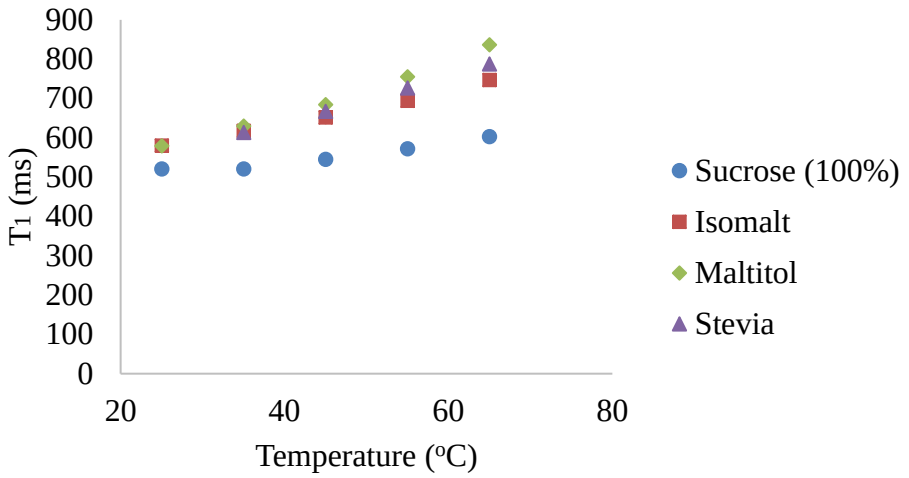


Figure 3. 11 Change in T1 relaxation times with respect to temperature

3.7.2.2 Spin-Spin (T_2) Relaxation Times

T_2 relaxation times of samples were measured and it was observed that biexponential model gave better fitting results. For biexponential fitting, as stated before XPfit software was used with the discrete component analysis function. From the biexponential fitting results; presence of 2 proton population (T_2a (shortest component) and T_2b (longest component) was detected and their contribution (RA_a and RA_b) to the overall signal was described as 'Relative Area'. Results are given in Fig 3.11-3.14. Similar to T_1 results T_2a and T_2b values were also significantly affected from temperature ($p<0.05$) and temperature resulted in an increase on the relaxation times.

