RHEOLOGICAL, TEXTURAL, PHYSICO-CHEMICAL AND SENSORY PROPERTIES OF LOW SUGAR APPLE MARMALADE

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ABSTRACT

RHEOLOGICAL, TEXTURAL, PHYSICO-CHEMICAL AND SENSORY PROPERTIES OF LOW SUGAR APPLE MARMALADE

Homemade low-sugar apple marmalade formulations were developed by partial replacement of sucrose with sweeteners such as stevioside and sucralose (25%, 50%) without using commercial pectin and chemical preservative additives. The objectives of this study were to formulate and optimize the composition of ingredients for the best quality low sugar marmalade production, to determine the rheological, textural and physicochemical properties and overall acceptability of formulated marmalades.

The concentration of sweeteners was found to have a significant effect on the physicochemical and rheological properties of the formulations. The hardness of the sweetener added marmalades decreased due to the reduction of total soluble solids (TSS). The marmalade samples had pseudo-plastic behavior exhibiting yield stress. Yield stress of the marmalade increased with increasing TSS content upon increasing sweeteners concentration. Herschel–Bulkley model was found to be the best model describing the time-independent rheological behavior of formulated marmalade samples. The consistency index decreased with raising the sweeteners substitution, whereas the flow behavior index showed increasing trend with the increase of the sweeteners content. From the sensory point of view, low sugar marmalades made by substituting 50% of the sugar content with stevioside have been shown to be as acceptable as marmalade containing only 500g of sucrose.

ÖZET

DÜŞÜK ŞEKERLİ ELMA MARMELATININ REOLOJİK, DOKUSAL, FİZİKO-KİMYASAL VE DUYUSAL ÖZELLİKLERİ

Ev yapımı düşük şekerli elma marmelatı formülasyonları, ticari pektin ve kimyasal koruyucu katkı maddeleri kullanılmadan, sukrozun stevia ve sukraloz gibi alternatif tatlandırıcılar (%25, %50) ile kısmen değiştirilmesiyle geliştirilmiştir. Bu çalışmanın amaçları, en iyi kalitede düşük şeker marmelat üretimi için içerik bileşimini optimize ve formüle etmek ve formüle edilmiş marmelatların reolojik, dokusal ve fizikokimyasal özelliklerini ve genel olarak kabul edilebilirliğini belirlemektir.

Tatlandırıcı konsantrasyonunun, formülasyonların fizikokimyasal ve reolojik özellikleri üzerinde önemli bir etkiye sahip olduğu bulunmuştur. Tatlandırıcı eklenmiş marmelatların sertliği, toplam çözünür katı maddelerin azalması nedeniyle azalmıştır. Marmelat numuneleri verim stresi sergileyen psödoplastik davranış göstermiştir. Tatlandırıcı konsantrasyonunun artması üzerine marmelatın verim stresi, artan TSS içeriği ile artmıştır. Herschel-Bulkley modelinin formüle edilmiş marmelat örneklerinin zamandan bağımsız reolojik davranışını tanımlayan en iyi model olduğu belirlenmiştir. Kıvam indeksi, tatlandırıcı ikamelerinin arttırılmasıyla azalırken, akış davranış endeksi tatlandırıcı içeriğinin artmasıyla birlikte artan bir eğilim göstermiştir. Duyusal açıdan bakıldığında, şeker içeriğinin %50'sinin stevia ile ikame edilmesiyle yapılan düşük şeker marmelatlarının, sadece 500 g sukroz içeren marmelat kadar kabul edilebilir olduğu gösterilmiştir.

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LIST OF SYMBOLS

a*	Redness-greenness
a_w	Water Activity
b*	Yellowness-blueness
DM	Degree of Methylation
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration
G'	Storage or Elastic Modulus
G''	Loss Modulus
HM Pectin	High-methoxyl Pectin
K	Consistency Index ($Pa.s^n$)
L*	Lightness-Darkness
LM Pectin	Low-methoxyl Pectin
LVR	Linear Viscoelastic Region
n	Flow Behavior Index
η_a	Apparent viscosity (Pa.s)
η_{ca}	Casson plastic viscosity (Pa.s)
R^2	Coefficient of determination
RMSE	Root Mean Square Error
RSAM	Reduced Sugar Apple Marmalade
t	Time (s)
TA	Titratable Acidity
TPA	Texture Profile Analysis
TSS	Total Soluble Solids (°Brix)

USDA	United States Drug Administration
τ	Shear Stress (Pa)
τ_o	Yield Stress (Pa)
τ_{oc}	Casson Yield Stress (Pa)
γ	Shear Rate (s^{-1})

CHAPTER 1

INTRODUCTION

Marmalade is a mixture brought to a suitable gelled consistency by adding sugar and water to the pulp, purée, juice and juicy extracts and/or edible parts of one or more fruit (Turkish Food Codex, 2006).

For hundreds of years, food preservation techniques have been applied to fresh and perishable fruits to extend their shelf life and increase their availability out-of-the season. Production of jams, jellies, marmalades, and fruit preserves are among those techniques. Marmalade, a common type of fruit-derived product, is known as a traditional delicacy. It is a semisolid food obtained by boiling fruit pulp with sugar, acid, pectin, and other ingredients like preservatives, coloring, and flavoring items until reaching the suitable consistency (Lal, Siddappa, and Tandon 1960; Baker et al. 2005). Due to high sucrose content with its sweetening effect and caloric value, marmalade is also a great source of energy and carbohydrate. However, a high sucrose diet has been associated with some health problems including diabetes, cancer, metabolic and cardiovascular diseases. Because of the negative connotations related to sugar consumption, low-calorie products are made by fully or partially replacing sugar with sweeteners depending on the properties required in the product.

It is technologically possible to reformulate marmalades to be a healthy alternative to traditional ones. Carbohydrate or non-carbohydrate artificial sweeteners, especially sorbitol, maltitol, xylitol, aspartame, acesulfame-K, saccharin, cyclamate, stevioside, sucralose, or combinations of these can be used in order to maintain or improve the properties of marmalades. The newly formulated product should meet the consumer's demands in terms of its textural, structural and flavor characteristics when compared with traditional products (Renard, van de Velde, and Visschers 2006). Currently, low sugar or sugar-free confections are also continuing to gain in immense popularity. Due to a steady increase in interest in a balanced diet and a healthy lifestyle, low sugar or sugar-free products have a place in the dietary choices of humans. At the same time, fruits are also providing essential nutrients in a healthy diet. They have a vital role for the health and maintenance of the body because of their concentrations of vitamins and minerals, and especially being good sources of dietary fiber and antioxidant. Gorinstein et al. (2001) studied the contents of dietary fiber in the whole apple, along with its pulp and its peel. They found that the peel of the apple is unusually a well-balanced and the richest source (0.91% fresh weight) in terms of total fiber, and also, insoluble fiber (0.46% fresh weight) and soluble fiber (0.43% fresh weight) proportions. Vetter, Kunzek, and Senge (2001) also emphasized that the phytochemicals and nutrients of apple pomace as well as having its functional characteristics like water holding, gelling, thickening and stabilizing abilities. It was demonstrated that apple with nutritional properties have a good potential in a variety of food formulations, as well.

Due to its functional diversity, especially pectin content, apple was selected as the most suitable fruits for the production of reduced sugar apple marmalade in this thesis.

The objective of this study was;

- to formulate the best quality low sugar apple marmalade production by optimizing the composition of ingredients and using sweeteners.
- to determine the rheological, textural and physicochemical properties and overall acceptability of low sugar apple marmalades.

This thesis covers 5 chapters. In the first part of this study (Chapter 2), the theoretical information about apple fruit and its composition, characteristics, nutritional values and health benefits was given in detail. Furthermore, the traditional marmalade and its production, ingredients used for the production were also explained by supporting researches. On the other hand, the apple marmalade made with using sweeteners were investigated based on the effect of the ingredients on the marmalade quality and properties. In the second part (Chapter 3), the methods used in the study were explained. In the third part (Chapter 4), reduced sugar apple marmalade properties were reviewed in detail. The results of physicochemical, rheological, textural and sensory characteristics were emphasized. In the last part of the thesis (Chapter 5), the main results obtained from the low sugar marmalade properties were given as a summary. Also, recommendations were mentioned for future researches.

CHAPTER 2

LITERATURE REVIEW

2.1. General Characteristics of Apple

Apples which are one of the most important fruit crops belong to the rose (Rosaceae) plant family. The scientific name for domesticated apple cultivars is also known as Malus domestica Borkh. (Forsline et al., 2003; Hancock, 2008). The major commercial group's of cultivars including Golden Delicious, Granny Smith, Fuji and Gala predominantly contribute to the world apple production. These varieties account for over 60% of the world's manufacturing output (Hancock, 2008). The average world apple production was 89.329.179 tonnes per year from 2006 to 2016 ("FAOSTAT" 2016). Figure 2.1 shows that Asia has the largest part in the production, followed by Europe. China is the leading apple producing country sharing approximately 40% of world production. Turkey has been ranked as the world's fifth largest producer with 2.638.207 tonnes of fruit (Table 2.1).

Table 2.1. Top ten producer countries by average production per year (Source: FAOSTAT, 2016)

Rank	Countries	Apple Production (tonnes)
1	China	35.531.994
2	United States of America	4.471.484
3	Iran	2.798.686
4	Poland	2.645.779
5	Turkey	2.638.207
6	Italy	2.284.018
7	India	2.155.843
8	France	1.730.634
9	Chile	1.581.531
10	Russian Federation	1.525.258

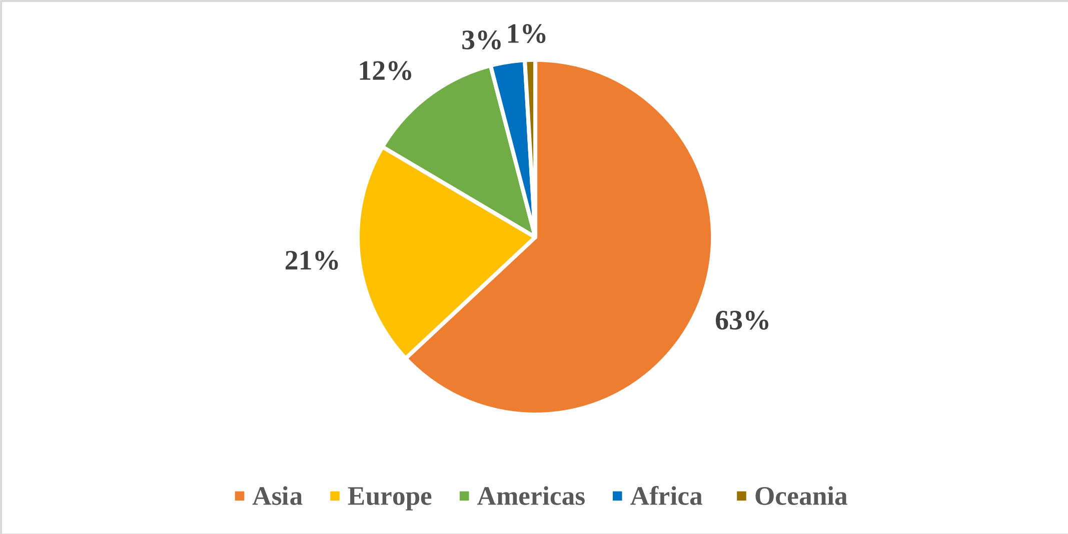


Figure 2.1. Production of Apples by Region
(Source: FAOSTAT, 2018)

Turkey is the rich source of apple crop because of the climatic conditions and ecological variation. Apples are traditionally cultivated almost all region of the country. Red Delicious (Starking) type of apple variety has dominated Turkey’s commercial production by about 1.5 million tons in 2017. Gala apple made up approximately 0.6 million tons of the crop produced in Turkey (Figure 2.2). During the period from 2004 to 2017, Gala apple production in Turkey trends is given in Figure 2.3.

The visual appearance of fresh apple fruits is an important quality determinant. Gala apples have the best quality with an attractive appearance, a sweet flavor, crisp texture and its refreshing aroma (Sturm et al., 2003; Vossen and Silver, 2000). The red color of the cultivars is due to the anthocyanin content, the main color pigment in apple (Veberic, Zadravec, and Stampar 2007; Iglesias, Echeverría, and Soria, 2008). Due to their higher water content among the other varieties, Gala apples are the most suitable apple crop for certain processes such as jam, jelly, marmalade, desserts, and drying (Hampson et al. 2003).

Apple trees can adapt to different climatic condition, so apple fruit is available in all-season and attract customers. But they may exhibit slightly different taste, shape, color and size according to the season that they have harvested. Thus, most of the researchers are mainly investigated the hardness, scent, stiffness, taste, color, shape and size of apples (Babojelić et al., 2007).

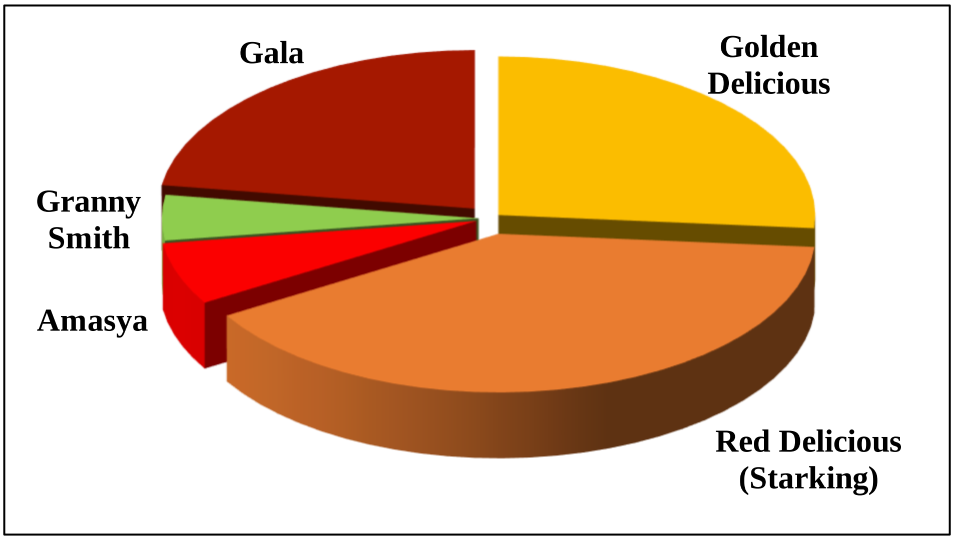


Figure 2.2. The Varieties of Apple Production in Turkey (Ton)
(Source: TURKSTAT, 2017)

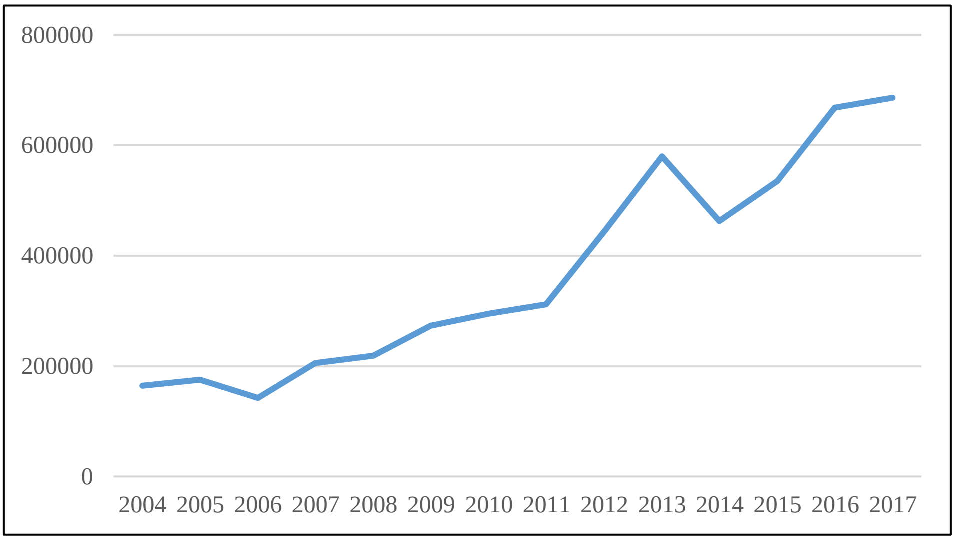


Figure 2.3. Change in Gala production by year
(Source: TURKSTAT, 2017)

External and internal properties of apples, i.e., morphology, biochemical or physical structures, show the quality of fruits. Internal quality of apples, that are classified as stiffness, sugar ingredients and flavor, play a role on the maturity and quality of fruits (Peng and Lu, 2006; Harsan et al., 2006). The quality test which depends on only consumers taste analysis, is not the only way of describing the value of fruit. Besides the quality, the biological value of fruits also important because of their nutritive ingredients fulfilling the needs of body (Babojelić et al., 2007). Apples are mainly composed of water (≈85%) (Table 2.2). They contain sugars (fructose > glucose > sucrose), organic acids (mainly malic acids), vitamins (the highest content of Vitamin C, 4.6 mg), minerals (ash = 0.20) (USDA 2018) and dietary fibers (≈2–3% and pectin < 50% apple fibers) (Karakasova et al., 2009).

Table 2.2. The Composition of Nutrient in Apple
(Source: USDA, 2018)

	Concentration (100 g)
Water	85.56
Energy(kcal)	52
Energy (kJ)	218
Protein	0.26
Total lipid (fat: g)	0.17
Total carbohydrates(g)	13.81
Total Sugars (g)	10.39
Fructose	5.90
Sucrose	2.07
Glucose (dextrose)	2.43
Total Dietary Fiber (g)	2.40
Pectin (g)	0.5
Ash, Total Minerals (g)	0.19
Potassium, K (mg)	107
Calcium, Ca (mg)	6
Magnesium, Mg (mg)	5
Phosphorus, P (mg)	11
Iron, Fe (mg)	0.12
Vitamin C, total ascorbic acid (mg)	4.6
Vitamin A (IU)	54
Vitamin E (α-tocopherol) (mg)	0.18

Apple is also significantly rich source of phenolic compounds which are known as “magic” ingredient in its content (Rong Tsao et al., 2003). In terms of the concentration of phenolic substances (the secondary metabolite class in plants), the apple has the second place among other fruits (Boyer and Liu, 2004a; Kalinowska et al., 2014). Ascorbic acid and dehydroascorbic acid are two type of vitamin C that exist in apple and have antioxidant effect as well (Davies, Partridge, and Austin, 2007; Campeanu, Neata, and Darjanschi, 2009). In recent years, flavonoids and phenolic acids of apples were studied and their effect on tumor cells proliferation was determined (Eberhardt, Lee, and Liu 2000). According to this study, antioxidant compounds (phenolic acids and flavonoids) were predominant compounds in apples compare to ascorbic acid. They showed a potential to prevent high amount of tumor cells proliferation in vitro. At the same time, phenolics determine the flavor, color, astringency of apples (Wu et al., 2007). Moreover, fiber is another component of apple and consist of pectins, lignins, celluloses and hemicelluloses. It has soluble and insoluble parts that are important for biological regulations. Lipid metabolism is related with soluble fiber, which reduces the low-density lipoprotein (LDL) cholesterol, the "bad" cholesterol. On the other hand, the water adsorption and intestinal regulation is associated with the insoluble fibers (Koutsos et al., 2015; Feliciano et al., 2010). Apple consumption has also beneficial health effects. Many studies have been shown that a higher intake of apple fruit, fresh or processed product, is linked to a decreased risk of some cancers, cardiovascular disease, diabetes. It may also provide significant weight loss (Boyer and Liu, 2004b; de Oliveira, Sichieri, and Venturim Mozzer, 2008). In addition, apples are a primarily major source that reduces the risk of Alzheimer’s disease and improves brain health (Howes and Simmonds, 2014). Due to high nutritional content, apples are mostly consumed as fresh around the world (Raudone et al., 2017). At the same time, the consumer also prefers to consume apples as dried form as well as in the marmalade, jam, jelly forms.

2.2. Jam, Jelly, and Marmalades

Jams, jellies, preserves, and marmalades are all produced by preserving the fruit with sugar and are thickened or jellied to some degree. These products have the following characteristics:

Jam is expressed as a mixture, which is brought to a suitable gel consistency with water and sugars, of one or several kinds of fruit its puree or pulp or the blend of both. In the 1000g finished jam production, the amount of fruit pulp or puree or the mixture of both must be no less than 350 g, excluding the following situations (Turkish Food Codex, 2006):

i) For redcurrants, blackcurrants, rowanberries, sea buckthorns, rosehips, and quinces, the amount should be no less than 250 g.

ii) For ginger, the amount should be no less than 150 g.

iii) For cashew apples, the amount should not less than 160 g.

iv) For passion fruit, the amount should not less than 60 g.

Jam is made from boiling the pulp of fruits (chopped or crushed) with the addition of enough sugar. Besides this, the jam should have a sufficiently thick, well-set consistency to keep the fruits in its position and shape. Generally, jams are more viscous and softer compared to jelly (Lal, Siddappa, and Tandon, 1960, D O'Beirne, 2003).

Jelly is a mixture brought to an appropriate gel consistency with sugars and/or the aqueous extracts or juice of one or several kinds of fruit. The juice or aqueous extracts or the mixture of both used in the 1000 g jelly production should be as much as the amounts specified in 1000g jam production. The amount of fruit's juice and/or aqueous extracts used in the jelly is calculated without incorporating the weight of the water used in the preparation of the extract (Turkish Food Codex, 2006). Jelly is made by boiling the fruit or fruit juice with or without water, and then, the boiled extract is strained. After the extract is mixed with sugar, the mixture is boiled until obtaining a transparent gel. Jelly should be clear, well-set and sufficiently tender after removing from its mould. Furthermore, it should provide for a fresh, delicious and the original taste of fruit. In jam, extra jam, jelly, extra jelly, marmalade, jelly-marmalade, and sweetened chestnut puree, the amount of soluble dry matter calculated by refractometer should be no less than 60%, excluding those products made by partially or fully replacement of sugars with sweeteners (Turkish Food Codex, 2006). In the reduced sugar preserves, the values of the soluble solid are in the range of 30-55% (D O'Beirne, 2003).

Preserve is prepared by cooking the fruit as a whole or in the large pieces form until becoming lightly gelled and transparent syrup. Fleshy and tender fruits should be used in the preserve. The "marmalade" term covers the products are obtained by using citrus fruits. Marmalade contains the slices or peels of the fruits as the suspended fruit pieces in the transparent jelly. (Lal, Siddappa, and Tandon, 1960). According to Turkish Food Codex, marmalade refers to a mixture of suitable gel made from one or more of the pulp, puree, juice, aqueous extract and peel of citrus fruits with sugar and water. In the production of the 1000 g end product, the amount of citrus fruit used must be no less than 200 g. At least 75 g of this must be obtained by the endocarp (Turkish Food Codex, 2006). The traditional marmalade is also a mixture, which is brought to a spreadable consistency, by adding sugars and water to the fruit pulp, juice, and juicy extracts. At the same time, the edible parts of plants including plant root, leaf, flower are also used in the marmalade. The amount of fruit pulp, puree, fruit juice and juicy extracts used in the production of 1000 g traditional marmalade should be at least 450 g. The fruit's aqueous extracts used in the traditional marmalades, the amount of the aqueous extracts is calculated without including the weight of water used in the preparation of the extract (Table 2.3).

Table 2.3. The Differences of Marmalade and Traditional Marmalade

(Source: Turkish Food Codex, 2006)

	Marmalade (1000 g product)	Traditional Marmalade (1000 g product)
Fruit	Citrus Fruits	Fruits and edible parts of plants
Fruit Amount	200 g	450 g
Consistency	Mixture	Spreadable Mixture
Soluble Dry Matter (%)	60	55

Food processing covers scientific and technological principles to slow down the biological mechanism or protect foods against spoilage caused by some factors, such as enzymes, temperature, light, moisture and the invasion of microorganisms (Simson and Straus, 2010). Many of the preservation techniques of foods are based on ancient times. The oldest techniques used for preserving fruit during the off-seasons include making candies, jams, jellies, marmalades and so on (Vilela et al., 2015; Lal, Siddappa, and Tandon, 1960). Marmalade as a traditional option for fresh fruit is obtained by boiling of fruit pulp with sugar, acid and pectin until suitable gel consistency.

According to Turkish Food Codex, traditional marmalade is defined as a mixture brought to a suitable gelled consistency made by adding sugar and water to the pulp, purée, juice and juicy extracts or edible parts of plants, such as plant root, leaf, flowers. Generally, in the 1000 g of final marmalade product, the pulp and/or purée quantity must be at least 450 g. Total Soluble Solids content of the product determined by using a refractometer should be no less than 55%, excluding the products made by partially or fully replacing sugar with sweeteners (Turkish Food Codex, 2006). In traditional marmalade, the total acidity should be limited to 15g/kg based on the anhydrous citric acid. Furthermore, insoluble ash in 10% HCl solution should be at most 20 g/kg. The amount of fruit, which is used by mixing different fruit varieties in jam, extra jam, jelly, extra jelly, marmalade, jelly marmalade, and sweetened chestnut puree products, is calculated depending on the percentage used in the mixture. The calculation is based on the permissible minimum amount of fruits. A preservative can be added if the content of soluble solid substance is less than 65% (Turkish Food Codex, 2002). However, the criteria do not apply because of repealing the regulation in 2002.

2.2.2. Preparation of Traditional Marmalade

Sugar has an important role in the production of traditional marmalade. Traditional marmalades are generally prepared by a high concentration of sugar, especially sucrose. It affects the total soluble solids content as well as the physical, chemical, sensorial attributes of marmalades. Sucrose reduces the water activity of products to approximately 0.8 and induces the pectin gelatinization by binding water for inhibition of the microbial activity (Basu and Shivhare, 2010). However, the high amounts of sucrose intake contribute to health problems such as obesity, cancer, heart disease, diabetes, and hypertension (Lauritzen, 1992; Vilela et al., 2015). The products prepared using sweetener instead of sugar have become alternative products for the food industry. (Basu, Shivhare, and Singh, 2013). In order to produce a desired low sugar marmalade product, it is not sufficient to reduce the amount of sugar only. At the same time, the concentration of pectin and the amount of sweetener to be added for the recipe are also important (Vilela et al., 2015). The desired gel structure is associated with the perfect composition of sugar, acid, and pectin. It is important to obtain the required gel structure. Therefore, the mixture is rapidly boiled to reduce its water content. The mixture is concentrated to ensure the gel consistency. (Simson and Straus 2010). Due to its significant effects on the structure and texture of the gel, sucrose is mostly preferred carbohydrate sweetener in food gels. (Bayarri, Durán, and Costell, 2004). The gelling ability of pectins with sugar and acid is influenced by the sugar types and the concentration of soluble solids. Sugar partially dehydrates the pectin molecule and assists their aggregation in the zones of cluster. When the sugar binds with water in the fruit, the pectin could create a model gel (pectin + sugar + acid + water) with a suitable strength and help for the formation of the acceptable texture in the marmalade. The separation of the free carboxyl groups is suppressed with the acid addition. The negatively charged pectin molecules are prevented from repelling each other. Thus, close contact between the pectin molecules is provided. The formation of hydrogen bonds between unseparated carboxyl groups is also allowed (Molyneux, 1971). The texture of the end product results in higher firmness and brittleness. (Mercer, 2002; Voragen, Schols, and Visser, 2003). As the sugar content rises, the firmness of the product also increases. However, an excessively high level of sugar leads to the crystallization of the sugar (Featherstone, 2016).

2.2.2.1. Sucrose

Sugars is an important ingredient for food products in the confectionary industry. It is part of the class of carbohydrates and chemically defined as $C_n (H_2O)_{n-1}$ or $(CH_2O)_n$. Sugar is commonly used name for sucrose that is also used as a sweetener in solid foods, e.g. jams and jellies, marmalades etc. Sucrose is a commercially available disaccharide found in many plants, especially sugarcane and sugarbeet (Colonna et al., 2006). Sucrose, which has a capacity to reduce the water activity, is commonly used for its sweetening effect in food systems (Simson and Straus, 2010).

The addition of sucrose provides a major conservative effect on the anthocyanin pigment as well as delaying or inhibiting effect of the enzymatic browning and color changes during processing (Suutarinen, 2002). At the same time, sucrose improves the characteristic flavors and aromas while minimizing the earthy tastes of fruit and vegetables. During marmalade production, the amount of dissolved solids is increased to about 70% by applying high mechanical forces. Due to the hydroxyl groups, sucrose has highly soluble in water. The solubility is increased with the existence of other dissolved solids. Thus, the efficiency of crystals produced from sugar syrups is reduced (Colonna et al. 2006). When the sugar syrup penetrates into the cell of fruit, water is removed from the fruit tissue with osmotic effects. At the same time, those effects also result in the collapse of the fruits (Suutarinen et al., 2000). Besides, the sugar uptake with the fruit tissue is affected by the sugar and its concentration. The added sugar allows to have a closer contact with the pectin chains by attracting water molecules. In recent years, osmotic dehydration is an efficient food preservation method used in the food industry in order to reduce the water activity of foodstuffs and to eliminate the negative effects of heat on the color and flavor (Chavan and Amarowicz, 2012; Yadav and Singh, 2014; Akbarian, Ghasemkhani, and Moayedi, 2014). The applications of osmotic dehydration generally used for enhancement of shelf life of jams, jellies, marmalades or desserts, reduction of the loss of aroma of dried and/or semidried foods, prevention of undesirable structural changes of frozen fruits, and improvement of nutritional and functional characteristics of all foodstuffs (Mavroudis, Gekas, and Sjöholm, 1998).

2.2.2.2. Pectin

Pectin is a molecular substance composed of complex polysaccharides, mainly found in the cell walls of all higher land plants. Due to being a natural compound of fruits and vegetables, pectin improves the structural and textural attributes of the product. The fundamental constituent of the pectin molecules is D-galacturonic acid monomers in methyl ester conformation linked by α-(1 → 4) glycosidic bonds. The different type of neutral sugars, namely L-rhamnose, L-arabinose, D- galactose, are attached to the side chains of the pectin polymers (Flutto 2003; Wang, Chen, and Lü, 2014).

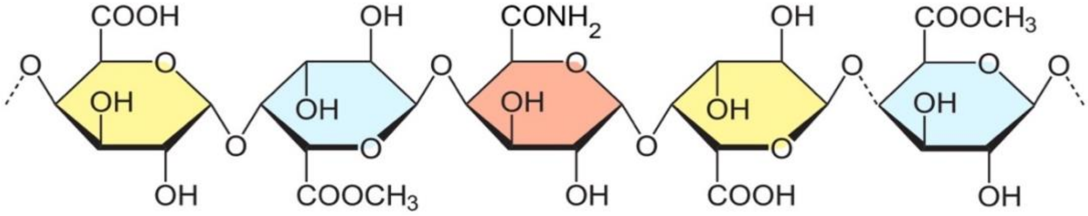


Figure 2.4. Structure of Pectin (Source: Silvateam, 2018)

Several studies have revealed that pectin is a hydrocolloid molecule having functional and structural diversity and characteristic features. Besides functionalities, pectin could be considered as a food hydrocolloid which is highly rich in terms of soluble dietary fibers. Most of the food hydrocolloids could be qualified as dietary fibers according to the Codex Alimentarius Commission (ALINORM 09/32/A) (Brownlee, 2011; Chawla and Patil, 2010; Li and Nie, 2016). It is suggested that an adequate daily intake of dietary fiber should be ensured as being an immense functional food ingredient of a healthy diet. Many health benefits are associated with the consumption of dietary fiber, such as reducing the serum cholesterol concentration, maintaining the level of blood sugar in diabetes, improving the function of digestive system, lowering the risk of cardiovascular disease, dropping the blood pressure and the prevention of some cancer types (Grigelmo-Miguel and Martín-Belloso, 1999; Wang, Chen, and Lü, 2014; Li and Nie, 2016). Pectin exists pervasively in all fruits and vegetables, but pectin content and quality depend on the type, ripeness and growing conditions of fruits (Fellows, 2004; Featherstone, 2016).

The production of pectin has been industrially obtained by the four steps listed below (May, 1990).

- Extraction from the raw plant material
- Clarification of the liquid extract
- Precipitation from the filtrate solution
- De-esterification of methyl ester groups with acid, alkali, or ammonia treatments dependent on the required pectin quality.

Additionally, changes of the structure of molecule could be done enzymatically. In recent years, many studies have been conducted to improve the extraction methods of the pectin by using enzymes rather than acid extractions (Baum et al., 2016). In general, pectin production at the industrial level is carried out by isolating it from apple pomace and citrus peels, namely lemon, orange, lime and grapefruit. Eventually, the extraction of pectin from citrus peels are provided by acid hydrolysis (May, 1990; Kaya et al., 2014). Table 2.4 shows the classification of fruit varieties according to pectin levels. The fiber content of apples is approximately 2-3 g / 100g and pectin constitutes less than 50% of the fiber content of apples. Apple pectin provides the textural stability in the end products by helping the formation of gel structure. The health benefits of apples are directly related to their fiber and phenolic contents. Aprikian et al. (2003) suggested that the combined effect of apple pectin and polyphenol content provides high impact on the cholesterol and lipid mechanism.

Table 2.4. The pectin content of raw plant material according to pectin levels

(Source: Finecooking, 2018)

High Pectin	**Low Pectin**
Chile peppers	Apricots
Citrus peels (Not flesh)	Blueberries
Concord grapes	Cherries
Cranberries	Citrus Flesh (Not peels)
Currants	Figs
Gooseberries	Melons
Quince	Peaches/nectarines
Sour plums	Pineapples
Tart apples	Raspberries
Tomatillos	Rhubarb
Tomatoes	Strawberries

Pectin is extensively used as a gelling agent in the manufacturing of fruit preserves. The gelation mechanism of pectin is considered under two major groups according to the degree of methylation (DM) which is the principal feature used for defining the pectin functionality (Vincken et al., 2003; Fraeye et al., 2010). DM expresses gel-forming ability and depicts potential gelling properties of pectin. The pectin types are categorized as low methoxyl (LM) pectins with DM<50% and high methoxyl (HM) pectins with >50% (Crandall and Wicker, 1986; Sila et al., 2009). High methoxyl pectin requires high amounts of sugar and high boiling temperature under acidic conditions in order to manufacture suitable products. Low methoxyl pectin requires the presence of calcium cations and the high gelling temperature. The strength of the gel formed depends primarily on the concentration of calcium ions because the products are usually made with lower amount of sugar (Crandall and Wicker, 1986; Voragen, Schols, and Visser, 2003). Thus, because of the characteristic features and conformational versatility, the pectin is widely used for gelatinization, thickening, emulsifying, and stabilization purposes in food, chemical, medical and pharmaceutical, textile, and a number of other industries (Wicker et al., 2014; Ciriminna et al., 2015).

Pectin coded as *E440a* for low and high methoxyl pectin or *E440b* for amidated pectins with Gras status approved as a safe food additive by The Commission of the European Union (Ciriminna et al., 2015). Commercial pectins are principally utilized for the formulation of a wide variety of gelling food products like jams, jellies marmalades, and low-calorie preserves. Pectin has effectively capable of water holding and gel-forming ability even if used at low concentration. It provides the desired uniform texture and smoothness by limiting the formation of water on the marmalade surface. Furthermore, in the products, pectin prevents the flocculation with ensuring a homogeneous distribution of the fruit particles (Ciriminna et al., 2015). Pectin can be mixed with food without influencing it's taste and flavor. Therefore, it is most commonly used as thickeners and stabilizers in the food industry, wherein pectin is used as a gelling or thickening agent in jams, fruit fillings, confectionery industry, and also as a stabilizer in beverage industry, dairy-based drinks, and baking products (Pagliaro et al., 2016).

2.2.2.3. Acid

In food preservation, organic acids have been used as food additives and preservatives for many years. They are either naturally occur in foods, or else purposively added to product during processing. Organic acids have roles in the extension of shelf life of highly perishable foods and inhibition of food deterioration. Some organic acids become more useful on the inhibition of spoilage microorganisms, while others typically act as the fungicides. The effect of organic acids is correlated with reducing the pH of substrate, degrading substrate transportation by causing deformation of the permeability of cell membrane. At the same time, the undissociated portion of the acid molecule is ionized and so, the internal components of the membranes become acidified. The undissociated molecule is related to the antimicrobial activity (Simson and Straus, 2010). Organic acids used for food preservation are citric, succinic, malic, tartaric, benzoic, lactic, and propionic acids.

Lemons contain some acids mostly used to provide several functions in many foods and drinks. For example, due to the ascorbic acid content, lemons are utilized in foods or fruits preservation as the antioxidant (Gulsen and Roose, 2001). Lemon juice is a rich source of citric acid, about 5% to 6%. Lemons and limes have the highest amount of concentrated citric acid compared to the other fruits. Citric acid has the highest inhibitory effect on thermophilic bacterial growth compared to acetic and lactic acids. Because the acid can be able to diffuse through the cell membrane of the bacteria, it can penetrate to the weak undissociated acid molecules in the membrane. (Penniston et al., 2008). All of the ingredients should be mixed thoroughly at the initial step of the marmalade process. After that, some critical ingredients may be added at the late steps to ensure a high-quality product in the process. Therefore, citric acid is added to the marmalade mixture after reaching its boiling point at the last step. It is also used for increasing the shelf life of the finished product. Furthermore, benzoic acid is widely used preservative since ancient times. As a food additive, sodium salt in benzoic acid demonstrates further effectiveness in acidic food-related systems where the pH is lower than 4.5. Hence the benzoates are principally used as an antimycotic agent in fruit juices, syrups, candied, fruit peel, pie fillings, ketchup and several sauces, pickled vegetables, relishes, and cheeses (Simson and Straus, 2010). The suitable daily intake for sodium benzoate is 5 mg/kg body weight (FDA, 1991). The benzoic acid or its sodium or potassium salts may be used as a maximum of 500 mg/kg-l in the reduced-sugar jam products. Moreover, the use of ethyl-, methyl-, or propyl-4-hydroxybenzoic acids and their sodium salts are also allowed at most 500 mg/kg (Ranken and Kill, 1993). According to Turkish Food Codex Regulation on Food Additives (2013), for only low-sugar and similar low-calorie or sugar-free products, the maximum amount of benzoic acids and benzoates should be 500 mg/l or mg/kg, as well. The amounts are expressed in terms of free acid.

2.3. Effect of Sugar Substitution on Marmalade

Raising awareness on healthy lifestyle has led consumers to look for the healthier, low calorie, safe, and easy to use nutritious foods. Low-calorie foods were developed specifically for consumers with health care problems beforehand. Then, their consumption has expanded from the prevention of some disease to health promotion, to weight control, and to fit the healthy lifestyle. (Hyvönen and Törma, 1983; Sandrou and

Arvanitoyannis, 2000; Khouryieh et al., 2005). Fortunately, the food and beverage industry recently offer alternative products made with sweeteners. (Parpinello et al., 2001). Low-calorie sweeteners used as sugar substitutes are added to many foods and beverages, not only reducing sugar intake and total calories but also maintaining food's palatability (Wiebe et al., 2011). Low-calorie foods of acceptable quality can be industrially prepared with using the non-caloric sweeteners either alone or in combination with sugar. The foods with the inclusion of alternative sweeteners should taste, and have textural and rheological attributes similar to the traditional foods (Hyvönen and Törma, 1983). The variability of their concentrations in marmalades give rise to the changes in rheology and texture and mouthfeel characteristics that perceived by consumers.

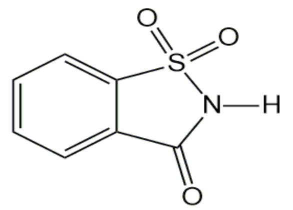
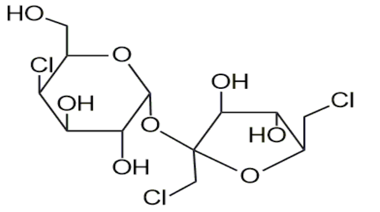
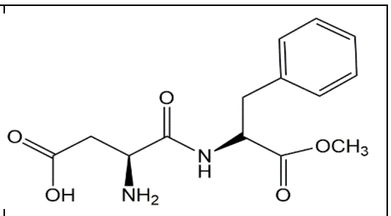
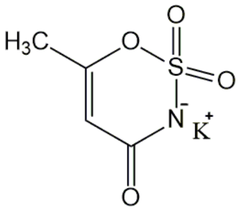
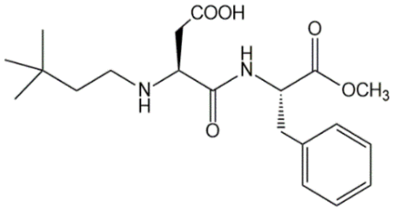
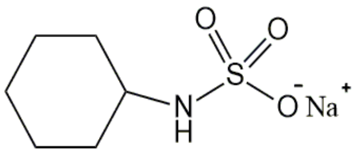
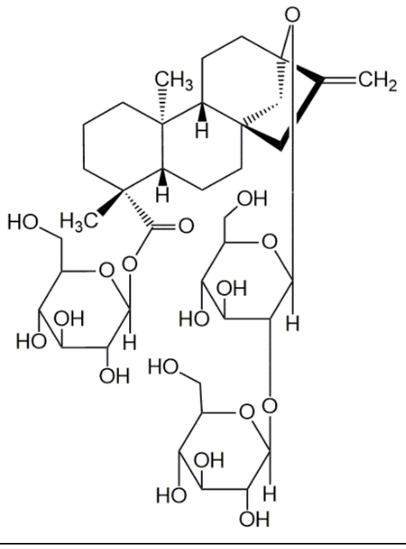
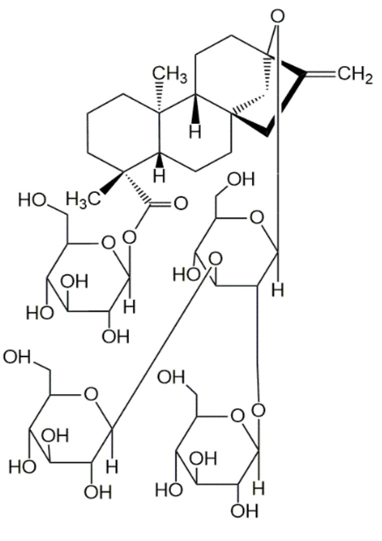
2.3.1. Artificial Sweeteners

The European Union (EU) has been developed a food safety system that called E-classification system to prevent risks generated from foods. In the first stage, the system was used for colorants and later on, it is used for stabilizers, preservatives, antioxidants, emulsifiers, gelling agents, sweeteners, thickeners and flavorings. This system is a kind of insurance for foods to protect their quality such as odor, texture, color or taste and prevent contamination from unhealthy microorganisms (Kallscheuer, 2018). Among food additives, calorie free sweeteners are used to mimic the taste of sugar.

Sweeteners are divided into two groups: those which have calories and provide nourishment (nutritive) and those that are calorie-free (artificial or non-nutritive). Monosaccharides, disaccharides and polyols are nutritive sweeteners and have almost similar sweetness degree as compared to sucrose. Artificial sweeteners contain various chemical groups that stimulate taste buds of tongue, so they have higher intensity of sweet taste perception than sucrose (Whitehouse, Boullata, and Mccauley, 2008). The Food and Drug administration have been approved six artificial sweeteners which are saccharin, aspartame, acesulfame potassium, cyclamate, sucralose, alitame, stevia (stevioside, Rebaudioside A etc.), neotame and advantame (Table 2.5).

These sweeteners are mainly used in the food industry to produce low-calorie food products. Unlike sugar, they do not trigger any insulin response in the body. They do not cause dental plaque (Kokotou, Asimakopoulos, and Thomaidis, 2012).

Table 2.5. Chemical Structure of Sweeteners

		
Saccharin	**Sucralose**	**Aspartame**
1,2-benzisothiazol-3 (2H)-on-1,1-dioxide	1,6-dichloro-1,6-dideoxy-β-D-fructofuranosyl-4-chloro-4-deoxy-β-D-galactopyranoside	N-L-α-aspartyl-L-phenylalanine-1-methyl ester
		
Acesulfame Potassium	**Neotame**	**Sodium Cyclamate**
6-methyl-1,2,3-oxathiazine-4(3H)-one 2,2-dioxide	N- [N-(3, 3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1–methyl ester	Sodium N-cyclohexylsulfamate
		
Stevioside	**Rebaudioside A**	
13-[(2-O-β-D-glucopyranosyl-β-D-glucopyranosyl) oxy]-ent-kaur-16-en-19-oic acid β-D-glucopyranosyl ester	13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl) oxy]-ent-kaur-16-en-19-oic acid β-D-glucopyranosyl ester	

Some important artificial sweeteners are summarized as follows;

- **Saccharin** was the first discovered sweetener named 1,2-benzisothiazol-3 (2H)-on-1,1-dioxide (Zygler, Wasik, and Namieśnik, 2009; Spencer et al., 2016). It is hydrolyzed to 2-sulfobenzoic acid and 2-sulfoamylobenzoic acid when pH is low. Three types of saccharin are available commercially; acid saccharin, sodium saccharin, and calcium saccharin. Compared to sucrose, saccharin is 200-700 times sweeter. It cannot be metabolized in the human body and it is excreted with urine. Calorie intake is zero. It has undesirable bitter-metallic taste. Due to its bitter taste, it is used with other sweeteners to mask the taste.
- **Aspartame** is the second discovered sweetener after saccharin. Its chemical name is N-L-α-aspartyl-L-phenylalanine-1-methyl ester where two amino acids (phenylalanine and aspartic acid) linked to methanol (Kroger, Meister, and Kava, 2006; Zygler, Wasik, and Namieśnik, 2009). The existence of methanol is a concern for consumers because of its toxicity. The form of aspartame can be changed according to pH. When pH is below 3, aspartame is hydrolyzed into aspartyl phenylalanine but above pH 6, it is changed into 5-benzyl-3,6-dioxo-2-piperazineacetic acid. Compared to sucrose, aspartame is 200 times sweeter. Also, it is not stable under heat, so it is not suitable for baking and cooking. It is metabolized in the human body, digested by enzymes and converted into amino acids and methanol. This sweetener is also used in foods including carbonated and non-carbonated beverages. However, if this sweetener is used, it is necessary to inform the consumers that the products contain phenylalanine. Patients with the disease named phenylketonuria should be careful by limiting the intake of this amino acid (Choudhary and Lee, 2018).
- **Acesulfame potassium** is a potassium salt of 6-methyl-1,2,3-oxathiazine-4(3H)-one 2,2-dioxide and 200 times sweeter than sucrose (Whitehouse, Boullata, and Mccauley, 2008; Mooradian, Smith, and Tokuda, 2017). It shows synergistic effect with other sweeteners (sucralose or aspartame) to mask their bitter tastes. Carbonated beverages are the products formulated by mixing acesulfame-potassium with other sweeteners (Kroger, Meister, and Kava, 2006). Also, it is very stable in a wide range of temperature and pH. Since the human body cannot metabolize acesulfame K, there is no caloric intake. Also, it does not cause any tooth decay.

- **Cyclamate** is another low-calorie sweetener which is a salt of cyclohexylsulfamic acid (DuBois and Prakash, 2012; Cabral et al., 2018; Chattopadhyay et al., 2014). It has two salt forms; sodium cyclamate and calcium cyclamate. The most widely known is sodium cyclamate that is used as non-nutritive sweetener. It is 30 times sweeter than sucrose and has unpleasant salty-bitter taste that can be easily recognized by consumers. Therefore, it is mainly used with saccharin to mask their tastes.
- **Neotame** is a derivative of aspartame where 3, 3-dimethylbutyl group added to aspartic acid and named N- [N-(3, 3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1–methyl ester (Whitehouse, Boullata, and Mccauley, 2008; Cabral et al., 2018). It becomes 6,000 to 10,000 time sweeter than sucrose. It is heat stable sweetener and has no adverse effects on tooth. Also, it can be directly metabolized in the human body. Depending on moisture content of the food, the stability of neotame changes as a function of temperature, pH and time.
- **Sucralose** is obtained after five-step reaction process from sucrose (Grice and Goldsmith, 2000; Frank et al., 2008). Three chlorine atoms are replaced with three hydroxyl atoms, so called 1,6-dichloro-1,6-dideoxy-β-D-fructofuranosyl-4-chloro-4-deoxy-β-D-galactopyranoside. The sweetness of sucralose is 600 times more than sucrose. Compared to other sweeteners, it has not bitter taste and used synergistically with other non-nutritive sweeteners. Food, beverages and drug industries are some fields that mainly used sucralose as a sweetener. Due to its stability, it can be used at varying temperatures. Sweetness level of sucralose is preserved during cooking and pasteurization. Thus, it is used in different food types including beverages, frozen desserts, shewing gum, processed fruits etc (Grotz and Munro, 2009). Sucralose are not metabolized and is excreted in two ways; most part by faeces and the remaining part by urine. Thus, calorie intake does not occur.
- **Stevioside**: *Stevia rebaudiana* (Bertoni) is one of 200 species that belongs to Asteraceae family (Gantait, Das, and Mandal, 2015; Geuns, 2003). The leaves of this plant contain **steviol glycoside** which provides sweet tastes. It has ten sweetening compounds which are stevioside, rebaudioside A, B, C, D, E, F, dulcoisde A, B and steviolbioside. Stevioside and rebaudiosade A are diterpene glycosides which widely used as natural sweeteners. Steviosides contains steviol

aglycone and three glycose molecules (Brahmachari et al., 2011; Christaki et al. 2013). Its sweetness is 200-300 times more than sucrose. It has a slightly bitter taste. On the other hand, rebaudioside A contains steviol compound with four glycoses. It is 250-400 times sweeter than sucrose and has no calorie content. Like stevioside, it does not give a bitter taste. Moreover, rebaudioside A sweetening potency is higher than stevioside. Stevia glycoside is not metabolized in the human body, so no calorie intake occurs. Due to heat stability, it can be cooked and baked. **Stevia** (commercial brand name) is a non-nutritive sweetener but technically it is not right to call as "artificial sweetener" because it is a natural product (Bülbül et al., 2019).

Studies on the health effect of artificial sweeteners:

In recent years, Sasaki, Kawaguchi et al. studied the genotoxicity of sodium cyclamate, saccharin, sodium saccharin and sucralose (Sasaki et al., 2002). Male ddY mice were orally treated with sweeteners (limit dose of 2000mg/kg). After 3 and 24h of treatment, comet assay was applied on glandular stomach, colon, urinary bladder, kidney, brain, lung and bone marrow. According to the results, sweeteners increase DNA damage in the gastrointestinal tract. Sodium cyclamate affected glandular stomach, colon, kidney and urinary bladder; saccharin only affected the colon; sodium saccharin affected the colon and glandular stomach; sucralose affected the glandular stomach, lung and colon. In another study, Maki et al. investigated blood pressure and heart rate of healthy men and women after rebaudioside A consumption (Maki, Curry, Carakostas, et al. 2008). 1000mg/day rebaudisiode A were consumed daily. According to results of this study, rebaudioside A administrated in healthy people for 4-weeks had no significant effect on their blood pressure. Measurements were also made while resting, seated but no changes were observed. Before this study, same examinations were made on rats. Dyrskog et al. studied the type 2 diabetes in rats (Goto Kakizaki) They investigated blood pressure of rats after 8-week ingestion of rebaudioside A but they did not observe any change in their blood pressure (Dyrskog et al. 2005). Similarly, Maki et al. studied (2008) type 2 diabetes mellitus in men and women (18–74 years of age) to examine glucose homeostatis by consuming 1000 mg/day rebaudioside A. After 16-week, there was not a significant change in blood pressure or glucose hemostasis by using chronic rebaudisoide A.

2.3.2. Advantages and Disadvantages of Using Artificial Sweeteners in Foods

Artificial sweeteners are mainly used in the food and beverage industry to develop low calorie and low sugar dietary foods (Kokotou, Asimakopoulos, and Thomaidis, 2012; Kroger, Meister, and Kava, 2006). Due to its intense sweet taste, a small amount of sweetener is enough to prevent use of large amounts of sugars. They do not have calorie content, also do not show insulin response compared to the sucrose.

Sweeteners may be used alone or as a mixture which are called as blends (Zygler, Wasik, and Namieśnik, 2009; Zhao and Tepper, 2007). Generally, blend of sweeteners is preferred to be used in food industry. For example, some sweeteners give unpleasant tastes which can restrict their utilization in foods and drinks. Saccharin and cyclamate are well-known examples to blend sweeteners where saccharin has a bitter taste that proportionally increased with its concentration. At high concentration, bitter taste is predominated and sweet taste is lost. Cyclamate is used to mask the bitter taste of saccharine, in contrary, saccharin is used to mask the unpleasant taste of cyclamate. The other widely used blends in food and beverages are; aspartame/ saccharin and aspartame/ acesulfame potassium. Sweetness and texture profile of foods can be reformulated by using blends of sweeteners which are mixed in certain proportions. Thus, the sweetness can be stabilized to produce new products. Artificial sweeteners have been shown to have some harmful effects on animals (Gupta et al., 2013). For example, saccharin has been found to cause cancer in animals. In recent years, cyclamate was not a reliable sweetener because of its carcinogenic effect. Additionally, if aspartame is consumed too much, it causes hereditary disease called phenylketonuria. The other sucrose-rich artificial sweeteners are related with the risk of cancer formation (breast, pancreatic and colon) (Larsson, Bergkvist, and Wolk, 2006; Dragsted et al., 2002; Kumar, Narayanan, and Ravi, 2015). On the other hand, natural based non-nutritive sweetener, stevia glycoside, is used as a sweetener in many countries. Many studies have been performed and it was demonstrated that it has no adverse effects on humans. It has therapeutic effects such as anti-hyperglycaemic, anti-hypertensive, anti-tumor, anti-oxidant etc (Jayaraman, Manoharan, and Illanchezian, 2008; Jeppesen et al., 2003).

2.4. Factors Effecting Marmalade Quality

The food industry should develop its industrial product portfolio by creating products in a wide range of dietary and sensory profiles to meet the needs of consumers. Raising the nutritional knowledge, as well as relatively increasing the quality of life, can be achieved by the development of new foods and preservation technologies. Formulation of new products by using different or substitutional ingredients should be similar to the traditional product in terms of rheological, textural, structural, and sensorial properties (Renard, van de Velde, and Visschers, 2006; Basu et al., 2011). Rheological properties influencing the overall acceptability are among the major factors specified by the consumer demands in addition to the sensory attributes of marmalade (Marjan and Javanmard and Endan, 2010; Sagdic et al., 2015). Food rheology considers the flow of a food matrix, the flow characteristics of individual food constituents displaying a complex rheological function, and the influence of processing methods on the structure of food and its features. Rheological studies are closely associated with the development of new products. In order to achieve a food product with an acceptable quality, the addition of ingredients at varied composition extensively requires the rheological understanding of their relation to food processing. Thus, the rheological characterization of the product is important in formulation of the product using different ingredients. In addition, rheological properties of food systems also contribute to optimizing the formulation procedure, along with detecting the functionality and quality control of ingredients (Fischer and Windhab, 2011; Sagdic et al., 2015). Rheological and mechanical measurements in combination with sensorial analysis are used to determine the different functionality of ingredients in the development and quality control of the new product. They are also correlated with sensory and texture changes (Basu and Shivhare, 2010). For the marmalade product, the relationships between food texture and structure of the gel are related to the variation of the ingredients or their concentrations. The changes in the gel are determined by texture analysis or by sensorial evaluation (Renard, van de Velde, and Visschers, 2006; Basu and Shivhare, 2010; Gao et al., 2011).

2.4.1. Quality of Traditional and Reduced Sugar Apple Marmalade (RSAM) Products

In general, the quality characteristics of marmalades are evaluated objectively by considering their texture, color and rheological behavior. Their gel texture and structure are evaluated subjectively by sensory and computer vision techniques (Javanmard et al., 2012; Gao et al., 2011). In the literature, there are different studies showing the effect of sweeteners on product properties. Basu and co-workers (2007, 2010, 2011, 2013) prepared fruit jams using various fruits. They investigated the effect of sugar types and concentration, pH, temperature, and pectin concentration on the rheological and textural parameters of the jams (Basu, Shivhare, and Raghavan, 2007; Basu and Shivhare, 2010; Basu et al., 2011; Basu and Shivhare, 2013; Basu, Shivhare, and Singh, 2013). They reported that mango jam showed pseudo-plastic characteristics with yield stress. It was shown that Herschel–Bulkley model was the best suited rheological model under studied conditions in which a wide variety of sugar and pectin range, temperature and pH level were used. When the sucrose concentration was increased to 60%, the loss and storage module increased. However, a gradual decrease in the modulus values was observed when sugar concentration was above 60%. Besides the rheological properties of fruit jams, textural properties were also obtained to determine the jam characteristics. Falguera et al. (2010) pointed out that the addition of calcium molecules to the jams enhanced the nutritional benefits in the end product, as well as promoting the gelation of pectin at the low amount of sugar concentrations.