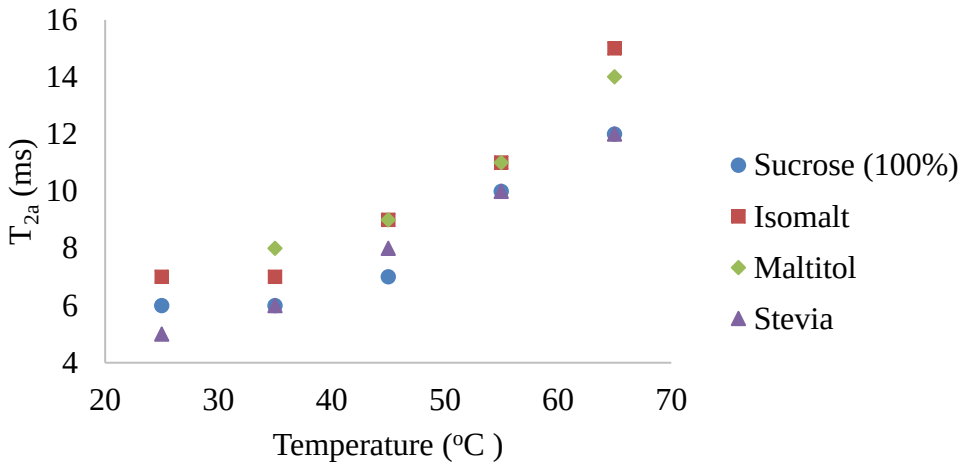


Figure 3. 12 Change in 1st proton population's T_2 wrt temperature

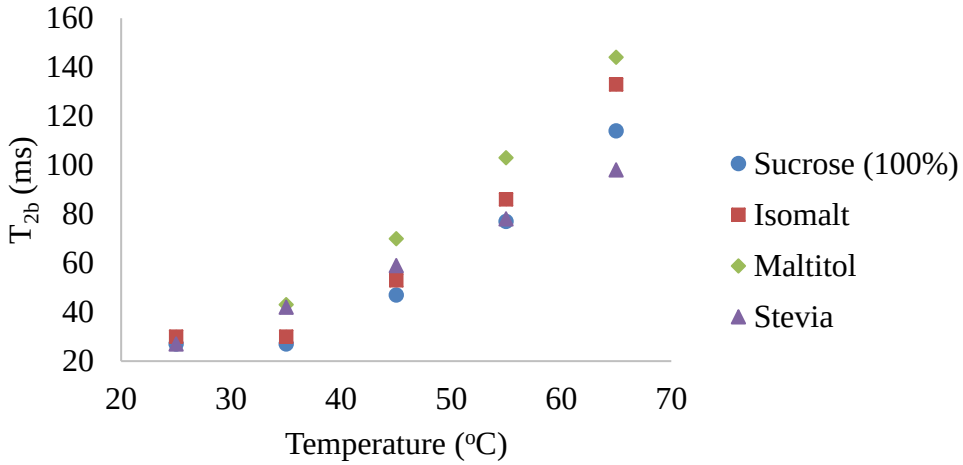


Figure 3. 13 Change in 2nd proton population's T_2 wrt temperature

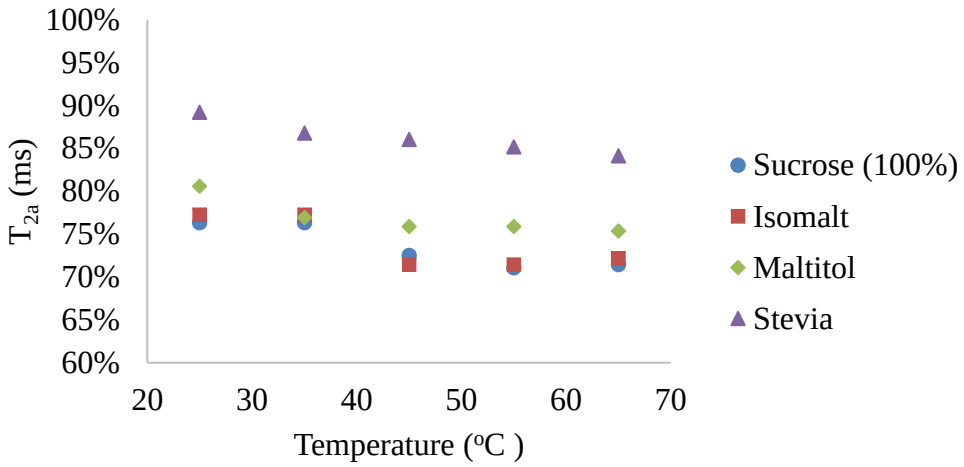


Figure 3. 14 Change in 1st proton population's contribution with respect to temperature

The longest T_2 component observed in the gels could have come from the more mobile water entrapped in the gel network whereas the shortest component could be attributed to the non-exchanging protons between gelatin and sugar or other sweeteners (Baris Ozel, Dag, et al., 2017; Oztop, McCarthy, McCarthy, & Rosenberg, 2012; Oztop et al., 2010; Williams et al., 2011). The 1st component usually being the shortest one is associated with non-exchanging protons. Maltitol had the longest the value for this component followed by isomalt ($p<0.05$) and stevia and sucrose samples were similar ($p>0.05$). The short component's T_2 value was also affected from temperature and increase in temperature resulted increase on the T_2 values. For the second and longest component maltitol was significantly found to different from the rest ($p<0.05$). As this component is associated with the more mobile water protons entrapped in the network it was reasonable to obtain similar values. Maltitol having the longest values is associated with maltitol being added in syrup form rather than powder form.

For the contribution of the 1st proton pool to the signal; stevia had the largest and significant contribution ($p>0.05$). Having the longest T_1 values due to a more

organized structure could have also affected this value. Maltitol was followed by stevia and isomalt and sucrose had the same contributions. In terms of temperature 25 and 35 ºC were found same ($p>0.05$) whereas they were different from 35-65 ºC ($p<0.05$).

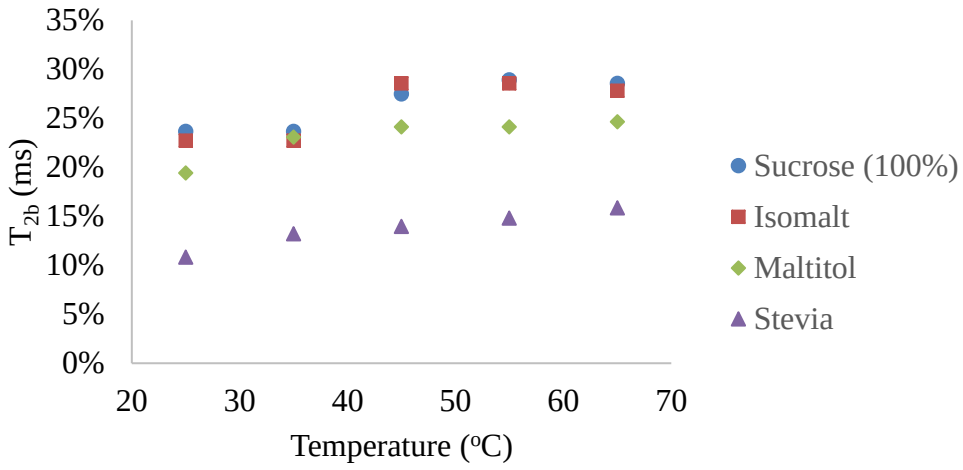


Figure 3. 15 Change in 2nd proton population's contribution with respect to temperature

Comparison of Relaxation Times from the High and Low Field Systems

ANOVA was conducted while taking into account the strength (frequency) of the magnetic field as another factor and T_1, T_2 values for both components and relative areas were evaluated. Since T_1 is known to be affected from magnetic field strength significantly there was an order of magnitude difference between the T_1 values on 2 systems. In other words T_1 values recorded at the high field system were around 600 ms whereas on the low field system they were around 60ms. Thus to conduct ANOVA, Box-Cox transformation with exponent 0.01 was applied. It was observed that T_1 values are longer at the higher field, which was an expected result. When the field strength was taken into account as a factor, sugar type was not found to be significant indicating that field did not have an effect on differentiating the sugar types wrt to T_1 and T_2. T_2 values were recorded by CPMG sequence, which is known to be a robust sequence for magnetic field inhomogeneities, and T_2 is normally not affected from field strength. Accordingly, no significant difference was found between the T_2 values of the 2 system ($p>0.05$) for both components. The only difference was on the relative area of the 1st population. The 1st components contribution was found to be higher in high field system. This was also not surprising as being a high field signal to noise ratio for the non-exchanging proton pools could be high.