Garrido et al. (2015) investigated the influence of different juice proportion, final soluble solid content, a wide range of pectin concentrations and pH values on the rheological and mechanical characteristics of apple jelly and maximized its overall acceptability as much as possible by optimization of formulation parameters. They found that the pectin gel strength increases while increasing pectin concentration which is the main factor affecting all the properties. Furthermore, soluble solid content is effective on the cohesiveness of the jelly while juice ratio has a significant effect on storage modulus and adhesiveness and also affects overall acceptability much more than other factors. Gajar and Badrie (2002) prepared a low-calorie christophene jam made with using various carrageenan concentrations and different pectin types and levels. The authors investigated the effects of the factors on the gel set and texture of jam during storage. The addition of aspartame, saccharin with aspartame, and sucralose in the christophene jam prepared as a low-calorie jam provided not only sweetness but also bulkiness. Besides this, saccharin just improved the sweetness. In this research, they evaluated that sucralose was the most favorite sweetener in comparison with the others and pectin was more effective on the set gel while the texture of the jam was not affected. Muhammad et al. (2008) studied the physicochemical and organoleptic properties of diet apple jam during 3 months of storage. They used Aspartame, cyclamate and saccharin individually or in combination (aspartame + cyclamate, cyclamate + saccharin and aspartame + saccharin). They prepared six different formulations for diet apple jam. They found that while the ascorbic acid content, moisture content, pH, and non-reducing sugar content decreased, the acidity, TSS and reducing sugar content increased during storage period. Besides, the apple jam prepared using aspartame (2.08g) + cyclamate (12.5g) with added sodium benzoate and the one made with combination of aspartame (2.08g) + saccharin (1.25g) with added sodium benzoate were the most preferred products and scored the highest by the panelists in the sensory evaluation. Tamer et al. (2010) conducted a study to produce low-calorie pumpkin dessert with the addition of aspartame and acesulfame-K artificial sweeteners. They evaluated the physical and chemical properties of pumpkin desserts and the effects of sweeteners on sensory properties of the end product. The authors concluded that the calorie value of the product by using aspartame and acesulfame-K could be reduced up to 55% without affecting the odor, taste, and texture of the pumpkin dessert. Vilela et al. (2015) reported that jams can be made by replacing sugar with sweeteners such as fructose, sorbitol, and fructooligosaccharides (FOS). They studied to develop the new formulation of a jam with suitable nutritional profiles by maintaining its textural and sensorial attributes compared to traditional ones. Eventually, they obtained that the sweeteners significantly influenced the quality attributes of the jam. It was stated that the jams made with fructose were similar to those prepared with sucrose. Besides, using fructose and FOS or sorbitol and FOS in combination led to a critical decrease in the energy value of jams. Rubio-Arraez et al. (2015) prepared an orange marmalade by using different proportions of healthy sweeteners (tagatose and oligofructose) and investigated their influences on the physicochemical, optical and rheological characteristics and microbiological stability of the marmalade during 45 days of storage. The authors found that the initial antioxidant capacity of the marmalades was enhanced when the 70% of oligofructose was used in the formulation. On the other hand, the marmalades, containing both oligofructose and tagatose at the same proportions, had high consistency and improved elasticity. The preference of the marmalades formulated with sweeteners was as acceptable as marmalade containing sucrose.

Another study also conducted by Rubio-Arraez et al. (2017) to develop a lemon marmalade formulation by replacing sucrose with sweeteners, namely tagatose, and isomaltulose. They monitored the antioxidant capacity, °Brix, pH, moisture, water activity, rheological, microbiological and optical attributes of marmalades during 60 days of storage. It was found that the new marmalade formulations had lower antioxidant capacity and lower consistency compared to those made with sucrose. Furthermore, the marmalades made with a higher ratio of isomaltulose (60%) had high luminosity compared to other samples. All lemon marmalades reformulated with sweeteners had better acceptability scores compared to the ones prepared with sucrose.

Belović and co-workers (2017) developed formulations to produce low-calorie jams with high dietary fiber content obtained naturally from tomato pomace. They prepared four jam formulations: Jam 1 formulation was prepared with sucrose without adding pectin. In Jam 2 and Jam 3 formulations, sucrose was partially substituted by stevioside. Jam 4 formulation was prepared for diabetic patients by fully replacing the fructose with stevioside. All the jam formulations had lower energy (87.1 to 193.7 kcal/100 g) and lower total carbohydrate content (17.23 to 43.81%) compared to the commercial jam. In addition to that, jams including tomato pomace had 15–20 times more dietary fiber compared to commercial apricot jam.

CHAPTER 3

MATERIALS AND METHODS

3.1. Raw Materials

Apples *(Malus domestica 'Gala')* were purchased from market place in Izmir, Turkey, at spring season, in 2017. Stevioside (Pure Stevia Extract 95% Rebaudioside-A) and Sucralose (Vitasweet®Sucralose) were kindly provided by Egepak A.Ş., Izmir, Turkey. Sucrose and lemons were purchased from a supermarket in Izmir, Turkey.

3.1.1. Sample preparation

Low sugar apple marmalade (homemade style) was prepared by following the steps presented in Figure 3.1. Firstly, apples were sorted and cleaned in order to get rid of all the foreign materials, including parts of branches and leaves, and defective portions. Fruits were washed with tap water and, stalks and cores were removed. Then, fruits were divided into four equal parts. Skin was peeled, and the fruits were boiled in water in a saucepan. The softened fruits were filtered through a sieve to obtain apple pulp. Then, the desired amount of sucrose and sweetener was added. The mixture was thoroughly stirred and boiled until the product became a marmalade form. Fifteen-ml of lemon juice was added into marmalade mixture just before removing it from the stove. Heating was stopped when total soluble content (TSS) reached to 60–65°Bx. Prior to filling, jars were steeped into the pot of boiling water for the sterilization process. The marmalades were hot filled into a hot jar. The jars were turned upside down on a clean towel, and they were completely cooled before storage. Samples were stored at 4 ± 2 °C.

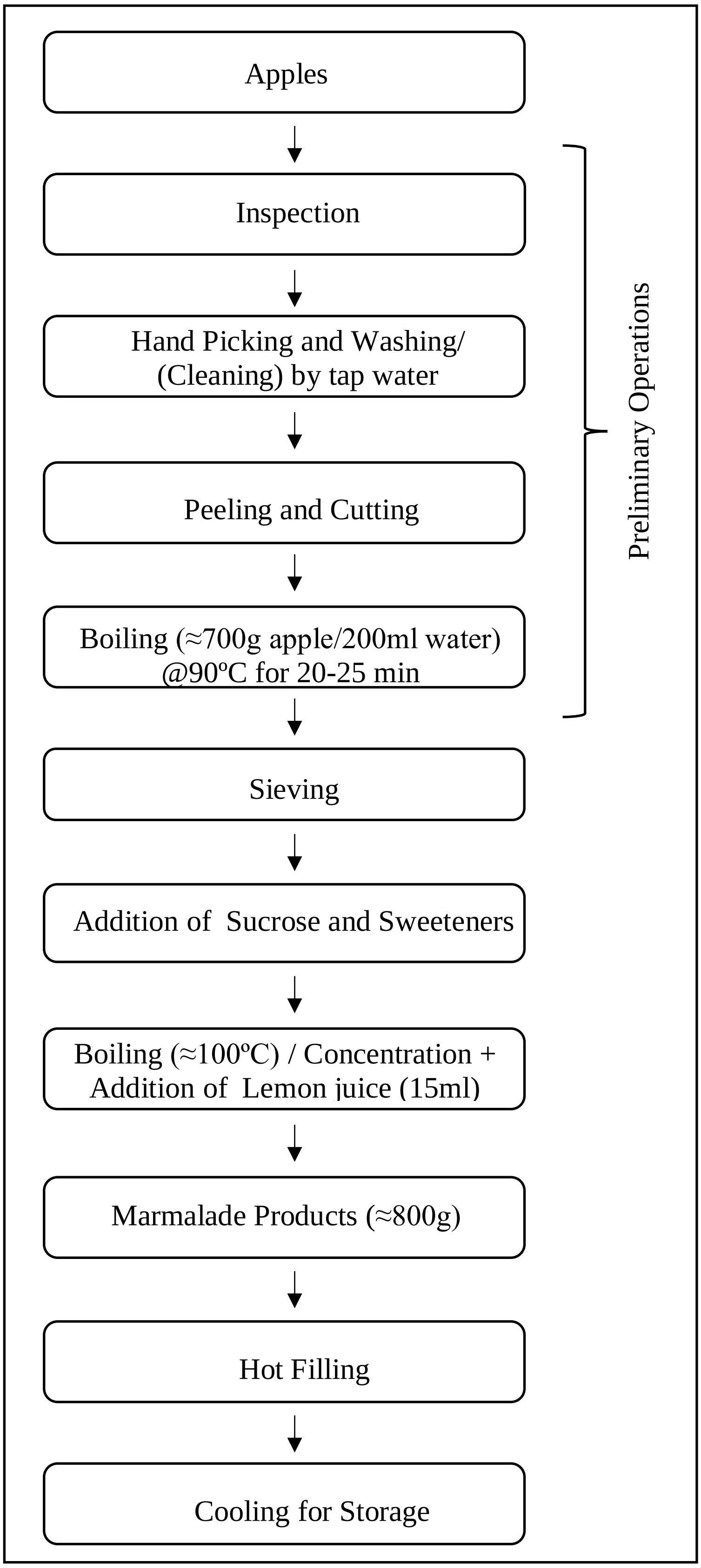


Figure 3.1. Schematic Representation of Processing Steps for the Production of Low Sugar Apple Marmalade (Homemade Style)

3.1.2. Experimental Design

Experimental design and analyses performed with marmalade samples were demonstrated schematically in Table 3.1.

Table 3.1. Experimental plan of apple marmalade preparation with alternative sweeteners (basis = 1000 g apple)

Formulation No	Sucrose level (g)	Sucrose Replaced (%)	Amount of Sucrose (g)	Stevioside (mg)	Sucralose (mg)
1	500	0	500	0	0
2	500	25	375	416.67	0
3	500	50	250	833.33	0
4	500	25	375	0	208
5	500	50	250	0	416
6	600	0	600	0	0
7	600	25	450	500	0
8	600	50	300	1000	0
9	600	25	450	0	250
10	600	50	300	0	500

In the preliminary experiments, the amount of sucrose required for marmalade production using 1 kg of apple was determined to be 500 g and 600 g. These quantities were determined by conducting preliminary sensory analysis. Marmalade samples prepared with sucrose were used as controls (500g, 600g). Depending on the prescription, 10 different types of marmalade formulations were produced, 2 of which were control samples. In each marmalade production, approximately 800 g of product was obtained.

Production of marmalade with alternative sweeteners (natural and artificial) was implemented by partially replacing sucrose in the formulation. Two levels of sucrose substitution (25%, 50%) was carried out with Stevioside (natural sweetener) and Sucralose (artificial sweetener), and the substitution was done on a weight basis of sucrose used. Relative sweetness factor of Stevia and Sucralose was considered for calculation of the amount needed for supplementation. The relative sweetness of Stevioside and Sucralose were 300 and 600, respectively. Apple marmalade prepared using alternative sweeteners, the partial replacement by adding of each additive was named as alternative sweetener quantity in the experimental design (Table 3.1). In order to meet the desired sweetness in the product, 500 g or 600 g sucrose was required to produce 800 g marmalade from 1 kg apple. Thus, sugar concentration in the marmalades were 62.5% and 75%. The reduced sugar marmalades were prepared by partially replacing the amount of sucrose with the sweeteners in the recipes shown in Table 3.2. For example, 125 g of sugar was removed from the recipe to replace 25% of sucrose in the product prepared using 500 g sucrose. This amount of sucrose was replaced with stevioside or sucralose by considering their sweetness index. In order to replace 125 g sucrose, 125/300 = 0.416 g stevia and 0.208 g sucralose were required.

Table 3.2. Formulations of Low Sugar Apple Marmalade Samples

Formulation No	**Low Sugar Apple Marmalade Formulation**
1	**500 g Sucrose** (Marmalade samples prepared with using 500g of sucrose only)
2	**Stevioside-25 + 75% Sucrose (500g)** (Marmalade samples prepared by replacing 25% of 500g sucrose with Stevioside sweeteners)
3	**Stevioside-50 + 50% Sucrose (500g)** (Marmalade samples prepared by replacing 50% of 500g sucrose with Stevioside sweeteners)
4	**Sucralose-25 + 75% Sucrose (500g)** (Marmalade samples prepared by replacing 25% of 500g sucrose with Sucralose sweeteners)
5	**Sucralose-50 + 50% Sucrose (500g)** (Marmalade samples prepared by replacing 25% of 500g sucrose with Sucralose sweeteners)
6	**600 g Sucrose** (Marmalade samples prepared with using 600g of sucrose only)

(Cont. on next page)

Table 3.2 (Cont.)

7	**Stevioside-25 + 75% Sucrose (600g)** **(**Marmalade samples prepared by replacing 25% of 600g sucrose with Stevioside sweeteners)
8	**Stevioside-50 + 50% Sucrose (600g)** (Marmalade samples prepared by replacing 50% of 600g sucrose with Stevioside sweeteners)
9	**Sucralose-25 + 75% Sucrose (600g)** (Marmalade samples prepared by replacing 25% of 600g sucrose with Sucralose sweeteners)
10	**Sucralose-50 + 50% Sucrose (600g)** (Marmalade samples prepared by replacing 50% of 600g sucrose with Sucralose sweeteners)

3.2. Methods

The measurements of the physicochemical, rheological, textural, sensorial and microstructural properties were obtained after production of low sugar apple marmalade samples.

3.2.1. Measurement of Physicochemical Properties of Marmalades

Water activity (a_w) of RSAM samples were measured by using Rotronic Hygrolab 3 bench top apparatus (Rotronic Hygrolab, UK) at room temperature. The marmalade sample was put into the small sample cup and loaded to the half-line of the cup. The sample cup was placed inside the equipment chamber. The water activity values were recorded within an approximately 3 min. All measurements were replicated 3 times. The average values of the data were calculated for each sample.

Total Soluble Solids (TSS) content was measured with a digital hand-held refractometer (ATAGO Pocket Refractometer, PAL-3, Tokyo, Japan) at 25°C. Initially, the refractometer was calibrated with distilled water. After that, the total soluble solids content of RSAM samples were determined according to the following method given below (Cemeroğlu, 2007):

- 10 g of sample weighted and dissolved in 10 ml distilled water. The solution was continuously stirred with a glass rod, then boiled approximately 2-3 min.
- After cooling at room temperature, the solution was transferred to 50 ml of volumetric flask and completed with distilled water.
- The solution was shaken thoroughly, and left for 20 minutes for settlement, and filtered through a cheesecloth.
- 1-2 drops were taken from the filtrate and then reading was done by a refractometer.
- The soluble solids content of solution was calculated by taking the dilution ratio into consideration according to following equation (3.1).

$$\% \text{ Soluble Dry Matter in water (g/ 100ml)} = B \times V / S \qquad (3.1)$$

B: Brix value in diluted samples

V: Volume (ml) to dilute the sample

S: Amount of Sample (g)

The ash content of apple marmalade was determined using a method described by Cemeroğlu (2007). 2 g of sample was weighed into each crucible after determining the tare of the crucibles which were brought to constant weight in a muffle furnace. A few (2-3) drops of 95% ethyl alcohol (ethanol) were then added on each sample. The crucibles were placed in the muffle furnace. Initially, the furnace was operated at 105 ºC. After the samples was ignited at 105 ºC for 1 hour, the temperature was gradually increased to 550 ºC in order to prevent the overflow of marmalade samples. After obtaining white ash (approximately 5-6 h) residue, the samples were cooled to 105 ºC in the furnace. The crucibles were placed in a desiccator for cooling to the room temperature and weighted on the electronic balance. The ash content is the inorganic residue remaining and it is expressed as a percentage of the total weight of marmalade incinerated. The experiment was done in triplicates.

Moisture contents of apple marmalade were obtained using vacuum oven method according to Cemeroğlu (2007). The empty glass petri dish and lid were dried in an oven at 105 ºC for 3 hours and then weighed. 3 g of sample was put into the dish and placed in a vacuum oven at pressure of 133 mPa and temperature of 70 ºC for 16-18 hours until reaching a constant weight. After that the samples were placed in a desiccator, cooled to room temperature and weighed. This procedure was repeated until the difference in weight between the initial and final weighing is less than 40 mg. The moisture content expressed as the percentage of the dry sample weight (Eqn. 3. 2).

$$\text{Moisture Content (\%)} = \frac{\text{(Weight of marmalade – Dry marmalade)}}{\text{(Weight of marmalade)}} \times 100 \qquad (3.2)$$

Total dry matter of the apple marmalade was also calculated using the following equation 3.3 or 3.4:

$$\text{Total Solids (\%)} = \frac{\text{(Weight of dish + Dry marmalade) – (Weight of dish)}}{\text{(Weight of marmalade)}} \times 100 \qquad (3.3)$$

or

$$\text{Total Solids (\%)} = 100 - \text{Moisture Content (\%)} \qquad (3.4)$$

The pH of the apple marmalade samples was determined by using a bench top (WTW Inolab 7310, Germany) pH meter calibrated with buffer solutions of pH 7 and pH 4. Ten gram of sample was weighed and filled up to 25 ml with distilled water. After the diluted sample was stirred with glass rod for about 2-3 minutes, pH of the sample was measured. Before each measurement, the electrode probe was rinsed with distilled water properly. All of the measurements were carried out at room temperature in three replicates (Cemeroğlu, 2007).

Ten g sample was poured into a flask and diluted with 25 ml distilled water. The diluted marmalade solution was titrated with a standardized 0.1 N sodium hydroxide (NaOH) up to pH value of 8.1. The volume of NaOH solution used to reach a titration endpoint was recorded. The percentage of titratable acidity of the apple marmalade samples (%) was calculated using the equation 3.5 (Cemeroğlu, 2007) and expressed as percentage of citric acid.

$$\text{TA (\%)} = (V) * (f) * (E) * 100 / M \qquad (3.5)$$

V: The volume of 0.1 N NaOH used for titration (ml)

f: Normality factor of NaOH solution (g)

E: Miliequivalent weight of citric acid for 0.1 N NaOH (0.006404 g)

M: Weight of the sample (g)

Color properties of RSAM were determined by means of CR 400 chromometer (Konica Minolta, Osaka, Japan) using Illuminant D_{65}. The instrument was adjusted utilizing a standard white tile. A cylindrical glass cell (5.5 cm in diameter) loaded with 50 g of samples was set on the top of the light source. The color values were expressed as CIE L*(Brightness), a*(redness-greenness) and b* (yellowness–-blueness) (CIE, 1976). Color measurements of all samples were conducted at room temperature. Three readings were taken at three different positions in the measurement screen with respect to L, a, b coordinates. Result indicates the mean and standard deviation of readings.

3.2.2. Rheological Measurements

The rheological measurements of reduced sugar apple marmalades were conducted at 30 °C by using AR 2000-ex rheometer (TA Instrument, New Castle, DE) equipped with Peltier Temperature Controller Unit. Temperature control system kept the temperature of the sample constant throughout the measurement. Samples were allowed to equilibrate to room temperature for at least one hour before testing. Measurements were performed using a 25 mm stainless steel parallel plate configuration system with a gap of 1 mm in a controlled-stress rheometer. Before any measurements, the instrument was calibrated to ensure the accuracy of test equipment.

The main components of a controlled stress rheometer are shown in Figure 3.2. The rheometer has a constant torque motor which works through a drag cup system. The movement of the measuring system fixed to the shaft are controlled by an angular position sensor. Samples are loaded between two parallel plates which top plate is mobile and the other is stationary. A rotational shear stress was performed on the sample by applying a torque on the top plate. The conversion from the applied torque value to a shear stress value were automatically recorded by the software of the instrument which is called a RheoWin Data Manager (RheWin Pro V.2.64).

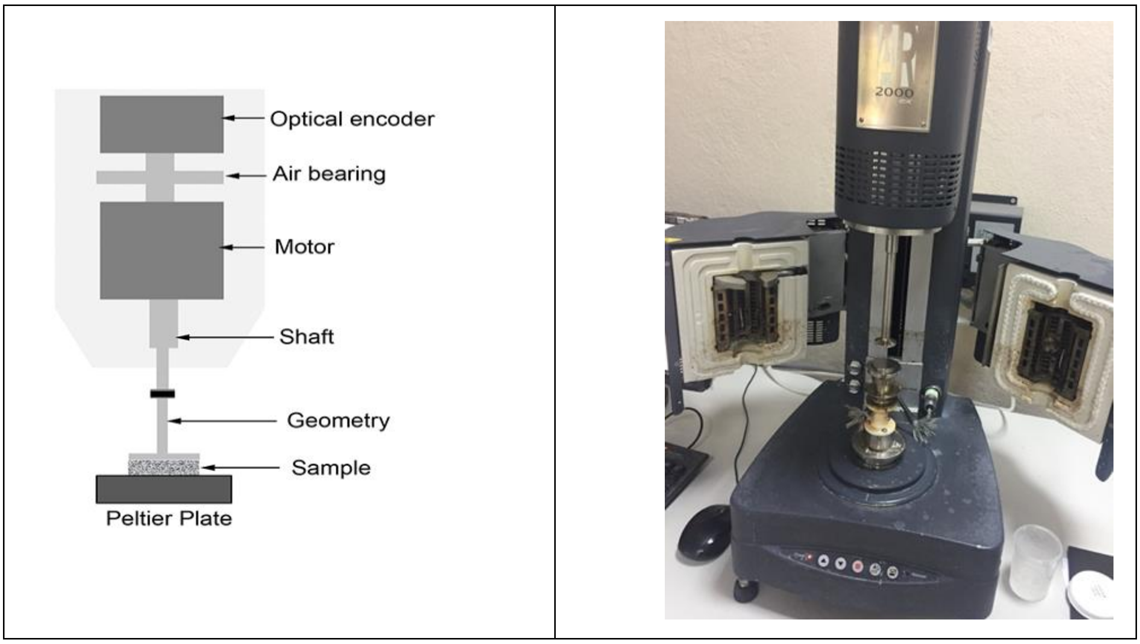


Figure 3.2. Schematic diagram and picture of a stress-controlled rheometer

The dynamic rheological measurements were conducted by following oscillatory stress sweep, stepped flow, oscillatory time sweep and oscillatory stress sweep tests.

Step 1: Oscillatory Stress Sweep Test

In the dynamic oscillatory test, the storage modulus (G′) and loss modulus (G″) were measured. The frequency dependent functions G′ (ω) and G″ (ω) are storage modulus and loss modulus, respectively. G′ is a measure of the energy stored and subsequently released per cycle of deformation per unit volume. It is the property that relates to the molecular events of elastic nature. G″ is a measure of the energy dissipated as heat per cycle of deformation per unit volume. G″ is the property that relates to the molecular events of viscous nature (Gunasekaran and Ak, 2000). In summary, the storage modulus represents storage of elastic energy, and the loss modulus represents the viscous dissipation of that energy. Oscillatory stress sweep test helps to determine the range of linear viscoelastic response (LVR) under oscillatory shear conditions (Figure 3.3). The stress sweep tests were carried out at a frequency of 1 Hz. Samples were initially stored at room temperature for minimum one-hour prior to testing. Viscoelastic properties of reduced sugar apple marmalades were performed at 30°C by using AR 2000-ex rheometer (TA Instrument, New Castle, DE) equipped with a temperature-controlled system.

Samples were placed between the parallel plates geometric configuration (diameter= 25 mm) with a gap of 1 mm. After loading the samples, there was a waiting time of 10 minutes to allow the samples to achieve thermal equilibration. For dynamic oscillatory test, storage modulus (G′) and loss modulus (G″) were measured at torque range 0.1-10000 μN.m. This test allowed to determine the general range of Linear Viscoelastic Region (LVR) of the samples. In subsequent testing, this shear stress (torque) range was adjusted appropriately to collect reliable data. The test was performed at 1.0 Hz frequency, and 20 points recorded per decade in the logarithmic manner. These conditions were selected as the most appropriate operating conditions for collecting data. All measurements were done triplicate. Data were analyzed and reported as averages of three replicates.

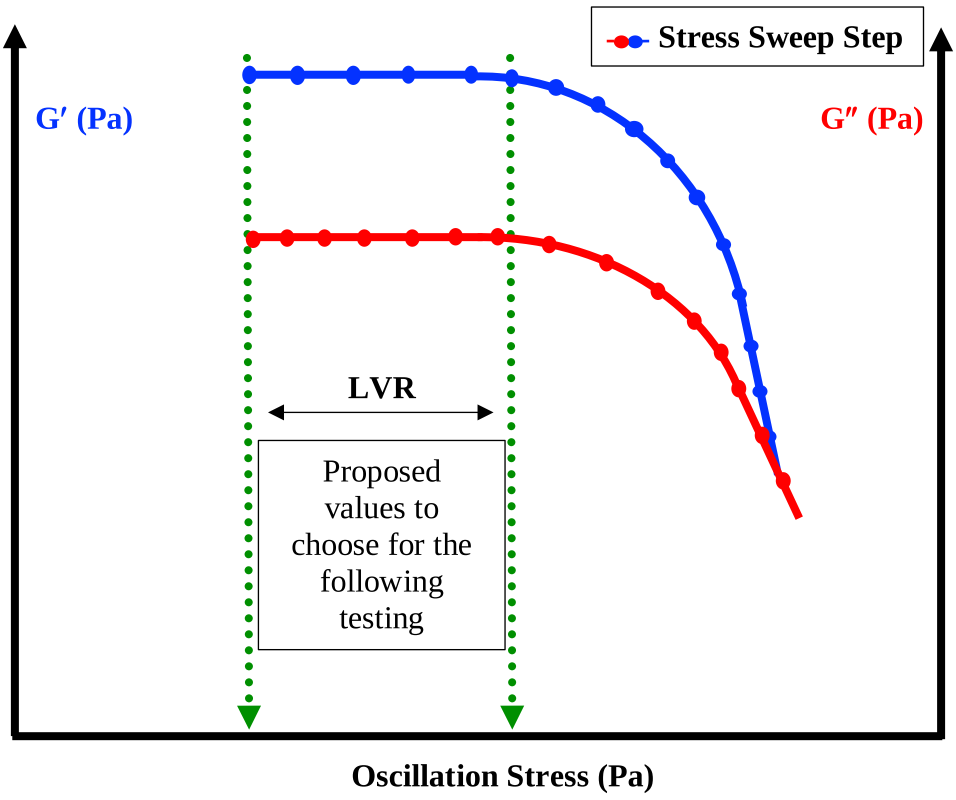


Figure 3.3. Linear Viscoelastic Region (LVR)

Step 2: Stepped Flow Test

Marmalade samples were required to be pre-sheared before collecting the data. Pre-shearing will help to determine a zero-time of shear, effectively eliminating any structure history prior to loading. Thus, Pseudo-Viscosity Profile of each marmalade samples was obtained by stepped flow test. Data were recorded as viscosity vs. torque/stress and converted to viscosity vs. shear rate (Fig. 3.4).

The measurement temperature was set at 30°C, and samples were allowed to equilibrate to 30°C for 10 minutes in the same manner as in step 1 before collecting the data. Torque ranges between 250-2500 μNm were determined for each sample formulation as follows: i) Formulation 1: 250- 2500 μNm ii) Formulation 2: 250-1750 μNm iii) Formulation 3: 250-1250 μNm iv) Formulation 4: 250-1750 μNm v) Formulation 5: 250-1250 μNm vi) Formulation 6: 250-2000 μNm vii) Formulation 7: 25-1500 μNm viii) Formulation 8: 250-1250 μNm ix) Formulation 9: 250-1750 μNm x) Formulation 10: 250-1500 μNm. 40 points per decade in logarithmic scale were selected to collect data in a low torque range with a more consistent manner. Data were plotted as viscosity versus shear stress and viscosity as shear rate.