CHAPTER 4

CONCLUSION AND RECOMMENDATION

In the scope of this study, gelatin based soft candies were formulated by using different sweeteners (isomalt, maltitol and stevia) at different substitution ratios (30:70, 50:50, 70:30) for the purpose of decreasing the caloric value of the candy product . Physical charactersation was also performed by conducting moisture content, water activity, color, firmness, springiness, differential scanning calorimeter, Low and High NMR Relaxometery experiments. The uniqueness of the study was to use and compare high and low-resolution time domain (TD) NMR for characterization and to understand the influence of the contribution of different ingredients on the final texture of the candies.

In terms of moisture content, maltitol samples had the highest MC results which were related to the form of the ingredient and also there could have occurred fewer interactions between sugar rich and polymer rich phases. Water activity results showed that the molecular weight difference and humectation characteristics had effect on the water activity and among all the formulations stevia samples had the highest molecular weight due to the rebaudioside A part compared with others due to low molecular weight and high solubility, resulting in higher highest a_w. Additionally, humectant characteristic of the isomalt were found to be higher than maltitol.

Furthermore, based on results obtained from the study, all formulations including the control sample (sucrose) did not exhibit Maillard browning reactions since all sweeteners were non-reducing. Thus, color results showed that, there were no change in the L^* and b^* values due to the Maillard reaction. L^* values were

different due to the turbidity of the sugar solutions while adding the polyols into the system.

Texture of candies depended on the sweetener type and in this study maltitol samples decreased the firmness value significantly and isomalt samples increased the firmness value significantly ($p<0.05$). Isomalt solubility was not high as sucrose and it could not attain sufficient H bonding with water. This might be due to the gelatin-isomalt strong network interaction than water isomalt dissolving network. Moreover, there was a significant decrease in springiness value of stevia samples with increase on the stevia concentration in the formulation ($p<0.05$). The decrease of the springiness value of stevia might have occurred due strong bond interaction in gelatin network.

T_g values were found to very low (Table 3.2) which was directly related with the water content of the confections. Furthermore, lower T_g is known to be with the stability of the samples. In this study, in terms of texture, stable candies were obtained according to T_g results.

The results of the LF- NMR Relaxometry conducted at METU indicated that substitution of sucrose with sweeteners definitely had an effect on T_1 values. Lowest T_1 values were obtained for control sample, which was attributed to hygroscopicity of sucrose. For T_2 relaxation times obtained in METU, biexponential model gave better description rather than mono exponential and relative contribution of the components (RAs) were also obtained from the T_2 data. These results were also observed in high field experiments. No correlation was found between the T_1 values obtained at the 2 systems in T_1 values at room temperature due to the inhomogeneities and being an the system at METU being a low field system. Moreover, for temperature range experiment conducted in Poland it was found that when an increase in the temperature, T1 relaxation times is were longer owing to the fact that, gelatin would melt at higher temperatures and the entrapped water will be more mobile with increasing temperature.

Additionally, stevia and maltitol samples had the longest T_1, thus, stevia samples could be more organized, and the crystallinity of stevia might be higher. This can be a further validated by other NMR experiments.

Biexponential results are also obtained in Poland from spin-spin relaxation times. Temperature experiments results were similar to the T_1 results. Increase in temperature resulted in an increase on T_2 times. However, at 25 and 35 ^{o}C results were found to different than 45, 55, 65 ^{o}C. This could be due to the phase transition of gelatin.

In overall, low calorie gelatin based candies were formulated and characterized and in terms of physical properties different sweetener showed different superiorities. Thus it is not possible to propose a single formulation that satisfies all physical characteristics.

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APPENDICES

APPENDIX A

Table A. 1 General Linear Model: Brix versus Sweetener Type; Sweetener Concentration

Factor	Type	Levels	Values
Sweetener Type	fixed	3	isomalt; maltitol, stevia
Sweetener Concentration	fixed	4	0; 30; 50; 70

Analysis of Variance for Brix, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Sweetener Type	2	14.2843	15.9495	7.9747	18.79	0.000
Sweetener Concentration	3	9.6697	8.7875	2.9292	6.90	0.002
Sweetener Type* Sweetener Concentration	6	12.2401	12.2401	2.0400	4.81	0.003
Error	21	8.9150	8.9150	0.4245		
Total	32	45.1091				

S = 0.651555 R-Sq = 80.24% R-Sq(adj) = 69.88%

Grouping Information Using Tukey Method and 95.0% Confidence

Sweetener Type	N	Mean	Grouping
Isomalt	11	66.4	A
Maltitol	12	66.0	A
Stevia	10	64.7	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Sweetener
Concentration N Mean Grouping
30 7 66.5 A
0 9 65.9 A
50 9 65.6 A B
70 8 65.0 B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Sweetener Sweetener
Type Concentration N Mean Grouping
stevia 30 3 67.5 A
isomalt 50 3 67.2 A
isomalt 30 2 66.8 A B
stevia 0 3 65.9 A B C
maltitol 0 3 65.9 A B C
isomalt 0 3 65.9 A B C
isomalt 70 3 65.7 A B C
stevia 70 3 65.6 A B C D
stevia 50 3 65.1 B C D
maltitol 30 2 65.0 B C D
maltitol 50 3 64.4 C D
maltitol 70 2 63.6 D

Means that do not share a letter are significantly different.