Apparent viscosity (η_a) of the apple marmalade samples was obtained from the peak-hold step in data analysis software of rheometer at 30°C. Since for non-Newtonian fluids, apparent viscosity is expressed as a function of shear rate. This test was performed at constant shear rate of 100 s^{-1} within 600 s. Each measurement was done in triplicate. Data were analyzed and reported as averages of three replicates.

Modelling of rheological data obtained in step 2:

The flow characteristics of materials can often be expressed by different types of rheological models as depicted in Figure 3.5. The collected data, i.e., the relationship between shear stress and shear rate data has been mathematically described by means of Power Law, Herschel-Bulkley, and Casson model.

Power Law Model

As a time-independent, the Power Law model (Eq. 3.6) defines the data for many food materials exhibiting shear thinning and shear thickening behavior. In order to describe the fluid behavior, the model contains two parameters.

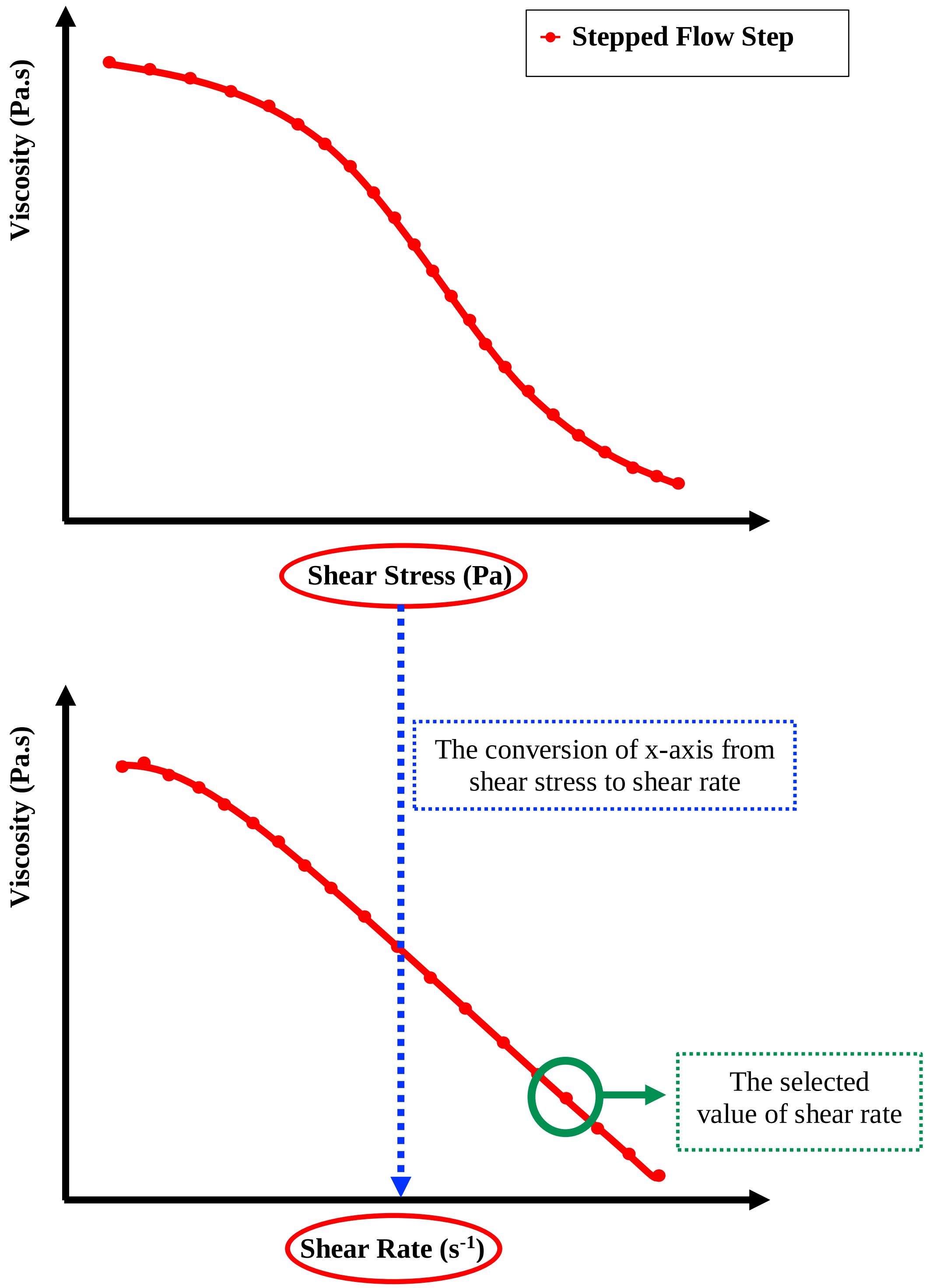


Figure 3.4. Diagram of Shear Stress and Shear Rate

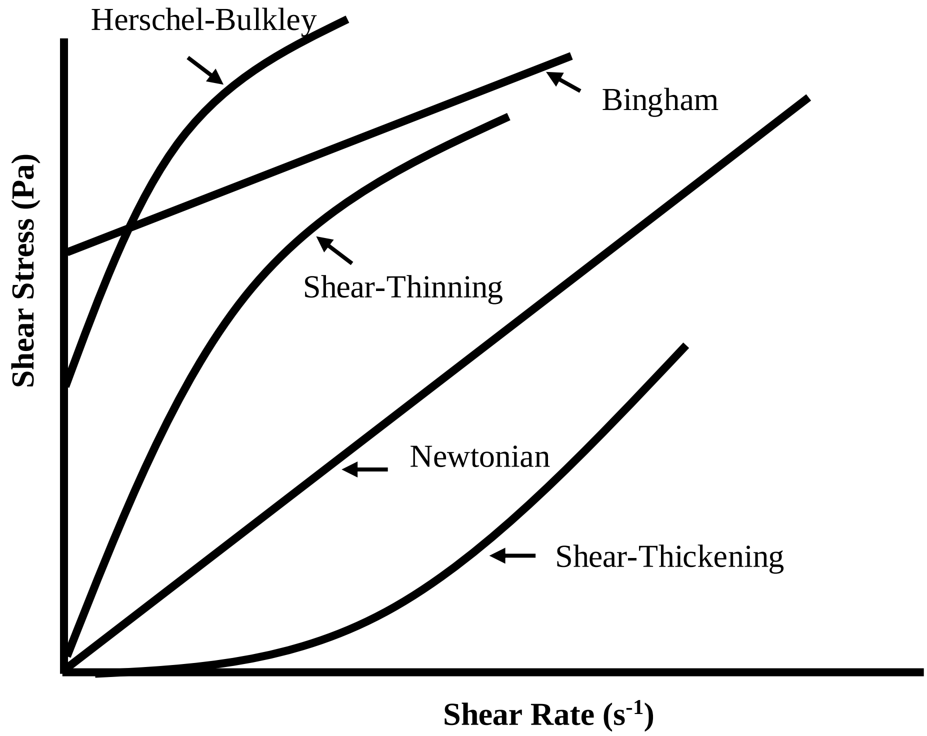


Figure 3.5. Types of Rheological Behavior Flow Curves

In the Power Law model, the relationships of shear stress-shear rate start with the origin. In the case of plotting on log-log coordinates of shear stress versus shear rate, the relationships between them become the linear plots (Rao 2007; Wagner, Mount, and Giles 2014).

$$\boldsymbol{\tau = K\,(\gamma)^n} \tag{3.6}$$

τ is shear stress (Pa), K is consistency coefficient (Pa.s^n), γ is shear rate (s^{-1}) and n is flow behavior index (dimensionless) (Ahmed, Ptaszek, and Basu 2017).

If n<1, the plot of shear stress versus shear rate is concave upwards which expresses shear thinning behavior.

If n>1, the plot of shear stress versus shear rate is concave downwards which represents shear thickening behavior.

If n=1, the plot is characterized by straight lines, which represent a Newtonian behavior, in terms of shear stress versus shear rate.

When taking the log of both sides of the Eq. 3.7:

$$\textbf{log } \tau = \textbf{log K + n log } (\gamma) \tag{3.7}$$

K and n parameters are obtained by plotting of log shear stress, τ, versus log shear rate, γ. K and n parameters are achieved by plotting of log shear stress versus log shear rate. From the log-log plots, the straight line is clearly obtained. The slope of the line is n, which can be directly read, and the intercept gives the value of log K. Since the model contains only two variables, which identify shear stress-shear rate values, the power law model has been mostly used in studies for the characterization of many foods. (Rao 2007).

Herschel-Bulkley Model

Herschel-Bulkley Model is commonly used to characterize the rheological behavior of certain food materials. If the food product is a semi-solid or concentrated, an additional force can be needed for initiating of the product flow, known as yield stress. In other words, the yield stress is typically defined as the minimum stress required for maintaining a steady shear flow. Sometimes, the experimental data are not fitted to Power Law model because of existence of the yield stress in foods. The power law model can also cover food materials with measurable yield stress. Thus, the model turns into Herschel Bulkley model which can be represented mathematically as follows:

$$\tau = \tau_o + \mathbf{K}\, (\gamma)^{\mathbf{n}} \tag{3.8}$$

τ is shear stress (Pa), **K** is consistency index (Pa s^n), γ is shear rate (s^{-1}) and **n** is flow behavior index (dimensionless), and τ_o is yield stress (Ahmed, Ptaszek, and Basu 2017).

In order to linearize the Herschel–Bulkley model, the yield stress, τ_o, is subtracted from the shear stress, τ, and the $(\tau - \tau_o)$ vs. shear rate is plotted on log-log coordinates.

$$\textbf{log } (\tau-\tau_o) = \textbf{log K + n log } (\gamma) \tag{3.9}$$

n and K values can be obtained by the logarithmic coordinates. K value is intercept and n value is determined from the slope of the plots. The Herschel Bulkley Model is mostly used for gels of various fruits with a yield point. (Ahmed, Ptaszek, and Basu 2017; Rao 2007). The Herschel-Bulkley rheological model describes the flow behavior of the food materials exhibiting shear thinning or shear thickening.

Casson Model

Casson model has been widely used to describe the properties of various food materials which demonstrate a yield stress (Rao 2007). The power law, Herschel-Bulkley and Casson equations are easy to use, and working well for modelling the steady simple shear flows (Barbosa-Cánovas et al. 1996).

The Casson model is a structure-based model and expressed as:

$$\boldsymbol{\tau^{0.5} = K_{0c} + K_c\,(\gamma)^{0.5}} \qquad (3.10)$$

$\boldsymbol{\tau}$ is shear stress (Pa), $\boldsymbol{\gamma}$ is shear rate (s^{-1}), $\mathbf{K_{0c}}$ is square of the intercept.

The plot of the square root of shear rate against the square root of shear stress yields a straight line. The slope of the straight line gives the K_c value and K_{0c} is determined from the intercept. The square of the intercept, $\boldsymbol{\tau}_{0c} = (K_{0c})^2$, is the yield stress of the Casson Model. The square of the slope, $(K_c)^2 = \eta_{ca}$, is the Casson plastic viscosity. Structure-based model applications for the rheological data can provide not only valuable information but also precious insight into the role of a dispersed system structure (Rao 2007). These rheological mathematical models were used to describe the flow behavior of each marmalade samples. The goodness of fit of each model was ascertained considering coefficient of determination (R^2), root mean square error (RMSE) and standard error (SE). The significance level at $p < 0.05$ was used throughout the study.

Step 3: Oscillatory Time Sweep Test

Oscillatory Time Sweep Test was performed to determine if the material properties are changing over the time of testing, i.e. the necessary amount of time to form a stable structure was determined. The shear rate and shear stress values were determined in the previous steps, i.e., oscillatory stress sweep test and stepped flow test, and used in the time sweep test. Thus, the measurements in the time sweep test were conducted at a shear rate of 0.05 s^{-1} and the shear stress of 20 Pa. The analysis was performed by setting the frequency to 1 Hz, sampling time to 5 sec and duration time of experiment to 15 minutes. The samples were pre-sheared for 10 min before testing. In this test, data for time and the storage modulus (G′) were collected. Then, the necessary amount of time to form a stable structure was determined from Figure 3.6.

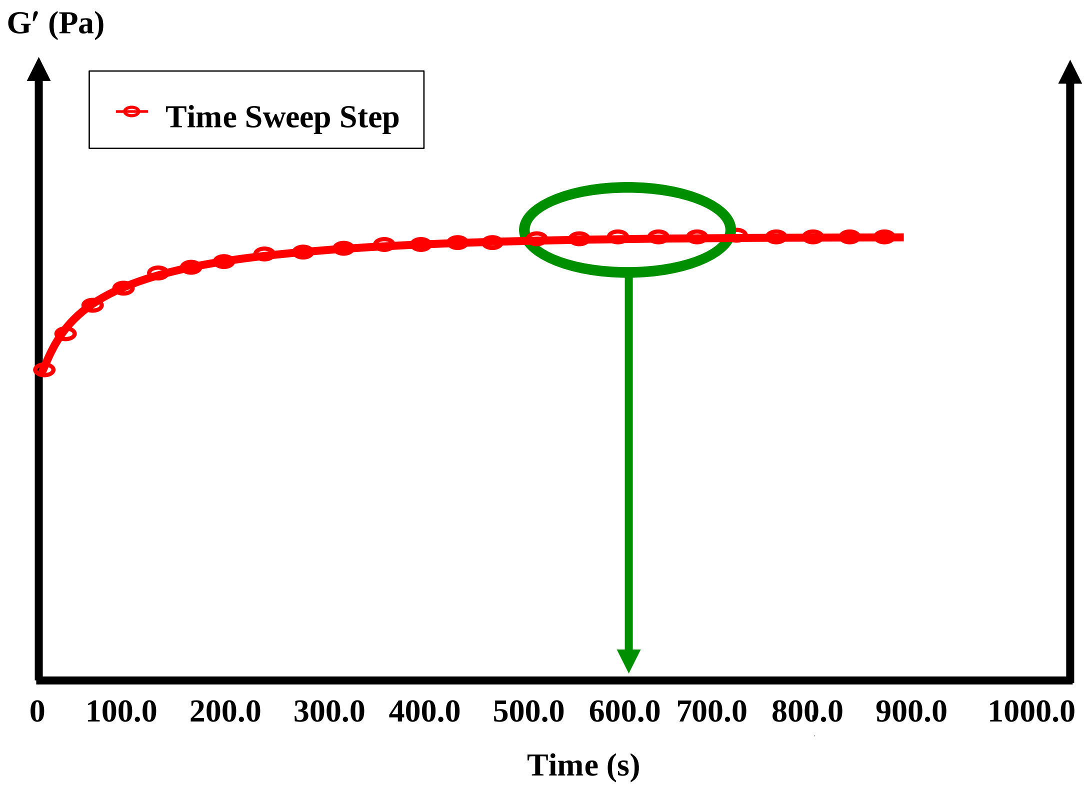


Figure 3.6. Determination of the time required to form a stable structure

Step 4: Oscillatory Stress Sweep Test

Oscillatory Stress Sweep test was carried out to determine the true linear viscoelastic region (LVR). In this test, the material is subjected to sinusoidally deformation with small amplitude oscillatory shear. The resulting deformation caused by mechanical forces is measured as a function of time. The linear and nonlinear regions can appear when increasing from small to large in the applied strain or stress amplitude at a constant frequency. The response of viscoelastic properties is measured by two materials which are the elastic storage and the viscous loss modulus. In the linear regime, the amplitude is small enough for the viscoelastic modulus. In the linear regime oscillatory shear tests, the amplitude is small enough not to destroy the material structure ($\gamma \approx 10^{-2}$-10^{-1} or $\gamma < 10^{-2}$ for the solutions of polymer). In order to understand the relationships among the rheological and the microstructural characteristics of the complex fluids, linear viscoelasticity is a useful theory. However, it is only used in cases of the small deformation (Hyun et al. 2011). When the applied of excessive forces to the sample, the elastic structure is broken down. Therefore, to analyze within the linear viscoelastic region (LVR), the strain or stress should be kept at low. As determining the true LVR, an amplitude sweep is conducted throughout the range of stress or strain. The modulus is stable while the structure of the sample is saved. The stress value in too high results in the breakdown of the structure and a decrease in the modulus. In the resulting curve, LVR length represents an indication of stability. The tests applied in this region are considered nondestructive. Because the forces are extremely low to change the microstructure of the sample. Therefore, all analysis of oscillation should be carried out in the linear viscoelastic region. For that, the specific range of shear stress was selected from step 1 beforehand for each marmalade samples to collect only safety data. The equilibration time period was determined as 10 min because the elastic modulus (G′) exhibited a more stable profile in the oscillatory time sweep test. The shear rate determined in the stepped flow step was adjusted to 0.005 s^{-1} in the conditioning step. The oscillatory stress sweep test was performed in a stress-controlled rheometer AR 2000-ex rheometer (TA Instrument, New Castle, DE) equipped with Peltier Temperature Controller Unit and parallel plate geometric configuration (diameter: 25 mm, gap: 1mm). Experiments were carried out at 30˚C. Data were collected and 20 points recorded per decade in the logarithmic manner. All tests were conducted in triplicate for each marmalade samples. Each data point was expressed as the mean values of three experimental replicates and standard error of the mean.

3.2.3. Texture Profile Analysis

The textural properties of marmalade samples were measured by using a texture analyzer (TA-XT Plus Texture Analyzer, Stable Micro System, UK) with a load cell of 5 kg in three replicates. Texture Profile Analysis (TPA) is composed of two cycles of compression. Before testing, the sample container was loaded with marmalade sample about 3 cm thickness and placed carefully on the center of the instrument's platform. The instrument settings for the measurements were done as trigger force of 0.05 N, pre-test of 2 mm/s, and post-test speed of 5 mm/s. The marmalade samples were compressed with a speed of 2 mm/s by the cylindrical probe (25.4 mm in diameter) in two times during each test. The depth of compression was kept constant at 20 mm throughout all measurements (Garrido et al. 2015). All measurements were performed at room temperature, using the fresh marmalade samples each time. Time and force applied by the probe were measured and the instrument automatically recorded the force–time curve. The resulting force-time curve in Fig. 3.7 is utilized to obtain textural attributes values. These are primary (hardness, cohesiveness, springiness and adhesiveness) and secondary parameters (chewiness and gumminess). The maximum force, known as hardness, required to compress the marmalade sample was detected directly from instrument's software. Adhesiveness (A_3), is the work required to overcome the forces of attraction between the sample and the surface of the probe, was determined as negative area for the first compression cycle. Cohesiveness was calculated as the proportion of the positive areas under the second compression (A_2) to the first compression (A_1) areas. Springiness was also calculated from the ratio between the distance of the second compression peak (L_2) and the distance of the first compression peak (L_1). Generally, gumminess and chewiness values are derived by calculation from the measured parameters. Gumminess, is described as the product of hardness and cohesiveness, was calculated easily by the formula "Gumminess = Hardness x Cohesiveness". Chewiness, is a measure of the work to masticate the food product, was also equal to the "Hardness x Cohesiveness x Springiness". The average values were used as mean and standard deviation.

3.2.4. Sensory Evaluation

Sensory analysis was performed to see which marmalade sample containing alternative sweetener was the most accepted as near as the marmalades prepared with only sucrose. For this purpose, sensory analysis was carried out using the acceptance test which consists of the nine-point hedonic scale. The hedonic test methods are also known as the degree of the liking of a food product (Lawless and Heymann 2010). The method procedures were adapted from Basu and Shivhare (2013).

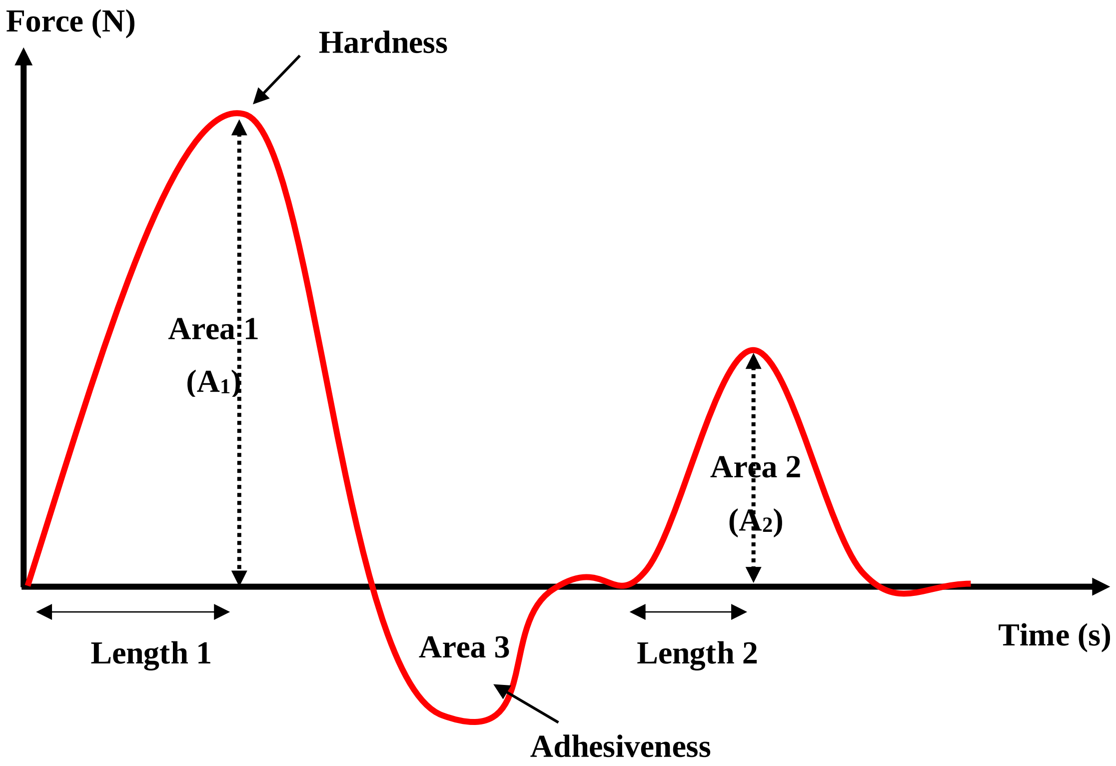


Figure 3.7. Typical Texture Profile Analysis Diagram

The trained panel consisted of twenty-one members (16 females and 5 males, ranging from 25 to 55 years of age) including students and academic staff. The instructions were given to the members who are familiar with sensory analysis techniques in advance. The sensorial parameters were determined as the appearance, taste, color, texture, and overall acceptability and explained to the panelists. Each of these attributes was assessed on a hedonic scale extending from 1 to 9 (9=like extremely, 5 = neither like nor dislike, 1 = dislike extremely) (Lawless and Heymann 2010). Panelists were asked to carefully examine the samples for appearance, taste, color, texture, and overall acceptability. The sensory evaluation sessions were conducted as 3 sections in the laboratory of Sensory Analysis of Department of Food Engineering at Izmir Institute of Technology (Izmir, Turkey).

The sensory evaluation was applied after 3 days of the production of RSAM samples. The samples were removed from the storage at +4 C about 2-3 hours before the analysis, and brought to room temperature. Different proportions of the samples (a tablespoon of each marmalade) were presented to the panel members at room temperature in white odorless plastic dishes coded by three-digit random numbers and all orders of serving were completely randomized. Plastic flat spoons and unsalted crackers were provided to the taste panelists. Unsalted crackers and/or drinking water were offered to cleanse their palates between test tasting and any time during the test as needed. Each panelist considered the marmalade samples given in 3 sections (10 samples per section, 1 section of ~30 min per day). The average values of the scores were used and the results were analyzed by analysis of variance (ANOVA). Panelists have filled in the form that is given on Appendix B.

3.2.5. Microstructure of Marmalades

Scanning Electron Microscopy (SEM) was used to provide information about the food microstructure using images at high resolution. SEM analyses are conducted in vacuum environments (Loehman 1993, Dinger 2005).

SEM was carried out on freeze-dried samples. Before the analysis, freeze-dried marmalade samples were fastened onto conducting sticky carbon tape and then coated with gold to impart electrical conductivity to the sample by Sputter Coater (Emitech K550X). RSAM samples were covered at 15 milliamps flux and under $6x10^{-2}$ mbar vacuum during 1.5 min. All samples were assayed and photographed with a Philips XL 30S FEG scanning electron microscope (SEM) operating at an accelerated voltage of 5 kV and magnification in the range of × 250–2500. SEM images were collected from different places on the RSAM samples. Philips XL 30S FEG Scanning Electron Microscope at IYTE-MAM is used for the analysis.

3.2.6 Statistical Analysis

Data analysis of rheological, textural and physicochemical analysis were carried out by using One-way analysis of variance (ANOVA). One-way analysis was performed to designate how significantly the different concentrations of apple marmalade which was prepared with both only sucrose and sucrose-sweeteners combinations affected the changes in the physicochemical, textural, optical characteristics and sensory scores of the marmalade. The difference on the properties between the marmalade formulations was compared with Tukey’s multiple comparison test using MINITAB 17 (Minitab Inc., State

College, PA, USA). The significance level at $p < 0.05$ was used throughout the study. In other words, the ANOVA test was conducted for all experimental values at 95 percent confidence interval.

For modelling, rheological data was analyzed by using commercial spread sheet (Excel, Microsoft Corp., 2015). All measurements were carried out in triplicate. The results were expressed as their means and standard deviations. The means were assessed in terms of Tukey comparison test.

CHAPTER 4

RESULTS AND DISCUSSION

4. 1. Physicochemical Properties of Low Sugar Apple Marmalade

Plant composition is affected by some factors such as region, soil type, variety, ripening state, irrigation and weather conditions. The composition has an effect on marmalade characteristics (Yildiz and Alpaslan, 2012). The results of physicochemical properties of low or reduced sugar apple marmalade (RSAM) production are listed in Table 4.1.

Water activity (a_w) of the marmalades ranged between 0.77-0.91. It was found that the water activity of the formulation 6 was the lowest compared to other formulations. Because the formulation 6 contained the highest percentage of sucrose (600g). It was found that the water activity content of the marmalade products was reduced with increasing sucrose content. Thus, the water activities of marmalades formulated with using only sucrose, i.e., 1 and 6, were much lower than that of formulations containing Stevioside and Sucralose, i.e. 2, 3, 4, 5, 7, 8, 9, and 10. At the same time, the water activity of marmalade 6 was lower than the formulation 1. An increase in the water activity of the product resulted from the increasing sweeteners concentrations. For the formulations where the 500g of sucrose was partially replaced (25% and 50% of sucrose) with Stevioside, the water activities were measured as 0.87 (formulation 2) and 0.91 (formulation 3), respectively. These values arose from the increase in Stevioside concentrations. Similarly, the water activities of formulations prepared with Sucralose in the same manner, i.e., 4 and 5, were found to be 0.87 and 0.91. This was because the formulation 5 had higher concentration of Sucralose (0.42 g) and lower sucrose content (250g) possessing higher free water content, compared to the formulation 4. On the other hand, for the formulations 7 and 8 prepared by replacing 25% and 50 % of 600 g sucrose with Stevioside, the water activity was determined to be lower, i.e. 0.84 and 0.88, than that of formulations considering 500g sucrose basis. Additionally, a significant difference was also observed between the formulation 9 and 10, because 25% to 50% of 600 g sucrose was replaced with the Sucralose in these formulations. As a result, water activity of apple marmalades increased with increasing sweetener concentration and decreased as sucrose content increased. All samples prepared in this study showed similar a_w values to that of lemon marmalades (Rubio-Arraez et al., 2017). Lemon marmalades were prepared by replacing sucrose by sweeteners such as tagatose and isomaltulose. Abid et al. (2018) reported that bacteria growth in pomegranate jam was observed at the a_w values higher than 0.86. Vilela et al. (2015) pointed out that the water activity value must be at least 0.8 for mold growth in strawberry, raspberry and cherry jams made by replacing sucrose with sweeteners. In our case, the water activity of the apple marmalade formulations was found to be higher than 0.8. One of the reason for the high values of a_w is probably because of hot filling of RSAM and the fact that water vapor can condense in the space area between the product and jar's lid. This can lead to an increase in the moisture content of the product. The other reason may be due to the low amount of sucrose in the formulations. A sufficient amount of sugar must be used in the formulations in order to reach the desired a_w. Sucrose is a compound that binds the water and reduces its participation in chemical reactions (Barbosa-Canovas et al., 2007). Thus, reducing the sugar content in the formulations, may cause a problem in the microbial stability of apple marmalades during storage. In this case, the pH (4.1 -3.0) of the reduced sugar products must be lowered by addition of citric or phosphoric acid, and antimicrobials (1000ppm of potassium sorbate or sodium benzoate, as well as 150ppm of sodium sulphite or sodium bisulphite) (Alzomora et al., 1995). Since, the main objective of this thesis is to develop a recipe for home-made style marmalade, neither preservatives nor chemical substances were used in the products. The microbial stability was ensured by lowering pH of the products using citric acid (lemon juice) only. Rubio-Arraez et al. (2015) stated that sweet orange marmalades, developed using sweeteners (tagatose and oligofructose) in different proportions, showed proper microbiological stability at pH values below 3.8. In our study, all pH values were lower than 3.8, which would ensure a proper microbiological stability of these products, as was observed in other fruit jams and marmalades made with lemon, strawberry, peach, plum or apricot (Carbonell et al., 1991; Garcia-Martinez et al., 2002; Rubio-Arraez et al., 2017).

Table 4.1. Physico-chemical properties of RSAM Products

Formulation No	Water Activity (a_w)	TSS (ºBrix)	Total Solid (%)	Total Ash Content (%)	pH	TA (%)
1	0.80 ± 0.00^{d}	65.78 ± 0.58^{b}	73.55 ± 0.77^{b}	0.28 ± 0.06^{a}	3.62 ± 0.01^{a}	0.24 ± 0.00^{f}
2	0.87 ± 0.01^{b}	61.63 ± 0.29^{d}	64.91 ± 0.69^{d}	0.26 ± 0.07^{a}	3.54 ± 0.00^{bc}	0.32 ± 0.00^{cd}
3	0.91 ± 0.01^{a}	52.49 ± 0.00^{h}	54.55 ± 0.55^{f}	0.27 ± 0.01^{a}	3.58± 0.00^{ab}	0.32 ± 0.00^{bc}
4	0.87 ± 0.01^{b}	60.16 ± 0.58^{e}	60.00 ± 0.41^{e}	0.28 ± 0.01^{a}	3.51 ± 0.01^{cd}	0.33 ± 0.01^{abc}
5	0.91 ± 0.01^{a}	50.29 ± 0.58^{i}	52.39 ± 0.75^{g}	0.30 ± 0.01^{a}	3.54 ± 0.00^{bc}	0.34 ± 0.00^{ab}
6	0.77 ± 0.01^{e}	74.79 ± 0.29^{a}	77.36 ± 0.02^{a}	0.30 ± 0.03^{a}	3.63 ± 0.01^{a}	0.23 ± 0.00^{f}
7	0.84± 0.01^{c}	62.31 ± 0.29^{cd}	69.29 ± 0.17^{c}	0.20 ± 0.08 ^{a}	3.55 ± 0.00^{bc}	0.29 ± 0.01^{e}
8	0.88 ± 0.01^{b}	53.79 ± 0.29^{g}	59.35 ± 0.60^{e}	0.25 ± 0.09^{a}	3.56 ± 0.00^{bc}	0.30 ± 0.01^{de}
9	0.83 ± 0.01^{c}	63.44 ± 0.50^{c}	69.71 ± 0.17^{c}	0.17 ± 0.04^{a}	3.47 ± 0.04^{d}	0.23 ± 0.00^{f}
10	0.87 ± 0.01^{b}	55.63 ± 0.29^{f}	58.65 ± 0.54^{e}	0.29 ± 0.02^{a}	3.51 ± 0.04^{cd}	0.35 ± 0.01^{a}

Results reported as mean ± standard deviations, (n=3). Tukey pairwise comparison test was applied for the significant differences in the same column. Means do not share the same letters are significantly different ($p \leq 0.05$). TSS: Total Soluble Solids content, TA: Titratable Acidity

The Total Soluble Solids (TSS) content of the RSAM samples was found to be the highest in the products containing only sucrose. The formulation 6 had a higher proportion of sucrose (i.e. 74.79 °Brix) than the formulation 1 (i.e. 65.78 °Brix). For the marmalade products made by using Stevia and Sucralose sweeteners, the TSS content varied between 63.44 and 50.29 °Brix. A decrease was observed in the TSS values with increasing sweetener concentrations. The formulation 2 had higher TSS values as it contained a smaller proportion of Stevioside sweetener, compared to the formulation 3. Besides, it was found that the increase in Sucralose concentrations significantly decreased the TSS content from 60.16 °Brix to 50.29 °Brix in the formulations 4 and 5, respectively. For the marmalade products formulated on the basis of 600 g sucrose, it was found that the total soluble solids content was also reduced with increasing sweeteners content. For example, the formulation 7 had less percentage of Stevioside concentration, that`s why the TSS content of the formulation 7 was significantly higher than the formulation 8. In the case of Sucralose, when its concentration is increased, the Total Soluble Solids content of the products e.g., formulations 9 and 10, was significantly reduced. The results of this study showed that Sucralose is reducing the TSS content of marmalades more compared to Stevioside when used at the same concentrations in product formulations. Basu, Shivhare, and Singh (2013) observed that in the mango jam prepared with sweeteners, the TSS content was reduced with an increase in the sweeteners substitution. COUNCIL DIRECTIVE 2001/113/EC of 20 December 2001 relating to fruit jams, jellies and marmalades and sweetened chestnut puree intended for human consumption, allows the soluble solids content to be lower than 60 ˚Brix when sweeteners are used in the formulation for these products, rather than sugars. The RSAM products prepared with sweeteners did meet the Directive criteria. Rubio-Arraez et al. (2017) found similar results in comparison with the TSS values of the reduced sugar lemon marmalade. They obtained the TSS values of the lemon marmalades in the range of 55 °Brix and 59 °Brix.

The total solids content of the RSAM products was in the range of 52.39 - 77.35 (%). The highest value was observed in the formulation 6 because the formulation was prepared using the highest sucrose concentration, i.e., 600 g. The lowest value in the total solids content was determined for the formulation 5 having 50% of the Sucralose concentration. In the same manner with the TSS content, the total solids content of the marmalade products was reduced with increasing sweeteners concentrations. For example, there were significant differences ($p<0.05$) between the formulations 2 and 3, although the formulations had the same sweetener, i.e., Stevioside. Similarly, the formulation 8 had a greater amount of Stevioside (1g) than the formulation 7, (0.5g) and had higher total solid content. For the formulations containing Sucralose sweeteners, the total solids content was also reduced with increasing sweeteners percentage. Generally, the total solids content for the formulations made by using 600g sucrose was found to be higher than those made with 500g sucrose. The dry matter is associated with extending the shelf life of jam and preserving the product throughout storage (Abid et al., 2018). They determined the dry matter of pomegranate jams with different amounts of sugar (10, 20 and 30%) and low-methoxylated pectin (0.2, 0.7 and 1.2%). The dry matter contents were higher for jams with 70% of fruits than those with 90% of fruits. It ranged between 49.7 and 59.4% which was in good agreement with our findings. It was desired that low sugar and healthy products were produced by using sweeteners while reducing sucrose content. Consequently, the sucrose content was reduced with increasing sweeteners percentages. Thus, more water molecules, which could not be bound by sucrose, could be present in the product and might affect the microbial stability of the product during storage. Thus, the content of high dry matter is important for food products.

The total ash content is directly related to mineral substances in food products. The total ash content of RSAM products were found to vary between 0.17-0.30 (%). The formulation 5 had the highest value of the ash content, whereas the formulation 9 had the lowest one. There were no significant differences between the ash content of all formulations ($p>0.05$). Thus, the total ash content was not significantly affected by the changes of the formulations. The total ash content values of the RSAM products showed similar results with the literature. For example, Gao et al. (2011) reported that there was no difference in the ash content of the four types of jam, ranging from 0.13 to 0.25. Tokbaş (2009) found that the total ash content of black mulberry jam ranged from 0.38 to 0.50. Also, Tosun (1991) reported that the total ash in the jams made with strawberry, rose, cherry, and apricot was 0.23%, 0.14%, 0.27% and 0.29%, respectively. Additionally, Kıvrak (2010) found that the total ash content of the cherry, strawberry and apricot jams, was in the range of 0.20% -0.36%, 0.11- 0.27%, and 0.30-0.56%, respectively.

The pH values of the marmalade products ranged between 3.47 and 3.63. The highest value was observed in the formulation 6, whereas the lowest one in the formulation 9. The pH values of the formulations containing only sucrose were not significantly different ($p<0.05$) from each other (formulations 1 and 6). For the formulations prepared with sweeteners, the pH values increased by increasing the Stevioside percentage in the formulations (# 2 and 3). On the other hand, there were no significant differences ($p<0.05$) in the pH values of the formulations 7 and 8 although the concentration of Stevioside was increased from 25% to 50%. The formulation 4 had a lower pH value because it included a lower percentage of Sucralose, compared to the formulation 5. Similarly, the pH was increased by increasing Sucralose concentrations in the formulations 9 and 10. Gajar and Badrie (2002) found the pH value to be 3.62 for the low-calorie christophene jam. They also reported that the pH value was in the recommended range of the pH of diabetic jams. Rubio-Arraez et al. (2015) prepared orange marmalades using sweeteners. They found the pH values of the marmalades in the range of 3 and 4. Similarly, the pH values of jams prepared with peach, plum, strawberry, and apricot (Carbonell, Costell, and Duran 1991; García-Martínez et al. 2002) were in the same range. The findings of this study are consistent with the results of these products.

The acidity in food products is associated with product stability and shelf life. The acidity value in jams results from the presence of organic acids in the fruits or adding acids while preparing jam (Kanwal, Randhawa, and Iqbal 2017). Also, the acid addition provides the desired gelation in marmalade, as well as improving the natural fruit flavor (Altuğ et al. 2001). The titratable acidity values of the RSAM products varied between 0.23-0.35 (as percent citric acid). It was determined that there was no significant difference ($p>0.05$) in the formulations (1 and 6) prepared with sucrose only. The titratable acidity was slightly increased by increasing Stevioside sweeteners concentrations in the formulations 2 and 3. Similarly, the acidity values were higher in the formulation 5 containing 50% of Sucralose concentration, compared to the formulation 4 containing 25% of Sucralose. For the formulations 7 and 8, the titratable acidity values were close to each other. The increase in the sweeteners percentage resulted in a slight increase in the acidity values. There were significant differences between formulations 9 and 10 in terms of the titratable acidity. The acidity values increased significantly from 0.23 (%) to 0.35 (%) while increasing the Sucralose content from 25% to 50%. The total amount of acidity, which depends on the types of jams, was higher in the cherry jams and also lower in the rose leaf jam (Kıvrak 2010). Kaplan (2006) reported that the titratable acidity of strawberry, rose, apricot and cherry jams were 0.48%, 0.26%, 0.53%, 0.71%, respectively. Tosun (1991) found that the titratable acidity was 0.18%-

0.66% for the strawberry jam, 0.12%-0.36% for the rose jam, 0.12%- 0.79% for the apricot jam, and 0.28%-1.64% for the cherry jam. The titratable acidity values of RSAM products are in agreement with the results of strawberry, rose, apricot and cherry jams.

4.2. Color Properties of Low Sugar Apple Marmalade

Color changes in the food systems depend on chemical composition of the food material and its structure (Basu and Shivhare 2013). The color of RSAM samples was measured and expressed as CIE color parameter such as L* (lightness-darkness), a* (redness-greenness), b* (yellowness-blueness). The value of L* indicates the lightness of the sample that changes from 0 (dark) to 100 (light), a* value represents the color changes from green (-) to red (+), b* value is the color changes from blueness (-) to yellowness (+). The color parameters of the marmalade samples are given in Table 4.2.

The value L* of the marmalade samples ranged from 22.70 to 29.55. While the formulation 5 had the highest values, the formulation 6 had the lowest values. Among other marmalades, the formulation 6 having the highest sucrose content had a darker color. This is because redness may have occurred during the gelation. Maillard reactions lead to many color and flavor changes in the products. Because the reactions are between sucrose and amino acids, redness or darkness can develop with thermal processing in the final product. In the apple marmalades prepared based on 500 g sucrose, the L* parameters of marmalade samples were significantly ($p<0.05$) different from each other. The marmalade containing 500 g sucrose (formulation 1) was brighter than those made with 600g sucrose (formulation 6). This is due to the fact that the sucrose content of the formulation 1 was less than formulation 6. Igual, Contreras, and Martínez-Navarrete (2010) indicated that high heat treatments could result in a sucrose caramelization, consequently, a darker color could occur in the jam product. Therefore, the parameters L* and b* had the lowest values, but the parameter a* also had the highest value in the marmalade formulations made by sucrose only. For the marmalade made with Stevioside sweeteners, L*value in the formulation 3 was greater than the formulation 2. A significant increase was observed in the L* value by the increased Stevioside substitution ($p<0.05$). Moreover, similar trend was obtained between the formulations 7 and 8 in terms of the color parameter of the value L*. Thus, it was found that raising the Stevioside percentages from 25% to 50% had a significant effect on the color of formulations. There was a similar behavior in the marmalade samples prepared using Sucralose sweeteners. It was found that the sweeteners concentrations had a significant effect on the formulations 4, 5, 9 and 10. The formulation 4 had a darker color than the formulation 5. The increase in the value L* of the marmalade formulations resulted from increasing the sucralose concentrations for the formulations 4 and 5. In addition, it was observed that the formulation 10 showed a lighter color than the formulation 9. The color parameters of formulations were significantly different from each other ($p<0.05$). In general, the marmalade formulations made by using Sucralose sweeteners appeared in a lighter color than those made with Stevioside. This may be due to the response of different sweeteners to the heating process. Sucralose is known to be highly stable at elevated temperatures that are often used in food, beverage, and drug manufacturing processes so that product sweetness levels can be maintained following cooking, baking, and/or pasteurization (Frazier 2007).

Table 4.2. Color Measurements of Low Sugar Apple Marmalade Formulations

Formulation	Lightness/Darkness L*	Redness/Greenness a*	Yellowness/Blueness b*
1	23.81 ± 0.15^{e}	-0.36 ± 0.13^{b}	6.08 ± 0.26^{f}
2	24.93 ± 0.04^{d}	-0.73 ± 0.07^{c}	6.72 ± 0.20^{e}
3	28.16 ± 0.18^{b}	-1.36 ± 0.08^{e}	8.63 ± 0.03^{ab}
4	26.51 ± 0.30^{c}	-1.27 ± 0.06^{de}	7.96 ± 0.18^{c}
5	29.55 ± 0.06^{a}	-1.96 ± 0.01^{f}	9.15 ± 0.18^{a}
6	22.70 ± 0.07^{f}	0.42 ± 0.10^{a}	6.17 ± 0.03^{ef}
7	24.77 ± 0.05^{d}	-0.37 ± 0.06^{b}	7.91 ± 0.09^{cd}
8	26.68 ± 0.27^{c}	-1.14 ± 0.02^{d}	6.59 ± 0.06^{ef}
9	24.81 ± 0.09^{d}	-0.32 ± 0.09^{b}	7.34 ± 0.49^{d}
10	27.74 ± 0.08^{b}	-1.27 ± 0.00^{de}	8.19 ± 0.18^{bc}

Results were expressed as mean ± standard deviations, (n=3). Tukey pairwise comparison test was applied for the significant difference. Each lower case letters in the same column show significantly differences in the color parameters between the formulations ($p \leq 0.05$).

It was found that a* value ranged from -1.96 and 0.42. As expected, a* was low since the reduced sugar apple marmalade samples had quite a light color. This was due to the fact that the pulp of the apple fruit is nearly white. While the formulation 6 had the highest value, the lowest value was observed in the formulation 5. It was found that the formulation 6 appeared to have the highest redness. This can be explained by the fact that the increased redness of the end product resulted from the Maillard reaction occurring between sugar and amino groups with heat treatments. The formulation 6 was significantly different from the formulation 1 ($p<0.05$). This can be associated with the fact that the formulation 6 contained the highest proportion of sucrose content than others. As a result, during the marmalade processing, a higher amount of sucrose interacted with amino acids, contributing to browning. On the other hand, the addition of sweeteners yielded lower values a*. Abid et al. (2018) stated that increasing proportions of pomegranate fruits in jam results in a decrease in a* value. The obtained jams (with higher amount of fruit) were less reddish which could be due to decomposition of the anthocyanins during cooking.

The color parameters of the value b* for the low sugar apple marmalade were found to be ranged from 6.08 to 9.15. The apple marmalade formulations containing sucrose only, i.e. the formulations 1 and 6 were observed to be slightly different from each other. They had similar values in terms of the color parameters of yellowness/blueness. On the other hand, there were significant differences between the formulations 2 and 3 because the increase in the Stevioside sweeteners concentrations contributed to the increase in the value b* of the formulations. In addition, the formulations 4 and 5 also followed the same trend when the amount of the sucralose sweeteners was increased. For the marmalade formulations in which 600 g sucrose was partially replaced with sucralose, the value b* was increased by raising the sweeteners percentages from 25% to 50%. In contrast, the formulation 7 containing Stevioside-25 had a significantly higher value than the formulation 8 (Stevioside-50) ($p<0.05$). The b* value of formulation 8 was very similar to the marmalade prepared using 600g sucrose only (formulation 6). There were no significant differences between the two formulations. Abolila et al., (2015) did not find a significant difference ($p<0.05$) in color scores between orange jams prepared with fructose, Stevioside and Sucralose. Likewise, Kerdsup and Naknean (2013) fully replaced sucrose by sorbitol created a product that showed the same acceptability in color and flavor as normal jam ($p>0.05$). Although, they did not detect any difference in L* and a* color parameters, they observed significant difference in the b* value when substituted sucrose with sorbitol. It was stated that these results were caused by the heating process. The results of our study are in good agreement with those of by Basu, Shivhare, and Singh (2013). They observed some physicochemical reactions during cooking of low calorie mango jam by replacement of sucrose with alternative sweeteners (stevioside and sucralose). The reactions such as acid degradation, Maillard reactions caused changes in the color of the final product. Especially for Maillard reactions, sucrose is an essential component. They found that the lack of available sucrose increased the values L* and b*, whereas the value a* did not change in the jam produced with higher concentrations of sucralose or stevioside. Peinado et al. (2015) found that the addition of citric acid with different percentages had a significant effect on the values a* and b* of the spreadable strawberry products reformulated with the mix of isomaltulose-sucrose.

In general, sugar content of apple marmalade samples had a significant effect on the color parameters. Both the values L* and b* were increased by increasing the sweeteners percentages. Thus, the higher L* and b* values for the low sugar apple marmalades indicated that they were lighter and more yellow as compared to that of prepared with only sucrose. In our study, the white flesh of the apple having no anthocyanin, may have contributed to the increase of b* and L* color parameters. On the contrary, a* values decreased with the sweeteners substitutions. The more negative a* values for the marmalades indicated that they were more greenish. In summary, the marmalade samples turned into more lighter, yellow and green when the sweeteners substitutions were increased from 25% to 50%.

4.3. Rheological Properties of Low Sugar Apple Marmalade

The rheological properties of the low sugar apple marmalade samples were measured at 30 ºC following the four steps procedure as described in the section 3.2.2.

4.3.1. Oscillation Stress Sweep Test

Rheological properties are important for the quality control, storage and processing stability and learning about molecular and conformational changes in food materials. Most food materials exhibit characteristics of both elastic and viscous behaviour and are called viscoelastic. The linear response to any type of deformation can be predicted using the relaxation modulus, G′, in the linear viscoelastic region (LVR) (Dogan and Kokini, 2006). In the linear viscoelastic region, the measured rheological properties are independent of the magnitude of the applied strain or stress. Oscillation stress sweep tests were carried out for each marmalade sample at 30 ºC to determine the suitable stress range in the linear viscoelastic region (LVR). Stress sweep was performed at constant frequency of 1 Hz in a wide range of torque values changing from 1 to 10000 µN.m. The effect of the addition sweeteners containing different concentrations on the storage (G′) or loss (G″) of moduli of the low sugar apple marmalades was measured in that range and the results of test were presented in Figures 4.1, 4.2 and 4.3 and given in Appendix A. As is seen, storage (G′) and loss (G″) moduli exhibited a similar behavior based on oscillation stress sweep test data for all formulations. It was observed that the values G′ were greater than values G″ for any given point during the test range. This demonstrated the dominance of the elastic modulus over the viscoelastic properties of the samples. While both the values G′ and G″ showed a stable pathway independently with the increasing stress value until a critical point, the dynamic modulus decreased in case of over-stress. The formulation 10 prepared by using sucralose-50) significantly contributed to the highest elastic and loss modulus degree in all formulations, as is clearly depicted in Table 4.3. The observations could be an indicator of different particles in nature of bonds or the structure of pectin network in the formulations. Also Figure 4.1 showed that the elastic and loss modulus of the formulation 1 had a higher degree than the formulation 6. This may be due to the deformation of the gel structure of the sample after putting the sample in the plate of the instrument. It can also be associated with the fact that the measurement of the modulus was made from a greater aqueous portion of the sample. There were slight differences in terms of dynamic viscoelastic properties in all formulations containing stevioside, as is depicted in Figure 4.2 and Table 4.3. Both elastic and loss values were similar to each other. The formulation 7 yielded a very close value to the control samples made by using sucrose only. Moreover, the lowest value of both elastic and loss moduli was observed in the formulation 3 containing stevioside-50 among all formulations containing stevioside sweeteners. This can be caused by the formulation exhibiting a fluid-like behavior because of the lower sucrose content. The power of the gel network in the marmalade was increased by the increase of the soluble solids content (TSS). Basu, Shivhare, and Singh (2013) reported that variations in the dynamic modulus with frequency sweep test were observed in the mango jams prepared with stevioside. Also, the authors obtained steeper slopes of dynamic modulus in the frequency test, compared to other TSS values. This was depicted as evidence to the liquid character due to the formulation having 50% soluble solids. Figure 4.5 demonstrated that the formulation 10 provided the highest values of both elastic and loss moduli among the formulations containing sucralose. The addition of sweeteners to the formulations contributed to higher viscoelastic properties of the marmalade samples with respect to those formulations containing sucrose. While the formulations 5 and 9 showed slight differences and remained very similar to each other, the formulation 4 had the lowest modulus degree (Table 4.3). This may be related to the sucrose content and the apple fruit used in the formulations. If the fruits had a higher amount of sucrose, this could have led to a highly strong gel structure in the marmalade samples even if the sucrose content was reduced. Generally, the amplitude of stress for G′ was selected in the range of 0.41-50 Pa for the linear viscoelastic region. On the other hand, LVR range for G″ was in the range of 0.41-200 Pa. In LVR region, each sample had a stable structure.

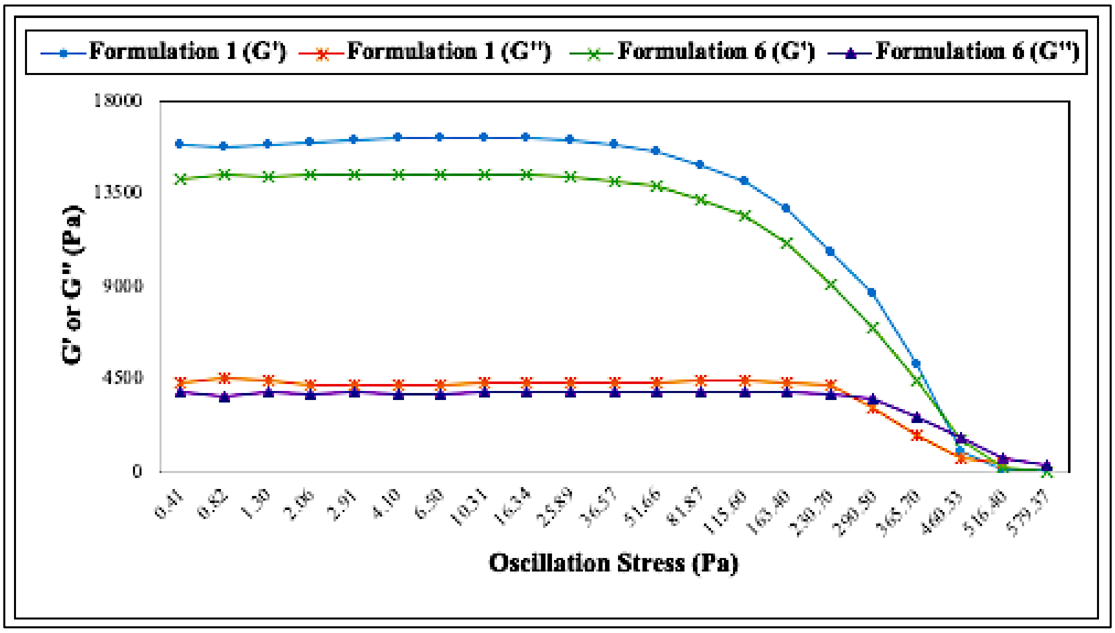


Figure 4.1 Oscillation stress sweep data of storage (G′) or loss (G″) moduli of Apple Marmalade Formulations prepared with 500 g and 600 g Sucrose

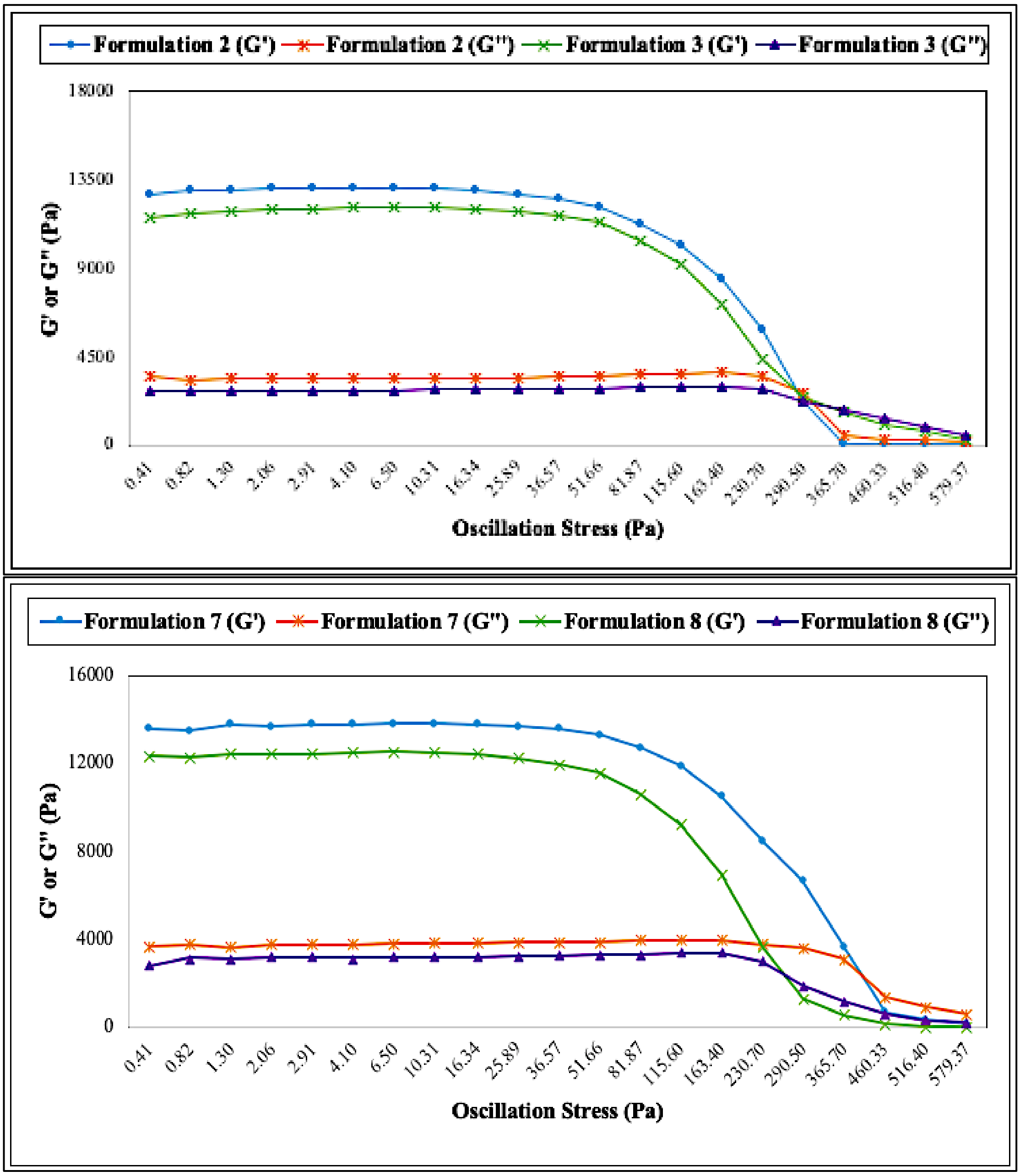


Figure 4.2 Oscillation stress sweep data of storage (G′) or loss (G″) moduli of Low Sugar Apple Marmalade Formulations prepared with selected Stevioside substitutions