Table A. 2 General Linear Model: Moisture Content versus Sweetener Type; Sweetener Concentration

Factor	Type	Levels	Values
Sweetener Type	fixed	3	isomalt; maltitol, stevia
Sweetener Concentration	fixed	4	0; 30; 50; 70

Analysis of Variance for Moisture Content, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Sweetener Type	2	63.158	63.145	31.572	63.63	0.000
Sweetener Concentration	3	35.587	31.530	10.510	21.18	0.000
Sweetener Type* Sweetener Concentration	6	34.440	34.440	5.740	11.57	0.000
Error	23	11.412	11.412	0.496		
Total	34	144.596				

S = 0.704391 R-Sq = 92.11% R-Sq(adj) = 88.33%

Grouping Information Using Tukey Method and 95.0% Confidence

Sweetener Type	N	Mean	Grouping
maltitol	12	35.1	A
isomalt	11	32.7	B
stevia	12	31.9	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Sweetener Concentration	N	Mean	Grouping
70	9	34.4	A
50	9	33.9	A
0	9	32.7	B
30	8	31.9	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Sweetener Type	Sweetener Concentration	N	Mean	Grouping
maltitol	70	3	36.9	A
maltitol	50	3	36.6	A
maltitol	30	3	34.0	B
isomalt	70	3	33.3	B
stevia	70	3	33.0	B
stevia	50	3	32.9	B
isomalt	0	3	32.7	B
stevia	0	3	32.7	B
maltitol	0	3	32.7	B
isomalt	30	2	32.7	B
isomalt	50	3	32.2	B
stevia	30	3	29.1	C

Means that do not share a letter are significantly different.

Table A. 3 General Linear Model: Water Activity versus Sweetener Type; Sweetener Concentration

Factor	Type	Levels	Values
Sweetener Type	fixed	3	isomalt; maltitol, stevia
Sweetener Concentration	fixed	4	0; 30; 50; 70

Analysis of Variance for Water Activity, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Sweetener Type	2	0.0082032	0.0067976	0.0033988	6.96	0.005
Sweetener Concentration	3	0.0201513	0.0224499	0.0074833	15.33	0.000
Sweetener Type* Sweetener Concentration	6	0.0217568	0.0217568	0.0036261	7.43	0.000
Error	21	0.0102540	0.0102540	0.0004883		
Total	32	0.0603654				

S = 0.0220972 R-Sq = 83.01% R-Sq(adj) = 74.12%

Grouping Information Using Tukey Method and 95.0% Confidence

Sweetener Type	N	Mean	Grouping
stevia	11	0.8	A
maltitol	11	0.8	A B
isomalt	11	0.7	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Sweetener
Concentration N Mean Grouping
0 9 0.8 A
70 9 0.8 A
30 7 0.8 A
50 8 0.7 B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Sweetener Sweetener
Type Concentration N Mean Grouping
stevia 50 3 0.8 A
maltitol 30 3 0.8 A
maltitol 0 3 0.8 A
stevia 0 3 0.8 A
isomalt 0 3 0.8 A
maltitol 70 3 0.8 A
stevia 70 3 0.8 A
isomalt 70 3 0.8 A
stevia 30 2 0.7 A B
isomalt 30 2 0.7 A B
maltitol 50 2 0.7 B C
isomalt 50 3 0.7 C

Means that do not share a letter are significantly different.

Table A. 4 General Linear Model: Color (L value versus Sweetener Type; Sweetener Concentration

Factor	Type	Levels	Values
Sweetener Type	fixed	3	isomalt; maltitol, stevia
Sweetener Concentration	fixed	4	0; 30; 50; 70

Analysis of Variance for Color (L value), using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Sweetener Type	2	50.052	77.007	38.504	36.92	0.000
Sweetener Concentration	3	515.996	469.755	156.585	150.14	0.000
Sweetener Type* Sweetener Concentration	6	171.273	171.273	28.546	27.37	0.000
Error	175	182.509	182.509	1.043		
Total	186	919.831				

S = 1.02123 R-Sq = 80.16% R-Sq(adj) = 78.91%

Grouping Information Using Tukey Method and 95.0% Confidence

Sweetener Type	N	Mean	Grouping
isomalt	65	46.3	A
maltitol	69	46.2	A
stevia	53	44.7	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Sweetener

Concentration	N	Mean	Grouping
0	48	48.4	A
70	54	45.8	B
50	47	45.2	C
30	38	43.6	D

Means that do not share a letter are significantly different.