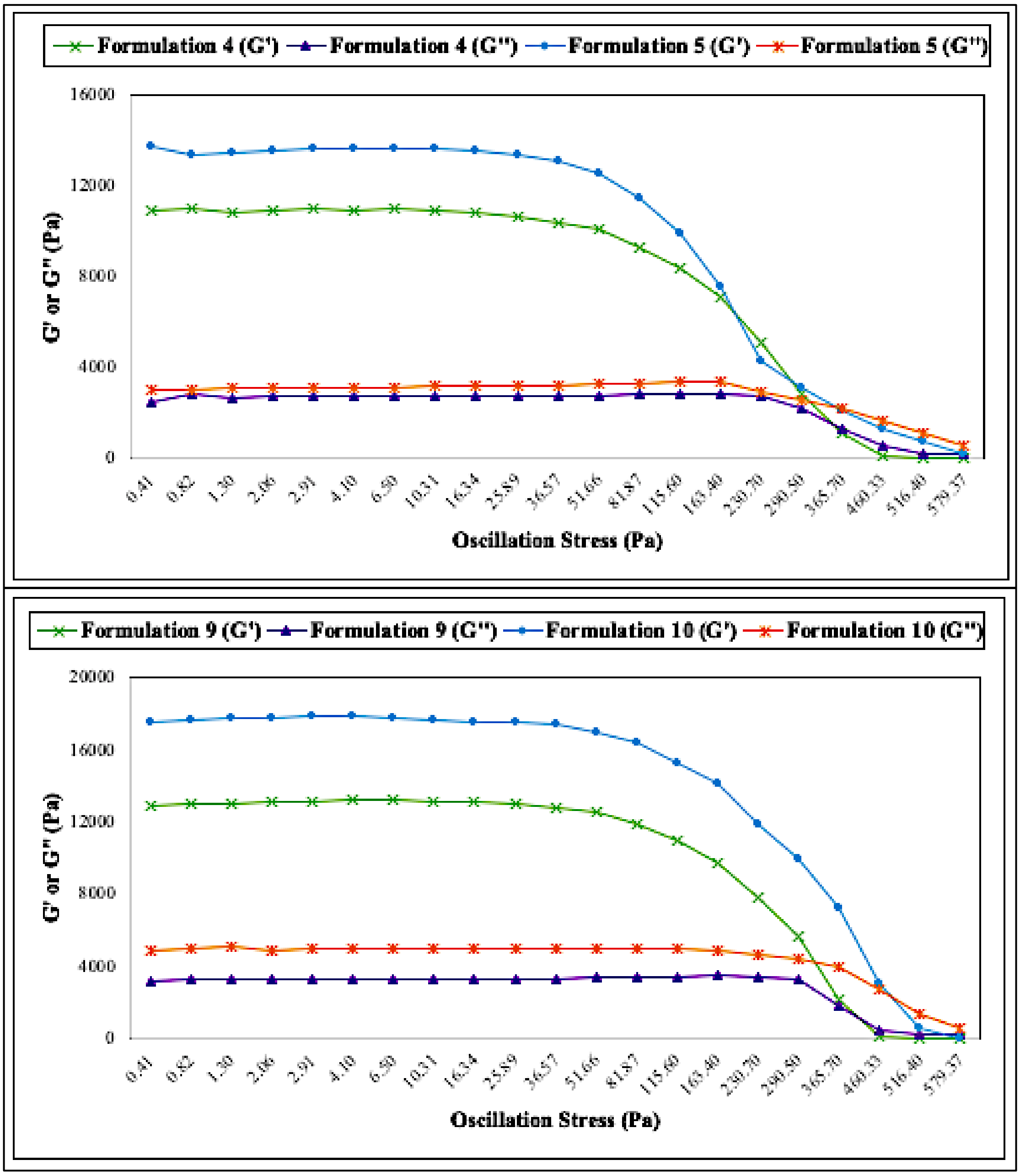


Figure 4.3 Oscillation stress sweep data of storage (G′) or loss (G″) moduli of Low Sugar Apple Marmalade Formulations prepared with selected Sucralose substitution

Table 4.3. ANOVA Results of Viscoelastic Properties of Low Sugar Apple Marmalade Formulations

	Step 1		**Step 3**	**Step 4**	
Formulation No	**G′ (Pa)**	**G″ (Pa)**	**G′ (Pa)**	**G′ (Pa)**	**G″ (Pa)**
1	11518.77 ± 6599.74^{b}	3327.49 ± 1682.14^{b}	13024.21 ± 298.51^{a}	13466.41 ± 236.75^{ab}	3585.68 ± 50.48^{ab}
2	8805.34 ± 5576.32^{cdef}	2564.346 ± 1445.71^{cd}	12287.49 ± 314.23^{a}	12155.13 ± 294.58^{ab}	3139.39 ± 132.22^{ab}
3	8085.76 ± 5100.28^{ef}	2129.68 ± 1159.71^{e}	11403.82 ± 672.00^{a}	10737.69 ± 159.82^{b}	2555.07 ± 154.28^{b}
4	7404.70 ± 4641.88^{f}	2066.97 ± 1159.32^{e}	12733.49 ± 324.77^{a}	14752.05 ± 197.30^{a}	3709.28 ± 78.28^{a}
5	9066.74 ± 5839.40^{cdef}	2388.72 ± 1337.96^{de}	12965.84 ± 516.90^{a}	12740.26 ± 300.46^{ab}	3044.23 ± 186.85^{ab}
6	10243.96 ± 5907.83^{bc}	2958.228 ± 1511.66^{bc}	13756.11 ± 297.68^{a}	13826.15 ± 191.14^{ab}	3647.96 ± 95.32^{ab}
7	9774.83 ± 5690.24^{cd}	2992.43 ± 1486.52^{b}	11763.04 ± 432.64^{a}	13919.49 ± 226.22^{ab}	3891.25 ± 119.92^{a}
8	8289.98 ± 5401.70^{def}	2372.977 ± 1358.32^{de}	12588.25 ± 477.15^{a}	12557.69 ± 260.12^{ab}	3276.35 ± 103.85^{ab}
9	9214.53 ± 5486.38^{cde}	2567.144 ± 1358.75^{cd}	15187.50 ± 371.28^{a}	13042.82 ± 153.30^{ab}	3292.68 ± 36.65^{ab}
10	13329.79 ± 6971.95^{a}	4043.71 ± 1822.16^{a}	15509.60 ± 478.73^{a}	13354.36 ± 127.09^{ab}	3850.07 ± 55.98^{a}

Results were expressed as mean ± standard deviations, (n=3). (a-f) values with the same superscript in the same column are not significantly different according to Tukey pairwise comparison test ($p \leq 0.05$).

4.3.2. Stepped Flow Test

In order to characterize the flow behavior of the low sugar apple marmalade samples, specific torque values ranging from 250-2500 μNm were selected to determine shear stress and shear rate data for each marmalade sample. The torque ranges were as follows: i) Formulation 1: 250- 2500 μNm ii) Formulation 2: 250-1750 μNm iii) Formulation 3: 250-1250 μNm iv) Formulation 4: 250-1750 μNm v) Formulation 5: 250-1250 μNm vi) Formulation 6: 250-2000 μNm vii) Formulation 7: 25-1500 μNm viii) Formulation 8: 250-1250 μNm ix) Formulation 9: 250-1750 μNm x) Formulation 10: 250-1500 μNm. Different rheological models, namely Power Law, Herschel-Bulkley (HB) and Casson, were applied and the results of the model parameters for the samples were presented in Tables 4.4 and 4.5 and given in Appendix B.

Considering all the experimental results, the low sugar apple marmalade samples containing different formulations had a shear thinning behavior (pseudo-plastic), because viscosity decreased with shear rate applied (Figure 4.4 and Table 4.6). The shear stress values increased with increasing shear rate values, as depicted in Figure 4.5. Minimum stress value of about 242.97 Pa is required for initiating the flow, indicating the yield stress. Yield stress was obtained for all the marmalade formulations and depicted in Table 4.4. The addition of sweeteners was obviously effective on the yield stress of the formulations. The yield stress values decreased with increasing sweeteners concentrations. This could be related to the sucrose content. Reduction of the sucrose concentration resulted in a decrease in the resistance to flow. Thus, mechanical forces applied to the marmalade samples were also decreased by the decreased sucrose. Tan et al. (2014) emphasized that the starch concentration which was increased from 15% to 25% led to an increase in the shear stress values because of the effect of sugar and starch as a thickening agent in the apple jam. In addition to this, yield stress values were highly affected by the addition of these agents to the formulations. Similar results were also obtained by Koocheki et al. (2009) in ketchup. The yield stress provided increasing values with the increase in the concentration of hydrocolloid in the product. The data of the relationship between shear rate and shear stress fitted well to the Herschel-Bulkley model to describe the flow behaviors of the low sugar apple marmalade exhibiting certain yield stress. In all cases, the coefficient of determination (R^2) were higher than 0.85 and root

Table 4.4. Herschel Bulkley and Casson Model for Rheological Behaviors of Low Sugar Apple Marmalade Formulations

	HB MODEL						CASSON MODEL					
	τ_0	n	K	R^2	RMSE	SE	τ_{oc}	K_c	K_{0c}	R^2	RMSE	SE
Formulation 1	406.49 ± 64.29^{a}	0.56 ± 0.07^{a}	25.85 ± 5.36^{a}	0.89 ± 0.07	0.09 ± 0.03	0.10 ± 0.04	414.26 ± 79.10^{a}	0.68 ± 0.31^{ab}	20.29 ± 1.96^{a}	0.88 ± 0.10	0.41 ± 0.17	0.48 ± 0.20
Formulation 2	304.08 ± 56.85^{abc}	0.61 ± 0.13^{a}	8.18 ± 4.85^{b}	0.88 ± 0.08	0.10 ± 0.04	0.12 ± 0.05	300.77 ± 67.61^{abc}	0.42 ± 0.49^{ab}	17.27 ± 1.93^{abc}	0.91 ± 0.07	0.39 ± 0.29	0.45 ± 0.30
Formulation 3	242.97 ± 36.57^{c}	0.73 ± 0.07^{a}	6.46 ± 2.23^{b}	0.91 ± 0.07	0.08 ± 0.03	0.09 ± 0.04	233.32 ± 36.17^{c}	0.45 ± 0.04^{ab}	15.25 ± 1.15^{c}	0.87 ± 0.09	0.23 ± 0.14	0.28 ± 0.16
Formulation 4	372.58 ± 53.88^{ab}	0.57 ± 0.10^{a}	11.18 ± 4.70^{b}	0.84 ± 0.10	0.11 ± 0.04	0.12 ± 0.05	380.51 ± 26.19^{abc}	0.31 ± 0.09^{ab}	19.50 ± 0.67^{ab}	0.89 ± 0.08	0.38 ± 0.17	0.43 ± 0.18
Formulation 5	271.50 ± 20.41^{bc}	0.64 ± 0.27^{a}	7.38 ± 4.32^{b}	0.91 ± 0.06	0.08 ± 0.03	0.09 ± 0.03	256.42 ± 20.56^{bc}	0.38 ± 0.17^{ab}	16.00 ± 0.65^{bc}	0.91 ± 0.02	0.26 ± 0.13	0.31 ± 0.14

Results were expressed as mean ± standard deviations, (n=3). (a-c) values with the same superscript in the same column are not significantly different according to Tukey pairwise comparison test ($p \leq 0.05$). RMSE: Root Mean Square Error, SE: Standard Error.

(cont. on next page)

Table 4.4 (Cont.)

	HB MODEL						CASSON MODEL					
	τ_o	n	K	R^2	RMSE	SE	τ_{oc}	K_c	K_{0c}	R^2	RMSE	SE
Formulation 6	353.88 ± 11.02^{abc}	0.46 ± 0.04^{a}	24.91 ± 4.81^{a}	0.90 ± 0.06	0.09 ± 0.03	0.10 ± 0.03	394.13 ± 10.83^{ab}	0.35 ± 0.07^{ab}	19.85 ± 0.27^{ab}	0.88 ± 0.08	0.57 ± 0.29	0.63 ± 0.31
Formulation 7	270.06 ± 51.79^{bc}	0.80 ± 0.10^{a}	16.52 ± 4.51^{ab}	0.90 ± 0.02	0.08 ± 0.01	0.10 ± 0.01	252.59 ± 58.75^{bc}	1.08 ± 0.32^{a}	15.82 ± 1.92^{bc}	0.93 ± 0.03	0.27 ± 0.08	0.32 ± 0.09
Formulation 8	269.12 ± 16.58^{bc}	0.82 ± 0.09^{a}	3.32 ± 0.92^{b}	0.86 ± 0.05	0.09 ± 0.01	0.11 ± 0.02	252.49 ± 11.57^{bc}	0.39 ± 0.07^{ab}	15.89± 0.37^{bc}	0.91 ± 0.05	0.23 ± 0.06	0.29 ± 0.07
Formulation 9	346.72 ± 56.55^{abc}	0.52 ± 0.14^{a}	10.49 ± 7.61^{b}	0.87 ± 0.05	0.10 ± 0.02	0.11 ± 0.02	360.01 ± 56.10^{abc}	0.19 ± 0.01^{ab}	18.88 ± 2.34^{abc}	0.88 ± 0.04	0.44 ± 0.13	0.49 ± 0.14
Formulation 10	315.65 ± 31.35^{abc}	0.57 ± 0.25^{a}	13.98 ± 4.37^{ab}	0.93 ± 0.06	0.07 ± 0.04	0.09 ± 0.04	315.03 ± 49.61^{abc}	0.55 ± 0.62^{ab}	17.70 ± 1.41^{abc}	0.93 ± 0.04	0.27 ± 0.12	0.32 ± 0.12

Results were expressed as mean ± standard deviations, (n=3). (a-c) values with the same superscript in the same column are not significantly different according to Tukey pairwise comparison test ($p \leq 0.05$). RMSE: Root Mean Square Error, SE: Standard Error.

Table 4.5. Power Law Model for Rheological Behaviors of Low Sugar Apple Marmalade Formulations

Power Law Model					
Formulation	**Flow behavior index, n**	**Consistency index, K (Pa s^n)**	**R^2**	**RMSE**	**SE**
1	0.10 ± 0.03^a	410.31 ± 69.26^a	0.91 ± 0.03	0.01 ± 0.00	0.02 ± 0.00
2	0.09 ± 0.04^a	275.10 ± 37.12^{ab}	0.93 ± 0.02	0.01 ± 0.00	0.02 ± 0.02
3	0.09 ± 0.04^a	229.15 ± 53.28^b	0.87 ± 0.11	0.01 ± 0.01	0.01 ± 0.01
4	0.10 ± 0.00^a	325.31 ± 27.12^{ab}	0.94 ± 0.05	0.01 ± 0.01	0.01 ± 0.01
5	0.09 ± 0.04^a	245.88 ± 21.26^b	0.87 ± 0.14	0.01 ± 0.012	0.02 ± 0.01
6	0.11 ± 0.03^a	333.53 ± 36.58^{ab}	0.94 ± 0.03	0.02 ± 0.00	0.02 ± 0.00
7	0.16 ± 0.03^a	262.14 ± 44.65^b	0.97 ± 0.01	0.01 ± 0.00	0.01 ± 0.00
8	0.13 ± 0.01^a	213.86 ± 18.04^b	0.95 ± 0.03	0.01 ± 0.00	0.01 ± 0.00
9	0.10 ± 0.04^a	285.62 ± 98.99^{ab}	0.89 ± 0.09	0.02 ± 0.01	0.02 ± 0.01
10	0.10 ± 0.06^a	301.98 ± 23.26^{ab}	0.92 ± 0.06	0.01± 0.00	0.01 ± 0.01

Results were expressed as mean ± standard deviations, (n=3). (a,b) values with the same superscript in the same column are not significantly different according to Tukey pairwise comparison test ($p \leq 0.05$).

mean square error (RMSE) were lower than 0.11 (Table 4.4). Compared to the Casson model, the Herschel Bulkley model had the lowest RSME and standard error (SE) values, as is seen in Table 4.4. The small RMSE values indicate the model better fit for the data (Unluturk et al., 2010). Since the low sugar apple marmalade samples exhibited the yield stress, the Power Law model was not suitable for describing the sample behavior (Table 4.5). Additionally, Power Law model resulted in very low flow behavior index (n) values. Therefore, the selected HB model was adequate to describe the flow behavior of low sugar apple marmalade samples having yield stress within the specified range. The determination coefficient between 0.80-0.90 was expressed as a good prediction. The excellent prediction was also defined as higher than 0.90 (Tamaki and Mazza, 2011). The rheological behavior of the low sugar apple marmalade samples was excellently predicted by the HB model parameters in the range of given shear rate with a determination coefficient, R^2>0.85. For only formulation 4, this value was determined as 0.84. The flow behavior index (n) of all the apple marmalade samples determined by the model was observed to vary from 0.46 to 0.82. The flow behavior index was increased by an increase in the concentration of sweeteners substitutions. Since the magnitude of the flow index was smaller than 1 ($n<1$) and the determination of coefficient (R^2) was higher than 0.85, it could denote that the low sugar apple marmalade samples exhibited a shear thinning behavior and described as Non-Newtonian fluids. The consistency index (K) of all formulations also ranged from 3.32 to 25.85. Consistency is a major quality factor in many semisolid foods such as purees and pastes. It indicates a strong interaction between the molecules in the sample structure and stability (Dogan and Kokini, 2007). The observation of this study was supported by Barbieri et al. (2018). They found that the consistency index was 39.40 $Pa.s^n$ for the gabiroba jam. Also, the consistency index was determined between 21-73 $Pa.s^n$ for the peach jam, as given by Falguera et al. (2010). Sagdic et al. (2015) stated that the value K was found as 17.6 $Pa.s^n$ for the rose hip marmalade at 25 °C. In other words, the consistency index varies depending on the components of jam formulations. Similarly, the consistency index decreased when the sweeteners concentrations were increased. The effect of the sweeteners addition on the formulations yielded lower values for the index. In other words, the consistency index decreased with a decrease in the total soluble solids (TSS) (Table 4.1). These findings confirm the results of the mango jam made with stevioside and sucralose sweeteners. Basu, Shivhare, and Singh (2013) reported that the Herschel-Bulkley model explained the rheological behavior of the mango jam samples containing those sweeteners very well. Also, changes in the TSS affected the parameters of the model. The flow behavior index showed an increasing trend with a decrease in the TSS; moreover, the consistency index decreased when the TSS values of the jam decreased, as is seen in the apple marmalade results. In the study conducted by Peinado et al., 2012, the strawberry products containing isomaltulose (30 Brix) and a blend of isomaltulose and fructose (50 Brix) caused a lower yield stress and consistency index, compared to other formulations containing sucrose or sucrose glucose blend.

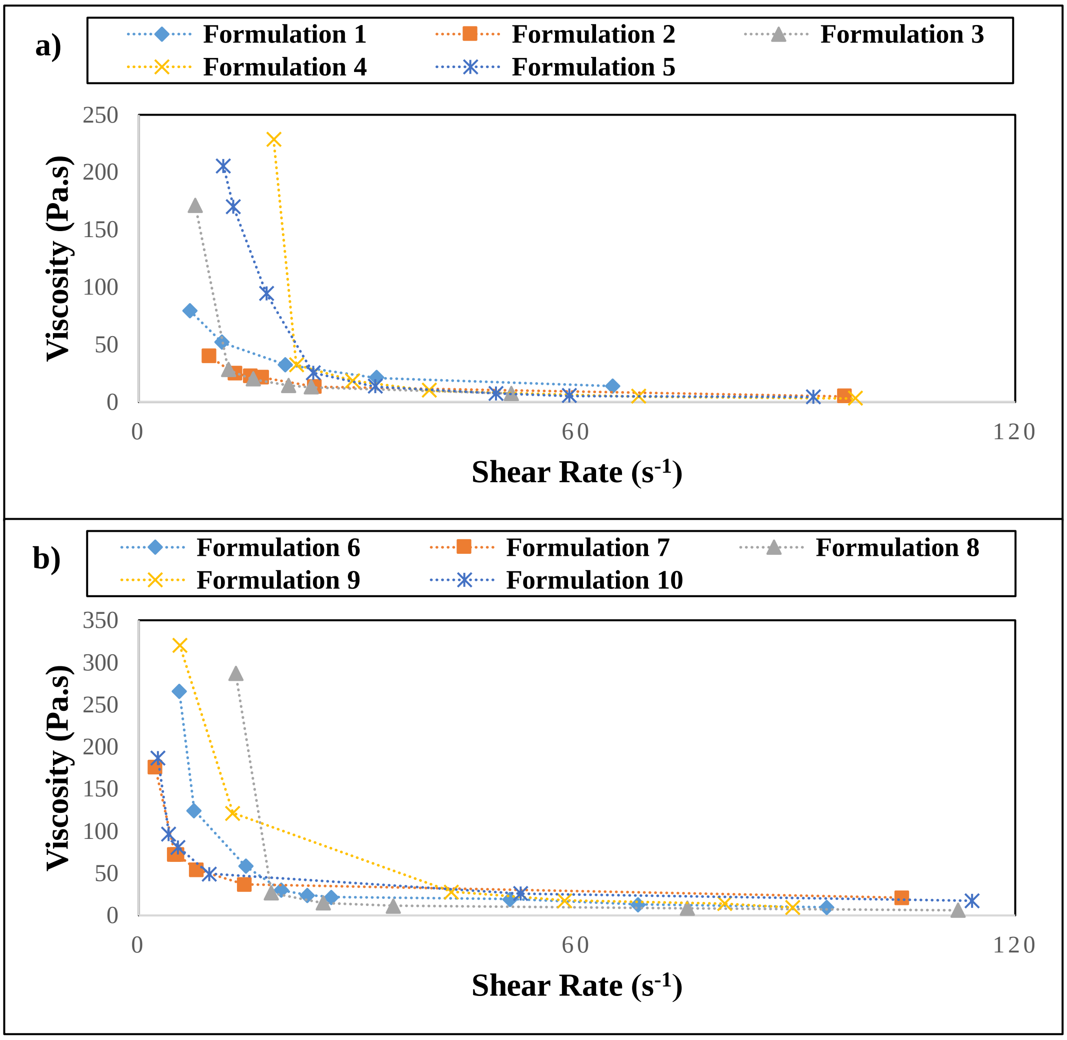


Figure 4.4. Average Values of Viscosity vs Shear Rate of Low Sugar Apple Marmalade Formulations a) The Formulations basis on 500g b) The Formulations basis on 600g

Table 4.6. Apparent Viscosity Data at Constant Shear Rate (100 s^{-1})

Formulation No	**Apparent Viscosity (Pa.s)**	**Formulation No**	**Apparent Viscosity (Pa.s)**
1	2.47 ± 0.89	**6**	4.51 ± 0.21
2	2.55 ± 0.28	**7**	3.32 ± 1.71
3	1.88 ± 0.57	**8**	1.26 ± 0.46
4	2.49 ± 0.45	**9**	2.87 ± 0.98
5	1.49 ± 0.36	**10**	2.98 ± 1.23

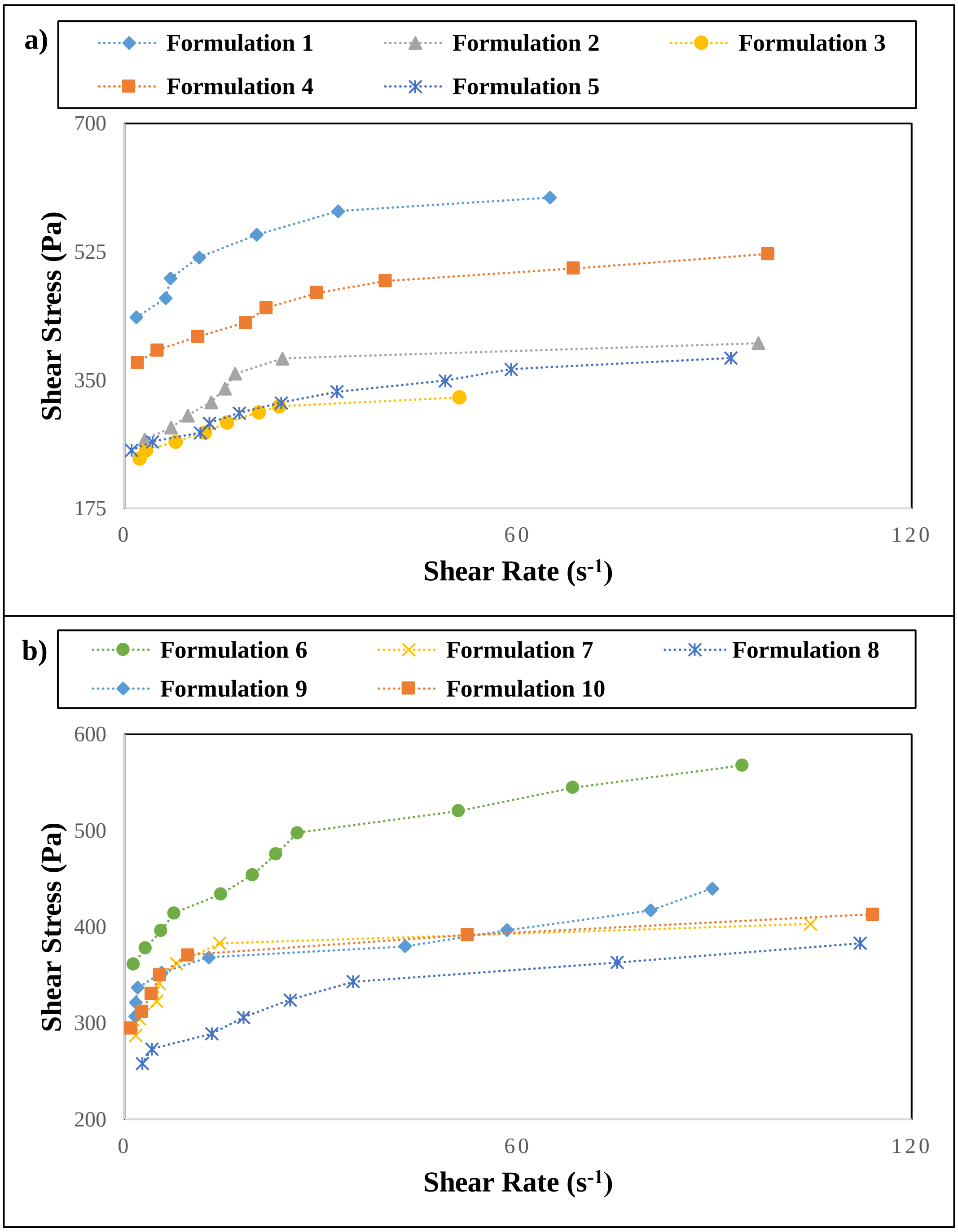


Figure 4.5. Average Values of Shear Stress vs Shear Rate of Low Sugar Apple Marmalade Formulations a) The Formulations basis on 500g b) The Formulations basis on 600g

4.3.3. Oscillation Time Sweep Test

Oscillation time sweep test was carried out at a constant frequency of 1 Hz to determine the change of viscoelastic properties over time. The test was performed at a shear rate of 0.005 and at a shear stress of 20 Pa obtained for each sample in previous steps within the linear viscoelastic region (LVR). Throughout 15 minutes (900s), elastic modulus data were collected as a function of time at 30 °C. As a consequence, the value of equilibration time was determined as 10 minutes for each sample to be used in the final step, since the elastic modulus remained more stable (around 10 minutes). From an industrial point of view, it is of great importance that the time required is short until reaching a steady state gel structure (Torres, Raymundo, and Sousa, 2013).

Elastic modulus (G′) demonstrated an increasing trend with the increase of time, as depicted in Figures 4.6, 4.7 and 4.8. Within the time range of 0-900 s, there were no significant differences between all formulations ($p>0.05$). However, the highest value of the elastic modulus was observed in the formulation 10 increasing approximately from 14000 to 16000 Pa while the formulation 3 had the lowest value in the range of approximately 10000-12000 Pa in all formulations given in Table 4.3. This may also be related to the types of apple fruits or the ingredients of the formulations. The formulation 10 could have larger fruit particles, compared to others. Also, this could affect the structure of pectin molecules. Genovese, Ye, and Singh (2010) reported that the increase in the concentration of particles contributed to the increase in the stiffness of the composite gel of the pectin/apple particles. Furthermore, the formulation 6 provided a higher value of modulus, compared to the formulation 1. The formation of the gel structure increased with increasing TSS values (Table 4.1) during the marmalade production and consequently, the gel showed a more elastic character than liquid-like material. There were slight differences in all formulations containing stevioside sweeteners. It was observed that the addition of sweeteners to the formulation was influential on the elastic modulus values of the low sugar apple marmalade samples. Moreover, the formulations prepared with sucralose sweeteners showed a semi-solid character because of their higher elastic modulus, compared to the formulations with stevioside. This denotes having different bonds in the nature of the formulations.

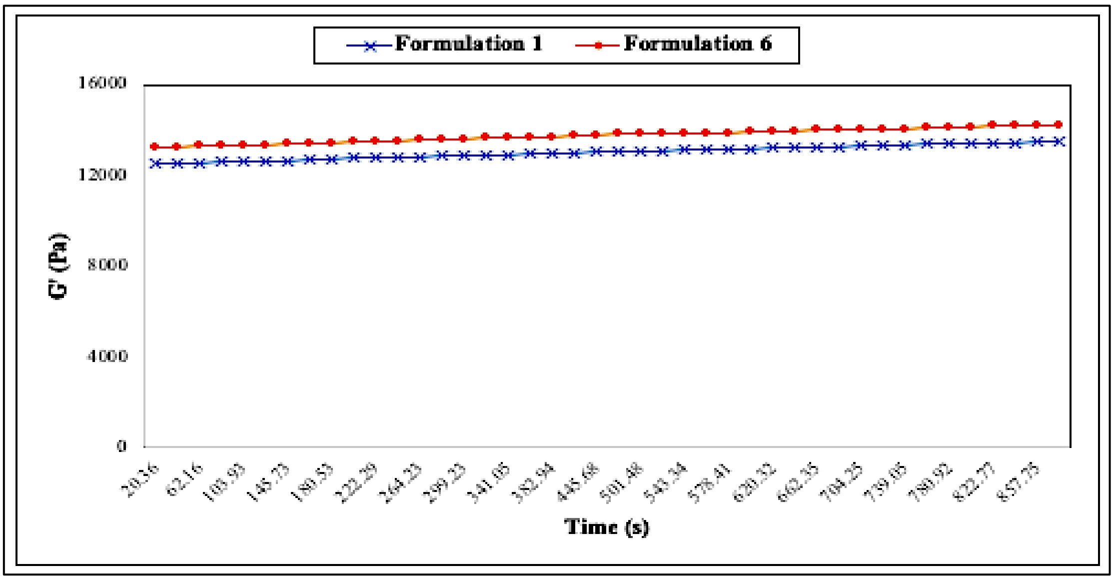


Figure 4.6. Time Sweep Test for Low Sugar Apple Marmalade Formulations prepared with 500 g and 600 g Sucrose

Torres, Raymundo, and Sousa (2013) investigated the effect of the addition of sucrose, xylitol and stevia on the rheological properties of the gels prepared with chestnut and rice flour. While the gels showed a stronger structure and the elastic modulus values had a noticeable increase without the additional component, the values significantly decreased in the presence of sucrose; moreover, the addition of xylitol to the gels resulted in a small reduction for the viscoelastic properties of the products. Additionally, no significant effect was observed in both moduli after adding the stevia to the gel formulation containing chestnut and rice flour. Not only the type of sweeteners but also the pH and pectin/starch concentration of the medium are also important in gelation process. Löfgren et al. (2005) stated that the ambient pH considerably affected the gelation process and viscoelastic properties. In their studies, pectin A formed the weaker gels in the presence of calcium at pH 3.5 and the values G′ were observed to increase slowly, compared to a rapid increase in the values at pH 3.0. Pectin B also showed an extremely weak gel structure in the presence of calcium at pH 3.5. In the study carried out by Tan et al. (2014), the elastic and loss modulus yielded a lower value for the jams containing starch with respect to the formulation containing non-starch in the apple jams formulated with/without starch. The pectin content which is very high in the apple pulp was a highly effective thickening agent. Moreover, Javanmard and Endan (2010) suggested that the stronger interactions of the apple pectin led to a greater viscous shear than the gels of the citrus pectin. Concerning the citrus pectin, the end product shows an easy brittleness and it has an insufficient spreadability due to having an elastic shear and being less viscous. In addition, they reported that the pectin of the citrus fruit provided a small viscoelastic region, compared to the apple pectin.

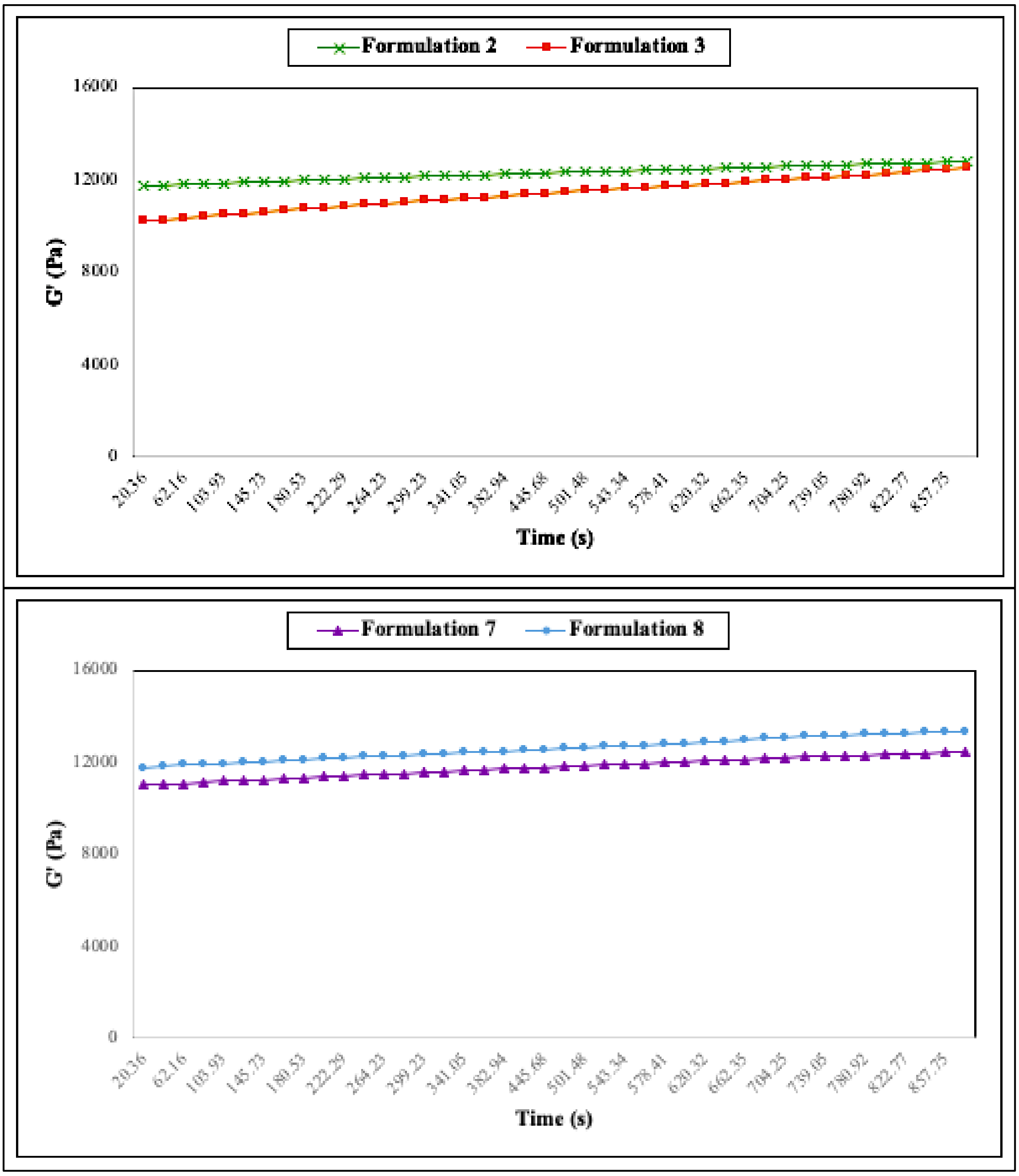


Figure 4.7. Time Sweep Test for Low Sugar Apple Marmalade Formulations prepared with selected Stevioside Substitutions

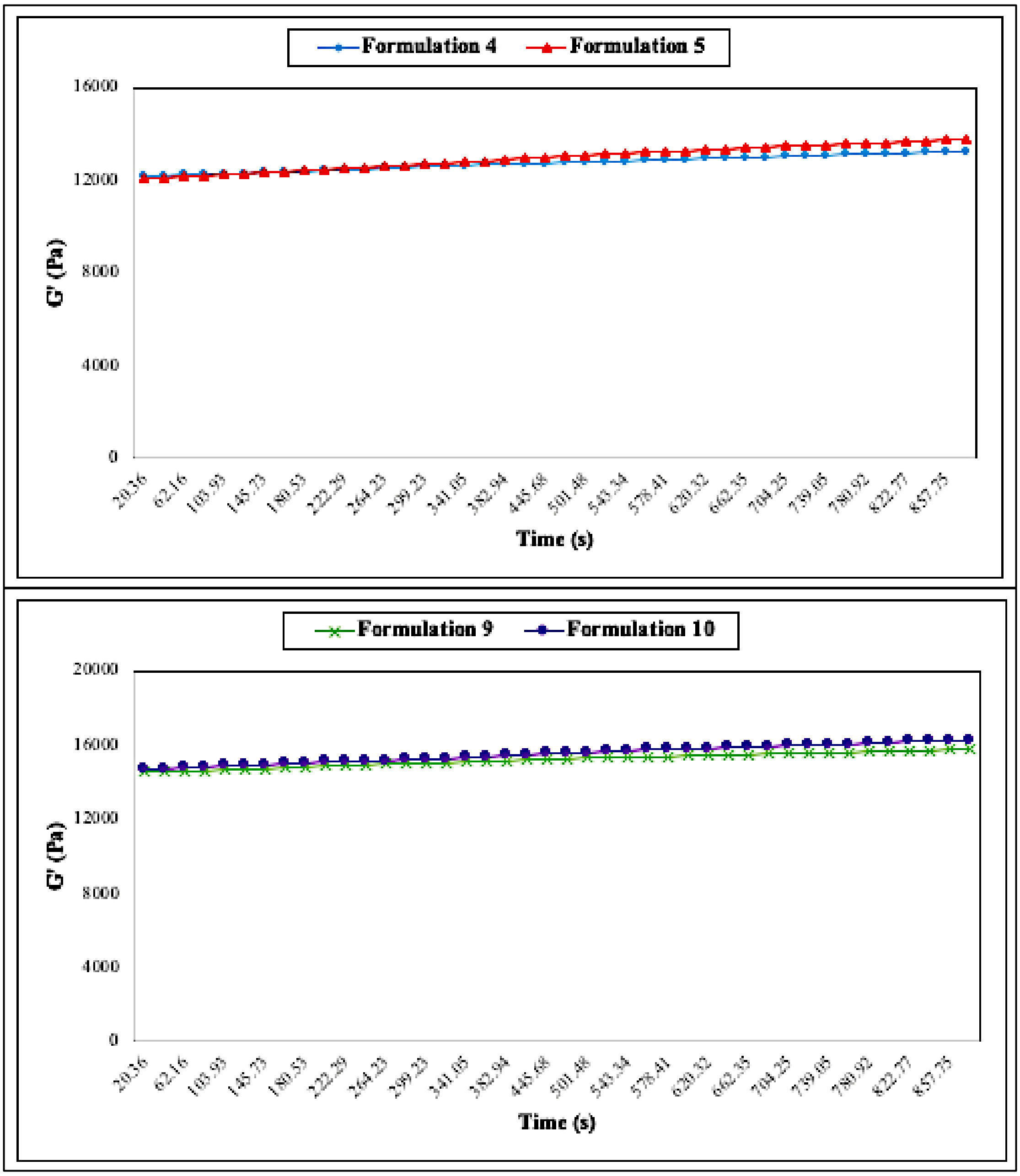


Figure 4.8. Time Sweep Test for Low Sugar Apple Marmalade Formulations prepared with Sucralose Substitutions

4.3.4. Oscillation Stress Sweep Test

Oscillation stress sweep test was performed in the range of stress values which was determined in the previous steps for each marmalade samples. Dynamic rheological viscoelastic properties of the low sugar apple marmalade formulations were measured within the linear viscoelastic region (LVR) ranging from 0.41 to 50 Pa for G′, and 0.41 to 200 Pa for G″. The results of both dynamic moduli showed similar behaviors largely independent of stress values, as is depicted in Figures 4.9-4.11. Storage or elastic modulus (G′) is related to the elastic quality, whereas loss modulus (G″) is also associated with the viscous quality of the products. For all samples, the elastic modulus (G′) was extremely higher than the loss modulus throughout the stress range, indicating a predominant contribution of the value G′ to the viscoelastic properties of the marmalade samples. In other words, the marmalade samples exhibited a dominant elastic/solid-like character. The firmness/consistency of the structure of the product was evaluated by the elastic modulus, which was obtained by the strength of gel (Garrido et al. 2015). When the stress values were mechanically increased, both the values G′ and G″ remained constant up to a certain stress point and then began to gradually decrease (Figures 4.9-4.11). This is an indicator of the break-down of the solid-like structure for the samples.

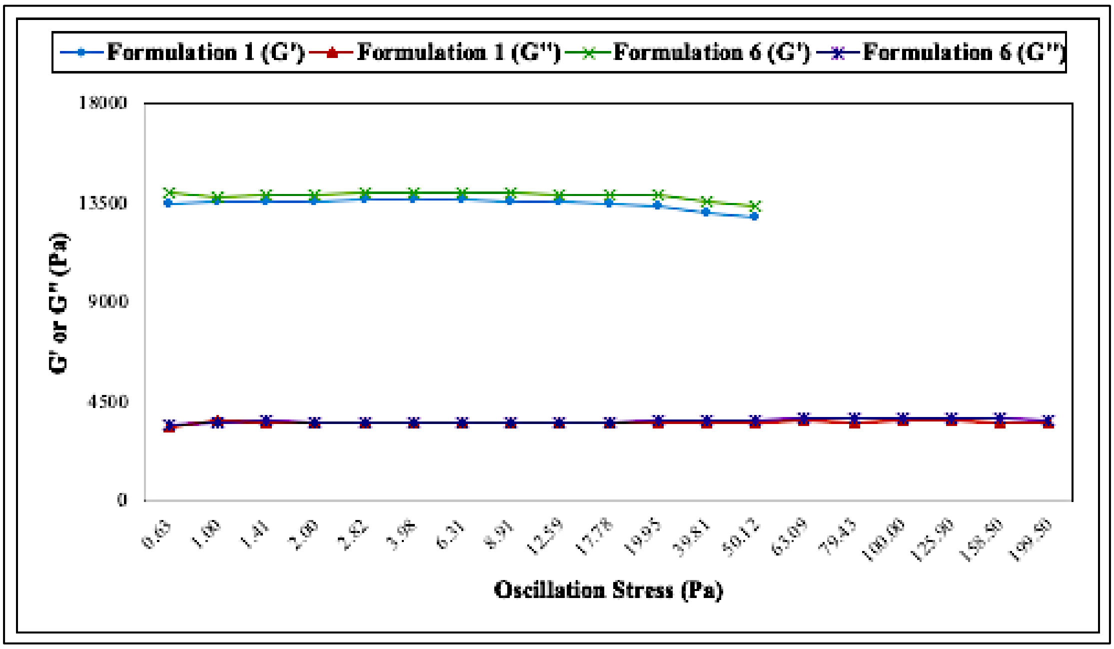


Figure 4.9. Viscoelastic Properties of Low Sugar Apple Marmalade Formulations prepared with 500 g and 600 g Sucrose

The formulation 4 made with sucralose-25 significantly contributed to the highest degree of both elastic modulus and loss modulus in all formulations. On the other hand, the lowest values of the modulus were significantly observed in the formulation 3, as is clearly seen in Figure 4.10 and Table 4.3. This may be explained as a reduction of the sucrose content, which resulted in the increase of the liquid-like character of the formulation. In the jam gelation process, the pectin molecule chains are aligned and stretched in sucrose and fruit pulp mix and consequently, the intermolecular formation of hydrogen bonding occurs in more available sites. In order to form a three-dimensional network, the pectin molecules are surrounded by hydrogen bonds. Nevertheless, it is provided to hold the sucrose within the structures of pectin network. Thus, an increased sucrose concentration and therefore an increase in TSS leads to the development of strong elasticity in the jam product (Basu et al. 2011). Similarly, the formulation 7 having a higher TSS degree led to higher values of elastic modulus, compared to the formulation 8. The results were in agreement with the results of the mango jam samples prepared by Basu et al. (2011). Table 4.3 showed that there were marked differences in all formulations preparing with sucralose. At the same time, the formulations containing sucralose sweeteners yielded higher values of G′ and G″, compared to the formulations containing stevioside sweeteners (Figures 4.10 and 4.11). This could be due to the different structure of the nature of bond in the sucralose, compared to the stevioside. On the other hand, there were no significant differences between the formulations 1 and 6 in terms of G′ values. Due to highest sucrose content, formulation 6 had the higher G′ value compared to the formulation 1. Thus, the gel strength of the formulation 6 was higher. The increase in the sucrose concentration increased the G′ and G″ values and decreased the water availability to form a hydrogen bond between the mixture of pectin, sucrose, and acid. Although the sucrose provided the stabilization to the structure of junction zones, over a certain concentration of sucrose reduced the gel quality and become a weaker gel structure of the pectin. The observation was supported by Basu et al. (2011). In their mango jam samples containing sorbitol, the sucrose concentration increased to above 60% resulting in an unstable structure in a firmer gel network of the pectin and a softer jam because of releasing more water molecules in the jam. In the study conducted by Löfgren, Walkenström, and Hermansson (2002), the high-methoxyl (HM), low-methoxyl (LM) pectin and their mixture gel structure rheologically were investigated and determined the viscoelastic properties. They expressed that changes in the sucrose concentration affected the gel strength between the HM and LM pectin, as well as the structure of the network. Torres, Raymundo, and Sousa (2013) studied the effect of the addition of sucrose, xylitol and stevia to the prepared chestnut and rice flours gel and evaluated the rheological properties of the formulation. The authors found that the addition of sucrose resulted in a significant decrease in the viscoelastic properties of the gels, whereas the xylitol addition had a small decrease; moreover, the presence of stevia showed no significant difference for both moduli in their measurement of the temperature, time and frequency sweep.

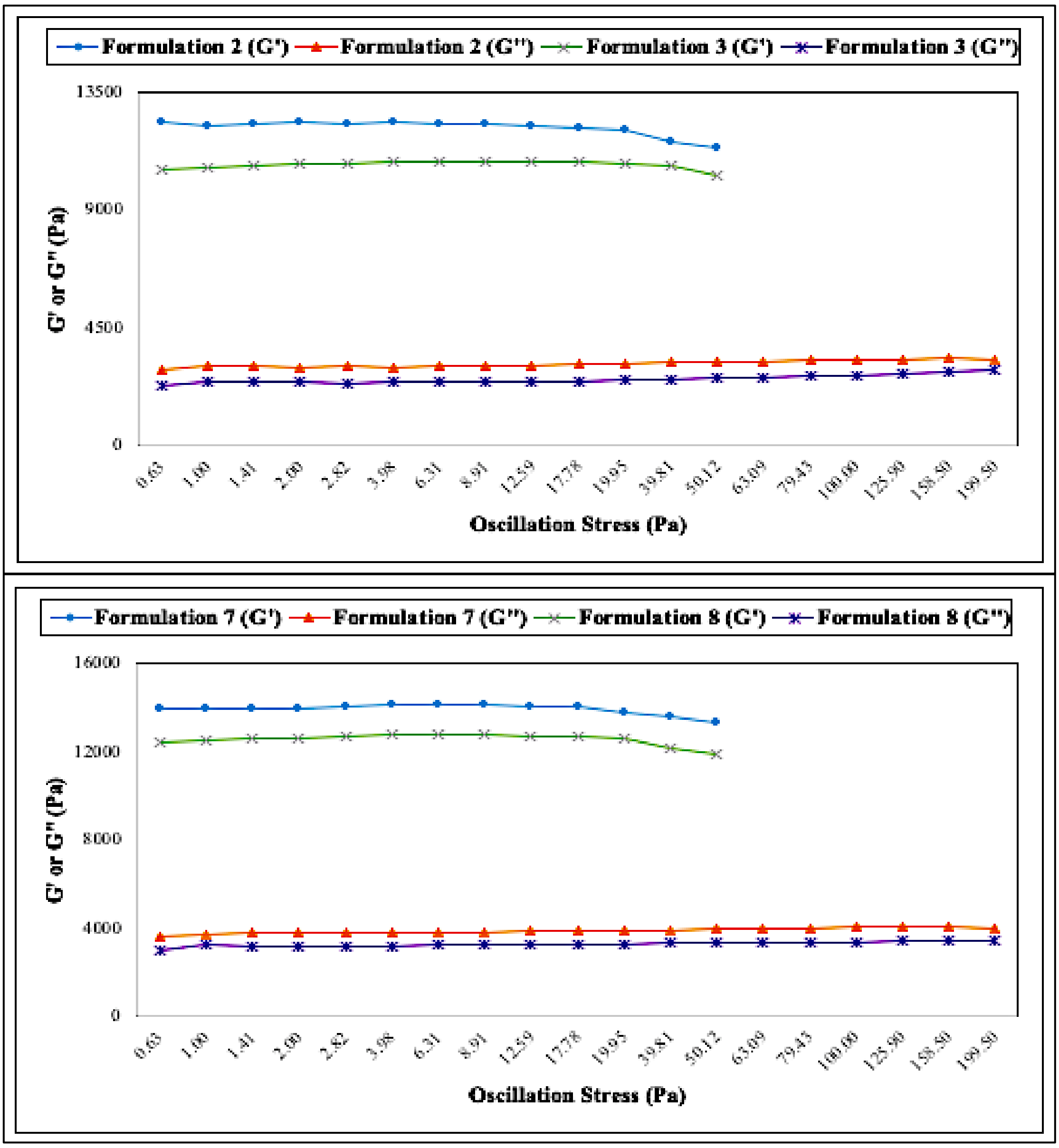


Figure 4.10. Viscoelastic Properties of Low Sugar Apple Marmalade Formulations prepared with selected Stevioside Substitutions

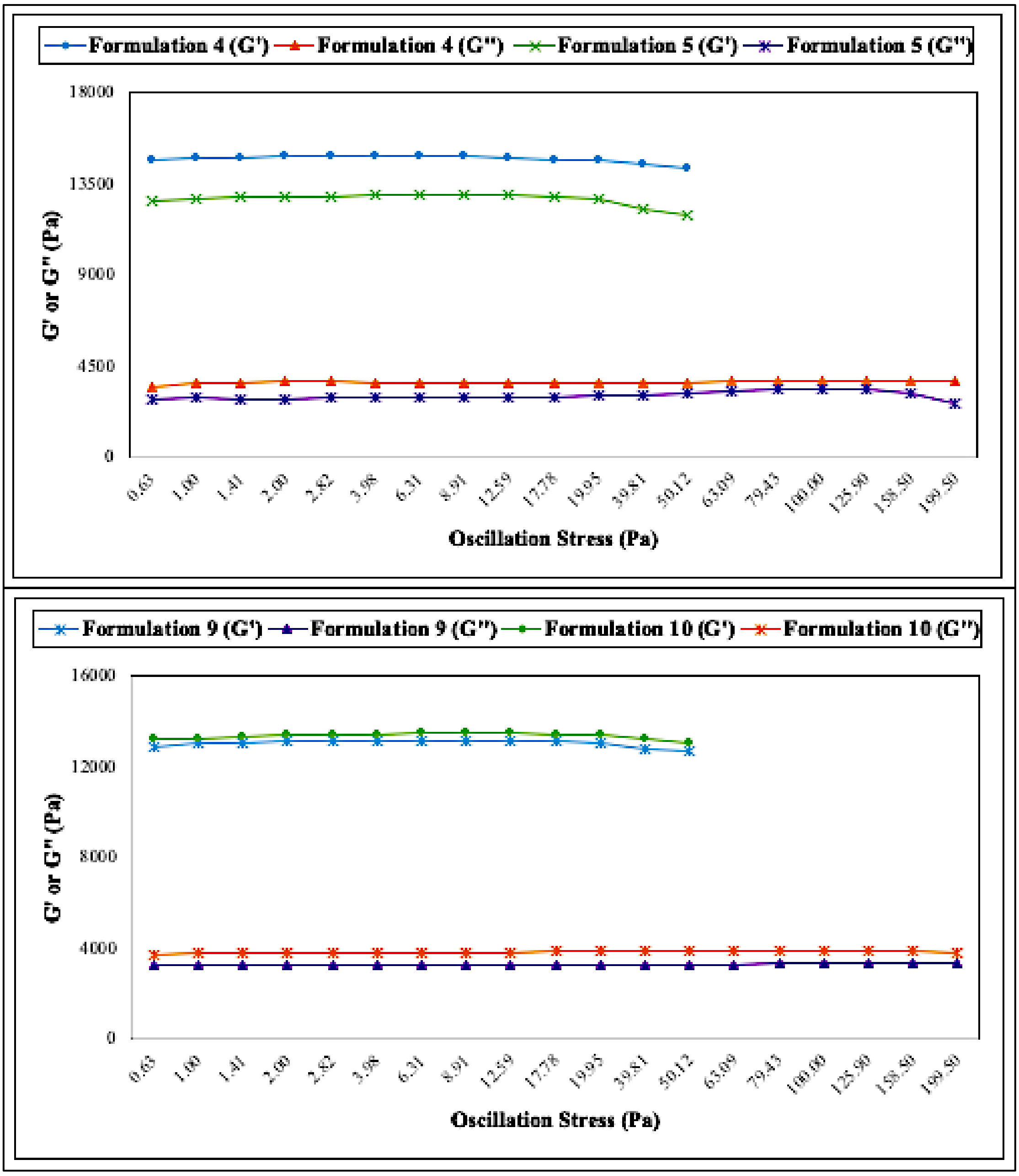


Figure 4.11. Viscoelastic Properties of Low Sugar Apple Marmalade Formulations prepared with selected Sucralose Substitutions

4.4. Textural Properties of Low Sugar Apple Marmalade

Food systems are mostly composed of a network with macromolecules and many small particles. Moreover, various intermolecular bonds and forces hold the systems together, as well. Interactions strongly affect the properties of foods including texture, structure, functionality and stability. The texture of the end product is strongly dependent on the changes in its structural history throughout the processing (Sikorski, 2002). Texture analysis can be regarded as a mimic of mastication in the mouth and it can be used to provide information on the oral processing behavior of semi-solid food for objective measurement of its textural characteristics (Naknaen and Itthisoponkul, 2015). In order to investigate the effect of the artificial sweeteners addition on the textural parameters of the marmalade samples; parameters such as hardness, adhesiveness, cohesiveness, springiness, gumminess and chewiness were measured and the results of the measurements were depicted in Table 4.7.