Type	Concentration	N	Mean	Grouping
isomalt	0	16	48.4	A
maltitol	0	16	48.4	A
stevia	0	16	48.4	A
isomalt	70	18	48.2	A
maltitol	70	21	46.2	B
maltitol	50	15	45.5	B C
isomalt	50	15	45.3	B C
maltitol	30	17	44.9	C
stevia	50	17	44.8	C
isomalt	30	16	43.3	D
stevia	70	15	42.9	D
stevia	30	5	42.7	D

Means that do not share a letter are significantly different.

Table A. 5 General Linear Model: Color (b value versus Sweetener Type; Sweetener Concentration

Factor	Type	Levels	Values
Sweetener Type	fixed	3	isomalt; maltitol, stevia
Sweetener Concentration	fixed	4	0; 30; 50; 70

Analysis of Variance for Color (b value), using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Sweetener Type	2	4.687	6.109	3.054	8.15	0.000
Sweetener Concentration	3	287.014	315.871	105.290	281.03	0.000
Sweetener Type* Sweetener Concentration	6	85.179	85.179	14.197	37.89	0.000
Error	182	68.187	68.187	0.375		
Total	193	445.067				

S = 0.612089 R-Sq = 84.68% R-Sq(adj) = 83.75%

Grouping Information Using Tukey Method and 95.0% Confidence

Sweetener Type	N	Mean	Grouping
maltitol	73	16.4	A
stevia	57	16.1	B
isomalt	64	16.0	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Sweetener

Concentration	N	Mean	Grouping
0	45	18.3	A
50	47	16.1	B
70	54	15.5	C
30	48	14.7	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Sweetener Type	Sweetener Concentration	N	Mean	Grouping
stevia	0	15	18.3	A
isomalt	0	15	18.3	A
maltitol	0	15	18.3	A
stevia	50	16	17.1	B
maltitol	30	21	16.1	C
stevia	70	15	15.8	C D
maltitol	70	21	15.7	C D
isomalt	50	15	15.7	C D
maltitol	50	16	15.6	C D
isomalt	70	18	15.1	D
isomalt	30	16	15.0	D
stevia	30	11	13.1	E

Means that do not share a letter are significantly different.

Table A. 6 General Linear Model: Firmness versus Sweetener Type; Sweetener Concentration

Factor	Type	Levels	Values
Sweetener Type	fixed	3	isomalt; maltitol, stevia
Sweetener Concentration	fixed	4	0; 30; 50; 70

Analysis of Variance for Firmness, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Sweetener Type	2	51132	45951	22975	223.62	0.000
Sweetener Concentration	3	136890	107975	35992	350.31	0.000
Sweetener Type* Sweetener Concentration	6	114643	114643	19107	185.97	0.000
Error	29	2979	2979	103		
Total	40	305646				

S = 10.1361 R-Sq = 99.03% R-Sq(adj) = 98.66%

Grouping Information Using Tukey Method and 95.0% Confidence

Sweetener Type	N	Mean	Grouping
stevia	12	662.3	A
isomalt	15	623.5	B
maltitol	14	577.9	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Sweetener

Concentration	N	Mean	Grouping
70	10	673.9	A
0	9	666.6	A
30	9	590.5	B
50	13	554.0	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Sweetener Type	Sweetener Concentration	N	Mean	Grouping
isomalt	70	3	761.6	A
stevia	30	3	686.6	B
stevia	50	3	674.2	B
stevia	0	3	666.6	B
maltitol	0	3	666.6	B
isomalt	0	3	666.6	B
maltitol	70	4	638.3	C
stevia	70	3	621.9	C
isomalt	30	3	576.3	D
maltitol	30	3	508.5	E
maltitol	50	4	498.2	E
isomalt	50	6	489.6	E

Means that do not share a letter are significantly different.

Table A. 7 General Linear Model: Springiness versus Sweetener Type; Sweetener Concentration

Factor	Type	Levels	Values
Sweetener Type	fixed	3	isomalt; maltitol, stevia
Sweetener Concentration	fixed	4	0; 30; 50; 70

Analysis of Variance for Springiness, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Sweetener Type	2	3712.83	3812.06	1906.03	1879.21	0.000
Sweetener Concentration	3	1880.30	1845.90	615.30	606.64	0.000
Sweetener Type* Sweetener Concentration	6	1503.98	1503.98	250.66	247.14	0.000
Error	55	55.78	55.78	1.01		
Total	66	7152.89				

S = 1.00711 R-Sq = 99.22% R-Sq(adj) = 99.06%

Grouping Information Using Tukey Method and 95.0% Confidence

Sweetener Type	N	Mean	Grouping
maltitol	22	89.5	A
isomalt	22	87.1	B
stevia	23	72.5	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Sweetener Concentration	N	Mean	Grouping
30	17	89.4	A
50	17	85.6	B
70	15	81.6	C
0	18	75.6	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Sweetener Type	Sweetener Concentration	N	Mean	Grouping
maltitol	30	5	96.2	A
isomalt	30	6	95.3	A B
maltitol	50	6	94.0	B C
isomalt	50	6	93.8	B C
maltitol	70	5	92.4	C
isomalt	70	4	83.8	D
stevia	30	6	76.8	E
isomalt	0	6	75.6	E
stevia	0	6	75.6	E
maltitol	0	6	75.6	E
stevia	50	5	69.1	F
stevia	70	6	68.6	F

Means that do not share a letter are significantly different.

Table A. 8 General Linear Model: Tg (C^0) versus Sweetener Type; Sweetener Concentration

Factor	Type	Levels	Values
Sweetener Type	fixed	3	isomalt; maltitol; stevia
Sweetener Concentration	fixed	4	0; 30; 50; 70

Analysis of Variance for Tg 2nd heating, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Sweetener Type	2	0,9566	0,4455	0,2228	0,81	0,459
Sweetener Concentration	3	18,2488	18,2272	6,0757	22,16	0,000
Sweetener Type* Sweetener Concentration	6	1,7359	1,7359	0,2893	1,06	0,422
Error	19	5,2088	5,2088	0,2741		
Total	30	26,1501				

S = 0,523593 R-Sq = 80,08% R-Sq(adj) = 68,55%

Grouping Information Using Tukey Method and 95,0% Confidence

Sweetener Type	N	Mean	Grouping
stevia	9	-43,4	A
maltitol	11	-43,6	A
isomalt	11	-43,7	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Sweetener

Concentration	N	Mean	Grouping
0	6	-42,1	A
30	9	-43,9	B
70	8	-44,1	B
50	8	-44,3	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Sweetener Type	Sweetener Concentration	N	Mean	Grouping
stevia	0	2	-42,1	A
maltitol	0	2	-42,1	A
isomalt	0	2	-42,1	A
stevia	30	3	-43,6	A B
isomalt	30	3	-43,8	A B
stevia	70	2	-43,8	A B
maltitol	70	3	-43,8	A B
stevia	50	2	-44,2	B
maltitol	50	3	-44,2	B
maltitol	30	3	-44,3	B
isomalt	50	3	-44,4	B
isomalt	70	3	-44,6	B

Means that do not share a letter are significantly different.

Table A. 9 General Linear Model: T_1 (ms) versus Sweetener Type, Sweetener Concentration

Factor	Type	Levels	Values
Sweetener Type	fixed	3	Isomalt, Maltitol, Stevia
Sweetener Conce	fixed	4	0, 30, 50, 70

Analysis of Variance for T1 (ms), using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Sweetener Type	2	1103.56	1103.56	551.78	63.05	0.000
Sweetener Conce	3	812.58	812.58	270.86	30.95	0.000
Sweetener Type* Sweetener Conc	6	537.30	537.30	89.55	10.23	0.000
Error	24	210.03	210.03	8.75		
Total	35	2663.47				

S = 2.95822 R-Sq = 92.11% R-Sq(adj) = 88.50%

Grouping Information Using Tukey Method and 95.0% Confidence

Sweetener Type	N	Mean	Grouping
Maltitol	12	73.8	A
Isomalt	12	62.4	B
Stevia	12	61.7	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Sweetener Concentration	N	Mean	Grouping
30	9	72.0	A
50	9	68.5	A
70	9	64.1	B
0	9	59.3	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Sweetener Type	Sucrose Content	N	Mean	Grouping
Maltitol	30	3	84.8	A
Maltitol	50	3	81.1	A
Maltitol	70	3	69.9	B
Isomalt	30	3	65.6	B C
Stevia	30	3	65.5	B C
Isomalt	70	3	63.3	B C
Stevia	50	3	62.9	B C
Isomalt	50	3	61.5	B C
Isomalt	0	3	59.3	C
Stevia	0	3	59.3	C
Maltitol	0	3	59.3	C
Stevia	70	3	59.0	C

Means that do not share a letter are significantly different.

Table A. 10 General Linear Model: RA_1, T_{21} (ms), T_{22} versus Sweetener Type, Sweetener Concentration

Factor	Type	Levels	Values
SweetenerType1	fixed	3	Isomalt, Maltitol, Stevia
Sweetener Conce1	fixed	4	0, 30, 50, 70

Analysis of Variance for RA_1, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F
SweetenerType1	2	0.180339	0.198647	0.099323	153.09
Sweetener Concentration	3	0.164099	0.165366	0.055122	84.96
SweetenerType1* Sweetener Concent.	6	0.139665	0.139665	0.023278	35.88
Error	18	0.011678	0.011678	0.000649	
Total	29	0.495781			

Source	P
SweetenerType1	0.000
Sweetener Concentration	0.000
SweetenerType1* Sweetener Concentration	0.000
Error	
Total	

S = 0.0254710 R-Sq = 97.64% R-Sq(adj) = 96.21%

Analysis of Variance for T_{21} (ms), using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
SweetenerType1	2	166.830	162.134	81.067	9.67	0.001
Sweetener Concentration	3	346.518	324.341	108.114	12.90	0.000

SweetenerType1* Sweetener Concent. 6 121.185 121.185 20.198 2.41 0.069
Error 18 150.833 150.833 8.380
Total 29 785.367

S = 2.89476 R-Sq = 80.79% R-Sq(adj) = 69.06%

Analysis of Variance for T_{22} (ms), using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
SweetenerType1	2	867.46	799.73	399.87	47.88	0.000
Sweetener Concentration	3	1763.31	1609.46	536.49	64.24	0.000
SweetenerType1* Sweetener Concent	6	698.10	698.10	116.35	13.93	0.000
Error	18	150.33	150.33	8.35		
Total	29	3479.20				

S = 2.88996 R-Sq = 95.68% R-Sq(adj) = 93.04%

Grouping Information Using Tukey Method and 95.0% Confidence for RA_1

SweetenerType1	N	Mean	Grouping
Stevia	9	0.7	A
Maltitol	10	0.6	B
Isomalt	11	0.5	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for RA1

Sweetener Concentration	N	Mean	Grouping
70	6	0.7	A
0	9	0.6	B
30	8	0.5	C
50	7	0.5	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for RA_1

Sucrose

SweetenerType1	Content1	N	Mean	Grouping
Stevia	70	2	1.0	A
Maltitol	70	2	0.7	B
Stevia	50	2	0.7	B C
Stevia	30	2	0.6	C
Stevia	0	3	0.6	C
Maltitol	0	3	0.6	C
Isomalt	0	3	0.6	C
Maltitol	30	3	0.5	D
Isomalt	70	2	0.5	D
Isomalt	30	3	0.5	D
Isomalt	50	3	0.4	D
Maltitol	50	2	0.4	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for T21 (ms)

SweetenerType1	N	Mean	Grouping
Maltitol	10	13.0	A
Stevia	9	8.4	B
Isomalt	11	7.8	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for T21 (ms)

Sweetener Concentration	N	Mean	Grouping
30	8	12.9	A
50	7	11.4	A
70	6	10.0	A
0	9	4.7	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for T_{21} (ms)

	Sweetener			
SweetenerType1	Content1	N	Mean	Grouping
Maltitol	30	3	20.0	A
Maltitol	50	2	15.0	A B
Maltitol	70	2	12.5	A B C
Stevia	70	2	10.5	A B C
Isomalt	30	3	9.7	B C
Isomalt	50	3	9.7	B C
Stevia	50	2	9.5	B C
Stevia	30	2	9.0	B C
Isomalt	70	2	7.0	B C

Isomalt	0	3	4.7	C
Stevia	0	3	4.7	C
Maltitol	0	3	4.7	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for T_{22} (ms)

SweetenerType1	N	Mean	Grouping
Maltitol	10	39.2	A
Isomalt	11	29.3	B
Stevia	9	26.9	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for T22 (ms)

Sweetener Concentration	N	Mean	Grouping
30	8	40.2	A
50	7	33.6	B
70	6	32.3	B
0	9	21.0	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for T_{22} (ms)

Sweetener

SweetenerType1	Concent1	N	Mean	Grouping
Maltitol	30	3	55.7	A
Maltitol	50	2	45.0	B
Isomalt	30	3	37.0	B C
Maltitol	70	2	35.0	B C D
Stevia	70	2	32.5	C D
Isomalt	50	3	29.7	C D E
Isomalt	70	2	29.5	C D E
Stevia	30	2	28.0	C D E
Stevia	50	2	26.0	D E
Stevia	0	3	21.0	E
Isomalt	0	3	21.0	E
Maltitol	0	3	21.0	E

Means that do not share a letter are significantly different.

Table A. 11 General Linear Model: T_1 versus Temperature Sweetener Type,

Factor	Type	Levels	Values
Temperature	fixed	5	25, 35, 45, 55, 65
Sweetener Type	fixed	4	Isomalt, Maltitol, Stevia, Sucrose

Analysis of Variance for T1, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Temperature	4	0.088356	0.088356	0.022089	22.52	0.000
Sweetener Type	3	0.089921	0.089921	0.029974	30.55	0.000
Error	12	0.011772	0.011772	0.000981		

Total 19 0.190048

S = 0.0313213 R-Sq = 93.81% R-Sq(adj) = 90.19%

Grouping Information Using Tukey Method and 95.0% Confidence

Sweetener

Type	N	Mean	Grouping
65	4	0.8	A
55	4	0.7	A B
45	4	0.7	B C
35	4	0.6	C D
25	4	0.6	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Sugar Type	N	Mean	Grouping
Stevia	5	0.7	A
Maltitol	5	0.7	A B
Isomalt	5	0.7	B
Sucrose	5	0.6	C

Means that do not share a letter are significantly different.

Table A. 12 General Linear Model: RAP1, RAP2, ... versus Sugar Type_1, Temperature

Factor	Type	Levels	Values
Sweetener Type_1	fixed	4	IM, Maltitol, Stevia, Sucrose
Temperature	fixed	5	25, 35, 45, 55, 65

Analysis of Variance for RAP1, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Sweetener Type_1	3	0.0528157	0.0528157	0.0176052	155.26	0.000
Temperature	4	0.0084306	0.0084306	0.0021076	18.59	0.000
Error	12	0.0013607	0.0013607	0.0001134		
Total	19	0.0626070				

S = 0.0106486 R-Sq = 97.83% R-Sq(adj) = 96.56%

Analysis of Variance for RAP2, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Sweetener Type_1	3	0.0528157	0.0528157	0.0176052	155.26	0.000
Temperature	4	0.0084306	0.0084306	0.0021076	18.59	0.000
Error	12	0.0013607	0.0013607	0.0001134		
Total	19	0.0626070				

S = 0.0106486 R-Sq = 97.83% R-Sq(adj) = 96.56%

Analysis of Variance for T2_1, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Sweetener Type_1	3	43.800	43.800	14.600	37.28	0.000
Temperature	4	156.500	156.500	39.125	99.89	0.000
Error	12	4.700	4.700	0.392		
Total	19	205.000				

S = 0.625833 R-Sq = 97.71% R-Sq(adj) = 96.37%

Analysis of Variance for T2_2, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Sweetener Type_1	3	8929.0	8929.0	2976.3	15.00	0.000
Temperature	4	27841.7	27841.7	6960.4	35.09	0.000
Error	12	2380.3	2380.3	198.4		
Total	19	39151.0				

S = 14.0840 R-Sq = 93.92% R-Sq(adj) = 90.37%

Grouping Information Using Tukey Method and 95.0% Confidence for RAP1

Sweetener Type_1	N	Mean	Grouping
Stevia	5	0.9	A
Maltitol	5	0.8	B
Isomalt	5	0.7	C
Sucrose	5	0.7	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for RAP1

Temperature	N	Mean	Grouping
25	4	0.8	A
35	4	0.8	A
45	4	0.8	B
55	4	0.8	B
65	4	0.8	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for RAP2

Sweetener Type_1	N	Mean	Grouping
Sucrose	5	0.3	A
Isomalt	5	0.3	A
Maltitol	5	0.2	B
Stevia	5	0.1	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for RAP2

Temperature	N	Mean	Grouping
65	4	0.2	A
55	4	0.2	A
45	4	0.2	A
35	4	0.2	B
25	4	0.2	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for T2_1

Sweetener Type_1	N	Mean	Grouping
Maltitol	5	11.8	A
Isomalt	5	9.8	B
Sucrose	5	8.2	C
Stevia	5	8.2	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for T2_1

Temperature	N	Mean	Grouping
65	4	14.0	A
55	4	11.3	B
45	4	8.7	C
35	4	7.0	D
25	4	6.5	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for T2_2

Sweetener Type_1	N	Mean	Grouping
Maltitol	5	110.2	A
Isomalt	5	66.4	B
Stevia	5	60.8	B
Sucrose	5	58.4	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for T2_2

Temperature	N	Mean	Grouping
65	4	134.0	A
55	4	96.3	B
45	4	65.5	B C
35	4	42.3	C D
25	4	31.7	D

Means that do not share a letter are significantly different.

Table A. 13 General Linear Model: T2a, T2b, RAa, T1BoxCox versus Field, Sweetener Type

Factor	Type	Levels	Values
Field	fixed	2	High, Low
Sweetener Type1	fixed	4	Isomalt, Maltitol, Stevia, Sucrose

Analysis of Variance for T2a, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Field	1	18.50	18.50	18.50	1.37	0.326
Sweetener Type1	3	124.68	124.68	41.56	3.08	0.190
Error	3	40.51	40.51	13.50		
Total	7	183.69				

S = 3.67471 R-Sq = 77.95% R-Sq(adj) = 48.54%

Analysis of Variance for T2b, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Field	1	30.03	30.03	30.03	0.95	0.402
Sweetener Type1	3	719.34	719.34	239.78	7.56	0.065
Error	3	95.09	95.09	31.70		
Total	7	844.47				

S = 5.63009 R-Sq = 88.74% R-Sq(adj) = 73.72%

Analysis of Variance for RAa, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Field	1	0.114724	0.114724	0.114724	56.87	0.005
Sweetener Type1	3	0.013499	0.013499	0.004500	2.23	0.264
Error	3	0.006052	0.006052	0.002017		
Total	7	0.134275				

S = 0.0449149 R-Sq = 95.49% R-Sq(adj) = 89.48%

Analysis of Variance for T1BoxCox, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Field	1	0.0010669	0.0010669	0.0010669	429.20	0.000
Sweetener Type1	3	0.0000081	0.0000081	0.0000027	1.08	0.475
Error	3	0.0000075	0.0000075	0.0000025		
Total	7	0.0010824				

S = 0.00157663 R-Sq = 99.31% R-Sq(adj) = 98.39%

Grouping Information Using Tukey Method and 95.0% Confidence for T2a

Field	N	Mean	Grouping
Low	4	11.5	A
High	4	8.5	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for T2a

Sweetener Type1	N	Mean	Grouping
Isomalt	2	14.8	A
Maltitol	2	13.0	A
Stevia	2	7.0	A
Sucrose	2	5.3	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for T2b

Field N Mean Grouping
Low 4 35.6 A
High 4 31.8 A

Means that do not share a letter are significantly different.
Grouping Information Using Tukey Method and 95.0% Confidence for T2b

Sweetener
Type1 N Mean Grouping
Maltitol 2 48.7 A
Isomalt 2 34.5 A
Stevia 2 27.5 A
Sucrose 2 24.0 A
Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for RAa

Field N Mean Grouping
High 4 0.8 A
Low 4 0.6 B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for RAa
Sweetener
Type1 N Mean Grouping
Stevia 2 0.8 A
Sucrose 2 0.7 A
Maltitol 2 0.7 A
Isomalt 2 0.6 A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for T1BoxCox

Field N Mean Grouping

High 4 1.1 A

Low 4 1.0 B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for T1BoxCox

Sweetener

Type1 N Mean Grouping

Isomalt 2 1.1 A

Maltitol 2 1.1 A

Stevia 2 1.1 A

Sucrose 2 1.1 A

Means that do not share a letter are significantly different.