Hardness parameter of the low sugar apple marmalade, which is a maximum force, ranged from 1.73 to 2.99 N. The highest values were obtained when the marmalade was prepared with 600 g sucrose only (formulation 6). During cooking of the marmalade, acid, sugar and pectin formed a strong gel structure. Due to having the maximum amount of sucrose in comparison with other formulations, the highest degree of hardness was observed in the formulation 6. Singh et al. (2009) stated that the sugar provided gel formation as well as retention of color and sweetening in fruit jams containing different fruit pulps combinations. Also, the authors considered that the firmness of jams could be relevant to the acid-ºBrix proportion. Basu, Shivhare, and Singh (2013) reported that the structure of networks was strongly connected to each other after reaching 60 ºBrix of TSS. Statistical analysis showed that there was no significant effect of the formulation changes with sweeteners on the hardness parameter. However, an increase in the sweeteners concentrations led to a decrease in the hardness of the marmalade. The formulation 3 had a slightly lower hardness value than the formulation 2 due to raising stevioside percentages from 25% to 50%. In the same manner, the hardness value of the formulation 7 was found to be higher than the formulation 8, which had the lowest hardness value of the marmalade samples. Among other formulations, it was understood that the formulation had a moister and softer structure. The increasing moisture content (approximately 40%) (Table 4.1) with sweeteners concentrations also supported that observation. In terms of textural hardness, a similar problem was found by Basu et al. (2011) in the mango jam substituted with sorbitol. They reported that the texture of their jam samples prepared completely by sorbitol substitutions was softer. Similar results were observed by Hyvönen and Törma (1983) for the strawberry jam containing xylitol and sorbitol substitutions.

Table 4.4. Textural Parameters of Low Sugar Apple Marmalade

Formulation No	Hardness (N)	Adhesiveness (j)	Cohesiveness	Springiness (m)	Gumminess (N)	Chewiness (j)
1	2.25 ± 0.10^{b}	6.84 ± 0.43^{b}	0.69 ± 0.05^{a}	0.96 ± 0.03^{a}	1.55 ± 0.04^{b}	1.48 ± 0.04^{b}
2	2.00 ± 0.13^{b}	4.90 ± 0.89^{bcd}	0.68 ± 0.06^{a}	0.95 ± 0.02^{a}	1.37 ± 0.16^{b}	1.30 ± 0.17^{b}
3	1.99 ± 0.09^{b}	4.84 ± 0.91^{cd}	0.68 ± 0.06^{a}	0.94 ± 0.03^{a}	1.36 ± 0.16^{b}	1.28 ± 0.20^{b}
4	1.80 ± 0.27^{b}	4.83 ± 0.79^{cd}	0.67 ± 0.05^{a}	0.92 ± 0.05^{a}	1.21 ± 0.24^{b}	1.11 ± 0.21^{b}
5	1.79 ± 0.05^{b}	4.54 ± 0.14^{cd}	0.66 ± 0.04^{a}	0.92 ± 0.04^{a}	1.19 ± 0.04^{b}	1.10 ± 0.08^{b}
6	2.99 ± 0.31^{a}	9.96 ± 0.83^{a}	0.75 ± 0.02^{a}	0.99 ± 0.00^{a}	2.25 ± 0.23^{a}	2.22 ± 0.24^{a}
7	1.75 ± 0.41^{b}	4.78 ± 0.97^{cd}	0.72 ± 0.05^{a}	0.96 ± 0.02^{a}	1.27 ± 0.35^{b}	1.22 ± 0.33^{b}
8	1.73 ± 0.13^{b}	4.03 ± 0.54^{d}	0.69 ± 0.05^{a}	0.94 ± 0.01^{a}	1.19 ± 0.09^{b}	1.12 ± 0.08^{b}
9	2.11 ± 0.02^{b}	6.17 ± 0.41^{bc}	0.69 ± 0.03^{a}	0.96 ± 0.03^{a}	1.45 ± 0.06^{b}	1.39 ± 0.11^{b}
10	2.00 ± 0.19^{b}	5.39 ± 0.46^{bcd}	0.68 ± 0.03^{a}	0.93 ± 0.01^{a}	1.36 ± 0.19^{b}	1.26 ± 0.17^{b}

Results were expressed as mean ± standard deviations, (n=3). (a-d) values with the same superscript in the same column are not significantly different according to Tukey pairwise comparison test ($p \leq 0.05$).

Basu, Shivhare, and Singh (2013) also reported that the increasing stevioside concentration resulted in a decrease in the hardness values of the mango jam prepared with stevioside substitutions. Moreover, their jam samples showed behaviors of thick liquids with the increase of the substitution from 25% to 100%. Similar trends were observed in the formulations using sucralose sweeteners. No significant difference was determined between the formulations prepared with sucralose in affecting the hardness. Although the sucralose concentration was increased from 25% to 50%, a decrease was determined in the hardness parameter of the formulation 5, compared to the formulation 4. There were slight differences between the formulations 9 and 10. The formulation 10 had a lower hardness value due to having a higher percentage of the sucralose concentration. In general, the sweeteners used in the marmalade preparation did not affect the hardness parameter of the samples. There was no significant difference in hardness of the marmalade prepared with different concentrations of stevioside and sucralose (Table 4.7). Likewise, the same trend was observed in the jam substituted with sorbitol. Basu et al. (2011) prepared the mango jam using different concentrations of sucrose (50%, 55%, 60%, 65% and 70%) at 1% pectin concentration and pH 3.4. Jams with sorbitol were prepared by partially or fully replacing 60%, 65% and 70% sucrose. They reported that the hardness of all the mango jam increased with the total soluble solids content; moreover, the value of hardness for the final mango jam sample decreased by increasing percentages of sorbitol. The pattern of hydrogen bonding formed by water in sorbitol is more obvious. Therefore, sorbitol also contains greater hydrogen bonding with water, compared to sucrose. In the samples made with sorbitol only, a weaker network formation with pectin was obtained and consequently, the formation of a less stable structure for the final jam sample was caused by increasing the sorbitol percentage. Their results were in agreement with the hardness results of the low sugar apple marmalade.

Adhesiveness as a textural characteristic, shows a negative force area in the curves of texture profile analysis. It is the work required to overcome the sticky forces between the sample and the probe. The adhesiveness results of the low sugar apple marmalade samples were obtained in a wide range from 4.03 to 9.97 J. As in the hardness, the lowest value of adhesiveness was observed in the formulation 8 (Stevioside-50), whereas the highest value in the formulation 6. During the return of the probe, maximum force was required for overcoming the attractive forces between the surface of both the probe and the food product. Therefore, the strong forces contributed to the highest increase in the adhesiveness value for the formulation 6 due to strong gel structure of the marmalade containing the highest degree of sucrose. For marmalade formulations containing sucrose only, it was observed that the formulations are significantly ($p<0.05$) different from each other. In the formulations 2 and 3, the increase in the stevioside sweeteners percentages resulted in a decrease in the adhesiveness value of the formulations. On the other hand, there was no significant differences in the adhesiveness value of the formulations 4 and 5, although the sucralose substitutions increased from 25% to 50%. In other words, the formulation 4 remained very similar to the marmalade formulation 5. Regarding 600 g marmalades reformulated with sweeteners, the increase of the stevioside sweeteners concentrations caused a marked decrease in the formulation 8, compared to the formulation 6. Similarly, the formulation 10 having high percentages of sucralose concentration showed lower values in adhesiveness, compared to the formulation 9. Similar results were obtained from the Cantaloupe jam prepared by substituting sucrose with different xylitol concentrations. Naknaen and Itthisoponkul (2015) observed that the increased xylitol concentration slightly reduced the stickiness/adhesiveness values in the cantaloupe jam. The authors indicated that the increased substitution yielded a lower stickiness and a softer gel structure for the jams. Vilela et al. (2015) found that the different sugar-sweeteners formulations (sucrose, fructose, fructooligosaccharides (FOS) and sorbitol) used in the strawberry, raspberry and cherry jam preparations significantly affected the adhesiveness of the strawberry jam samples. In terms of adhesiveness, the formulations containing sorbitol and FOS were observed to be significantly different from other formulations for the strawberry, raspberry and cherry jams. Because FOS could result in an increase in the compactness of microstructure during the jam preparation. Abid et al. (2018) prepared various pomegranate jam formulations by substituting the pectin extracted from the peel, commercial pectin and dry and lyophilized peel of pomegranate fruits. The addition of pectin positively contributed to the adhesiveness of the jam samples. It was possible that the more adhesive pomegranate jams were made by increasing the pectin concentration.

Another texture parameter, cohesiveness, which is expressed as a ratio of the areas of positive forces under the compressions (A_2/A_1), gives how well the product resists a second deformation, compared to under the first deformation behavior. It indicates the strength of internal bonds in the sample. In terms of cohesiveness parameter, there were not any significant differences among all formulations. In other words, the formulations which were made by adding sweeteners were not significantly effective on the values of cohesiveness parameters. Furthermore, the results of cohesiveness followed a similar trend to the results of hardness. Measurement results of the low sugar apple marmalade were found to range from 0.66 to 0.75, as depicted in Table 4.7. The highest value of cohesiveness was observed in the formulation 6 as expected, whereas the lowest value in the formulation 5. Cohesiveness values were yielded the lowest with increasing sweeteners concentrations. The formulation 5 had a more deformable and a moister structure. The observation was supported by the hardness values of the marmalade samples. Garrido et al. (2015) also indicated that the brittleness of the material highly increased with the decrease in the cohesiveness values. As the percentages of stevioside sweeteners increased, a gradual decrease was determined in the formulation 3, compared to the formulation 2. Similarly, the formulation 7 had a higher cohesiveness value than the formulation 8. For the marmalade formulations made using sucralose sweeteners, the cohesiveness values decreased in the formulation 5, compared to the formulation 4 as the sweeteners percentages increased from 25% to 50%. With the same trend, it was found that the formulation 10 had a lower value than the formulation 9 due to the decrease in the cohesiveness value by the increased substitutions. This is due to the fact that the formation of the pectin network became extremely weak and less stable due to reducing the amount of sugar. In the study conducted by Royer et al. (2006), it was observed that the increase in the hardness and cohesiveness of the apple pomace and quince jelly resulted from the addition of further quince to the formulations. Quince fruit having the high pectin concentration supported the jellification and affected the texture of the final product. Belović et al. (2017) obtained similar results from four low-calorie jam formulations. Since the sufficient amount of sugar was not present for gelling with natural pectic components obtained from the tomato pomace, the jam made by partially replacing the sucrose (50%) with stevioside led to a highly soft formulation. Increasing the pectin concentration from 1% to 1.25% in their jam formulations caused an increase in the structure stiffness. Thus, the values of textural firmness increased with the addition of pectin.

Springiness is a parameter for determining the texture profile of the products. It is closely related to the elasticity of the samples. After a deformation occurs during the first compression, springiness demonstrates how well the sample physically spreads back. In other words, it is the rate at which a deformed sample returns to its original size and shape. In general, it was found that there were no significant differences in the springiness properties of all formulations. The addition of the sweeteners to the formulations led to a decrease in the springiness of the low sugar apple marmalade samples (Table 4.7). As expected, the formulation 6 contained the highest value of springiness property (0.99 m) due to its high hardness values. Increasing the stevioside percentages in the process from 25% to 50% yielded a lower value in the springiness for the formulation 3, compared to the formulation 2. The formulation 8 also exhibited similar behaviors when the stevioside concentrations were changed between those values. This trend may be due to the deformation of pectin or less stable network structure. For both the formulations 4 and 5, the addition of sucralose sweeteners reduced springiness values of the formulation with respect to those with non-sweeteners. Although the sucralose sweeteners concentration was increased, the springiness of both the formulations 9 and 10 did not significantly differ from each other. When optimum conditions were exceeded or not provided, it was found that the elasticity of the samples decreased with deformation of the pectin network. Peinado et al. (2015) suggested that hydrolysis of the pectin molecule could have an impact on the structure of gel and its elasticity. Furthermore, mechanical properties were significantly affected by the variables only, which were pectin and citric acid and interactions of these at the same time. The use of different types of sugar (isomaltulose, sucrose and fructose) was significantly effective on the spreadability of strawberry formulations at high pectin concentrations.

Another parameter of texture examined in this study was gumminess, which is defined as the product of the values of hardness and cohesiveness. It is the energy needed to disintegrate a semisolid food until it is ready to swallow. The results of gumminess parameter for the low sugar apple marmalade samples ranged from 1.19 to 2.25 N, as is seen in Table 4.7. Gumminess values of the formulations were reduced by the increase of the concentrations of both sweeteners. However, according to the results of the ANOVA analysis, the gumminess values of all formulations, except the formulation 6, were not significantly affected by the addition of sweeteners substitutions. The formulation 6 was significantly different with the value 2.25 N from others. It was found that for both formulations 2 and 3, there were slight differences in the gumminess with the increased stevioside substitutions. Moreover, an increasing trend in the stevioside percentages caused a slight decrease in the gumminess of the formulation 8, in comparison with the formulation 7. The low sugar marmalade made by using sucralose substitutions showed similar behaviors with respect to those reformulated by stevioside sweeteners. Increasing the sucralose percentages in marmalade production from 25% to 50% resulted in the decrease of the gumminess values of the formulation 5, compared to the formulation 4.

In the same manner, it was found that there was no significant impact of the increase of the sucralose concentrations for both of the formulations 9 and 10.

The last parameter of the textural characteristics is chewiness, which is expressed as the product of the values of gumminess and springiness. In other words, it can be described as an energy required for masticating the food. The chewiness results of the low sugar apple marmalade ranged from 1.10 to 2.22 J, as depicted in Table 4.7. The chewiness values of the marmalades followed the same trend as other parameters. It was observed that the increase in both sweeteners concentrations in the marmalade samples decreased the values of chewiness with respect to the samples to which sucrose was added only. Concerning the chewiness property, it was found that the marmalade formulated with 600 g sucrose was significantly different from others. The one-way ANOVA results indicated that the addition of the sweeteners to the apple marmalade samples did not significantly change the chewiness values of those. Concerning the apple marmalades containing stevioside sweeteners, when the sweeteners concentrations were increased, a slight decrease was determined in the formulation 3, compared to the formulation 2. Also, the formulation 8 had a lower chewiness degree than the formulation 7 due to increasing the stevioside percentages from 25% to 50%. This may be due to the presence of a lower amount of sucrose. Thus, pectin and water interactions were supported by increasing the stevioside concentration. Cai et al. (2017) prepared fish gelatin with the addition of xylitol and stevia sweeteners. Although the addition of sweeteners did not significantly affect the springiness values of gelatin, an increase in the stevia concentration from 0% to 5% caused a significant decrease in the hardness, cohesiveness, and chewiness. It was found that stevia had less pronounced effects than xylitol. Due to its higher solubility and OH groups, the xylitol had a little effect on the gel texture of gummi-type candies when used even at high concentrations to replace sucrose. The addition of sucralose sweeteners to the marmalade formulations showed the same trend in the chewiness value with respect to those to which stevioside was added. For the formulations 4 and 5, there were slight differences in the chewiness of the formulations; moreover, the formulation 5 had the lowest value in chewiness. Similarly, increasing the sucralose concentration in the process from 25% to 50% led to a decrease in the chewiness value of the formulation 10, compared to the formulation 9. But in general, there was no significant difference in the chewiness parameters of samples prepared with stevioside and sucralose ($p<0.05$).

4.5. Sensory Evaluation of Low Sugar Apple Marmalade

Sensory evaluation, or sensory analysis, is the process of evaluating consumer products by the senses of sight, smell, taste, touch and hearing. Sensory evaluation is a necessary part of both product development and quality control. The analysis determines the quality and acceptability of the product to meet the consumer perception. In order to assess the quality of the low sugar apple marmalade samples prepared with different sweeteners and percentages, the sensory parameters determined with respect to appearance, texture, color, taste and overall acceptability. The average scores of the attributes were statistically reported in Table 4.8. Furthermore, the sensory properties of low sugar marmalades were also presented in the spider chart (Figure 4.12).

The formulation 1 prepared using 500 g sucrose and the formulation 3 made by replacing of 50% of sucrose with stevioside sweetener achieved the highest scores. The control samples made by using 500 g (formulation 1) and 600 g (formulation 6) sucrose only were significantly different from others especially for texture and overall acceptability among all attributes. While the formulation 1 was selected as the most favorable one in the texture, the formulation 6 had the least acceptance due to a highly firmer and more granular structure. Since the sucrose content was extremely high in the formulation 6, the water molecules were bound to the sucrose and the network of pectin, acid, sucrose was strongly interconnected. This caused a highly apparent increase in the hardness of the structure. The results were in agreement with the instrumental results of textural properties. Similarly, it was found that the formulation 1 provided the highest degree although the formulation 6 was the lowest one in the overall acceptability parameter. Besides, the formulation 1 showed the highest score for all the attributes, while the formulation 6 relatively had the highest degree for only color attributes. Except for those samples, there were no significant differences with regards to two parameters in all formulations containing sweeteners. For the texture of the samples, the formulation 4 scored the lowest, whereas the formulation 9 showed the highest degree. Nevertheless, the addition of sweeteners to the formulations was also effective on the appearance and color parameters. The formulation 5 yielded the lowest score for color properties of the marmalade samples. Color scores of the marmalades were reduced by the decrease in the concentration of the total soluble solids (TSS). Similar trends were observed in the color

Table 4.5. Sensory Evaluation of Low Sugar Apple Marmalade

Formulation No	Appearance	Taste	Color	Texture	Overall acceptability
1	7.76 ± 1.04^{a}	6.86 ± 1.62^{a}	7.52 ± 1.21^{a}	7.43 ± 1.08^{a}	7.57 ± 1.12^{a}
2	7.10 ± 1.64^{ab}	6.05 ± 1.86^{a}	7.29 ± 1.38^{abc}	6.57 ± 1.66^{ab}	6.90 ± 1.76^{ab}
3	7.29 ± 1.52^{ab}	6.52 ± 1.69^{a}	7.38 ± 1.24^{ab}	6.86 ± 1.39^{ab}	7.00 ± 1.48^{ab}
4	6.90 ± 1.34^{ab}	5.76 ± 1.67^{a}	6.48 ± 1.44^{abc}	6.10 ± 1.48^{ab}	6.19 ± 1.60^{ab}
5	5.81 ± 2.27^{b}	6.29 ± 1.90^{a}	5.86 ± 1.77^{bc}	6.33 ± 1.65^{ab}	6.29 ± 1.76^{ab}
6	6.71 ± 2.08^{ab}	5.43 ± 2.11^{a}	6.24 ± 1.73^{abc}	5.48 ± 2.06^{b}	5.76 ± 2.10^{b}
7	7.14 ± 1.56^{ab}	6.14 ± 1.31^{a}	7.24 ± 1.18^{abc}	6.67 ± 1.96^{ab}	6.71 ± 1.45^{ab}
8	6.38 ± 1.96^{ab}	5.86 ± 1.71^{a}	6.10 ± 2.21^{abc}	6.33 ± 2.20^{ab}	6.33 ± 1.46^{ab}
9	7.05 ± 1.40^{b}	6.33 ± 1.59^{a}	6.71 ± 1.71^{abc}	7.05 ± 1.83^{ab}	6.95 ± 1.28^{ab}
10	5.86 ± 2.15^{b}	5.81 ± 2.18^{a}	5.71 ± 2.28^{c}	6.10 ± 2.43^{ab}	6.19 ± 2.18^{ab}

Results reported as mean ± standard deviations, (n=3). The significant differences were specified by Tukey pairwise comparison test ($p \leq 0.05$). Means do not share the same letters are significantly different. Each lower case (a, b, c, d) indicates differences between the sample formulations in the same column.

score of the formulation 10. Moreover, the formulations 5 and 10 demonstrated a lighter color and less stable structure due to a lower amount of sucrose, resulting in the lowest appearance and color score among all formulations. Also, it was observed that not all the formulations significantly differed from each other in terms of taste sensations. The formulations 4 and 6 had the lowest scores in sweetness, whereas the formulations 1 and 9 contributed to higher scores, compared to other formulations.

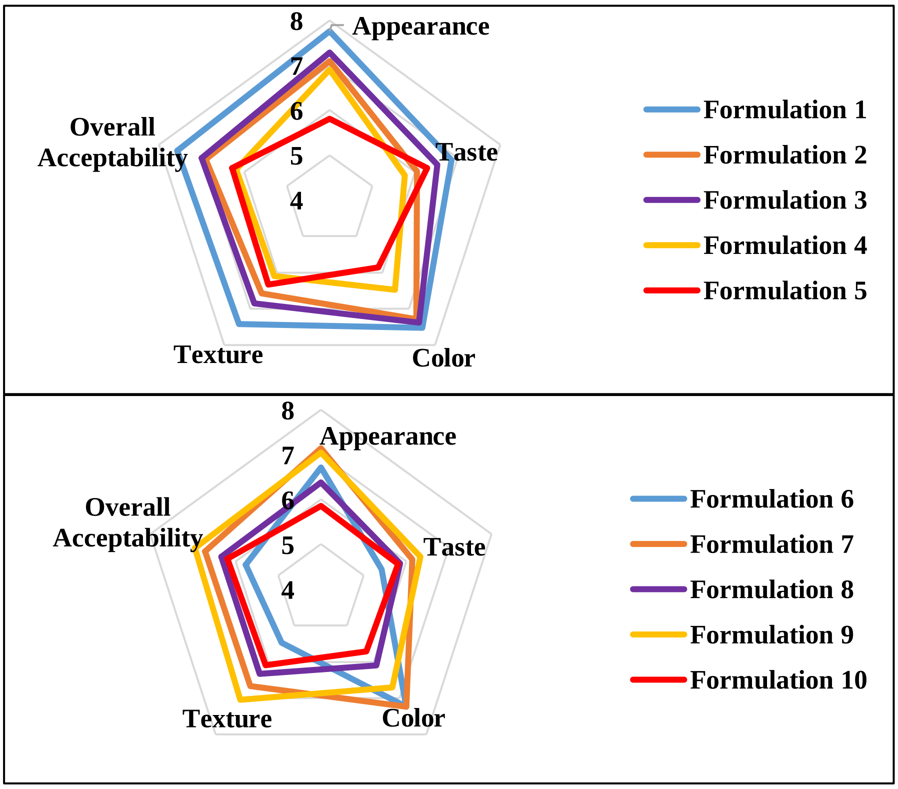


Figure 4.12. Sensory Attributes of Each Marmalade Formulations

- **Appearance of Low Sugar Apple Marmalade Samples**

In order to understand the processing effects on the product and consequently the influence of consumer acceptance, the investigation of appearance is a major stage for food quality. Food appearance is not only the color specification of a product, it comprises a wider area in relation to quality changes in the product (Hutchings, Ronnier Luo, and Ji, 2013; Murray and Baxter, 2003). According to the test scores, it was observed that the sample made with 500g sucrose was the most favorable sample for the appearance parameter as depicted in Table 4.8 and Figure 4.13. Significant differences were determined between the formulation 1 and others in affecting the parameter ($p<0.05$). On the other hand, the highest score was observed in the formulation 3 among the samples made by using sweeteners; moreover, it was determined that there were slight differences between the formulations made with different concentrations of stevioside and those made with sucralose (25%). On the other hand, the lowest scores were observed in the formulations prepared by replacing 50% of sucrose with sucralose. There was no significant difference between the formulations 5 and 10. Also, the formulation 5 was the lowest one with a value of 5.81. This can be due to less sucrose content in the formulations. Compared to the sucrose, the sweeteners allowed water to interact with the pectin molecules. Thus, the samples appeared to have a softer and moister structure because of a weak network structure of pectin. This observation resulted in the greenish/yellowish color of the product. In addition to this, it could be explained by the fact that the low scores were caused by the color of the product. Although all of the formulations prepared with 500g sucrose relatively resembled a pentagon shape, the shape of others was distorted (Figure 4.13).

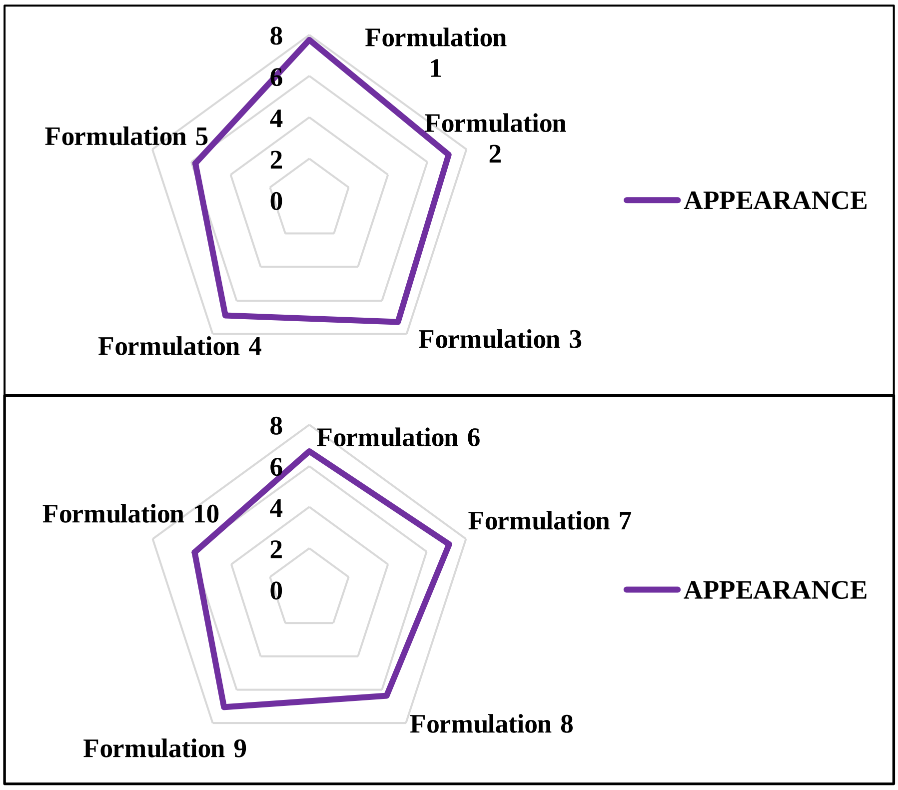


Figure 4.13. Appearance Attributes of Low Sugar Apple Marmalade

- **Taste of Low Sugar Apple Marmalade Samples**

During the development of the food product, the impacts on sensory characteristics (taste, flavor), as well as the effects on health for consumer's perception and acceptance are important. The sensation of a taste is associated with personal impressions and taste experiences, depending on the age, preferences, habits and environmental conditions (Guiné, Ramalhosa, and Valente, 2016). The taste parameter results of the low sugar apple marmalade were found to range from 6.86 to 5.76, as in Table 4.8. The spider plot (Fig 4.14) also showed that the highest score of marmalades was determined in the formulation 1, whereas the formulation 6 had the lowest one. This is because the formulation 6 was extremely sweet due to higher sucrose content. Although the panelists who like higher sweetness gave the formulation high scores, the samples remained in the lowest degree. Indeed, the addition of sweeteners to the formulations did not significantly affect the taste parameters of all formulations. As expected, this is a desirable situation. Thus, jams made with sweeteners can be depicted as a mimic to those made with sugar. Among the samples containing stevioside sweeteners, the formulation 3 had the highest degree in the taste sensation (Fig 4.14). Nevertheless, the least favorite was determined to be the formulation 8 in those samples. While the formulation 9 was observed to have the highest score, the lowest score was observed in the formulation 5 for the taste parameters of the samples reformulated by sucralose sweeteners. According to the feedback from the panelists, some formulations with stevia sweeteners caused a bitter taste in the mouth when their concentrations were increased. In the study conducted by Gwak et al. (2012), the samples with different concentration levels were prepared by using eight bulk sweeteners and four intense sweeteners. The authors also investigated whether the sweeteners had similar sensory qualities to sucrose. They found that sucralose followed a similar pathway with sucrose and showed a lower bitterness with respect to stevia. Also, stevia led to a continual taste in the mouth. However, the sample with the second highest score for taste attributes contained stevia sweeteners (formulation 3 developed by replacing 50% of sucrose). This can be associated with the fact that the apple taste and addition of sucrose both mask the bitter taste of stevia. Alizadeh et al. (2014) made fruit-based milkshake by substituting sucrose with stevia. The results of the sensory properties of their samples showed that the sample containing stevia concentration (75%) had the best quality of overall; moreover, it was observed that the samples containing stevia (100%) were similar to the control samples in terms of the "mean liking score".

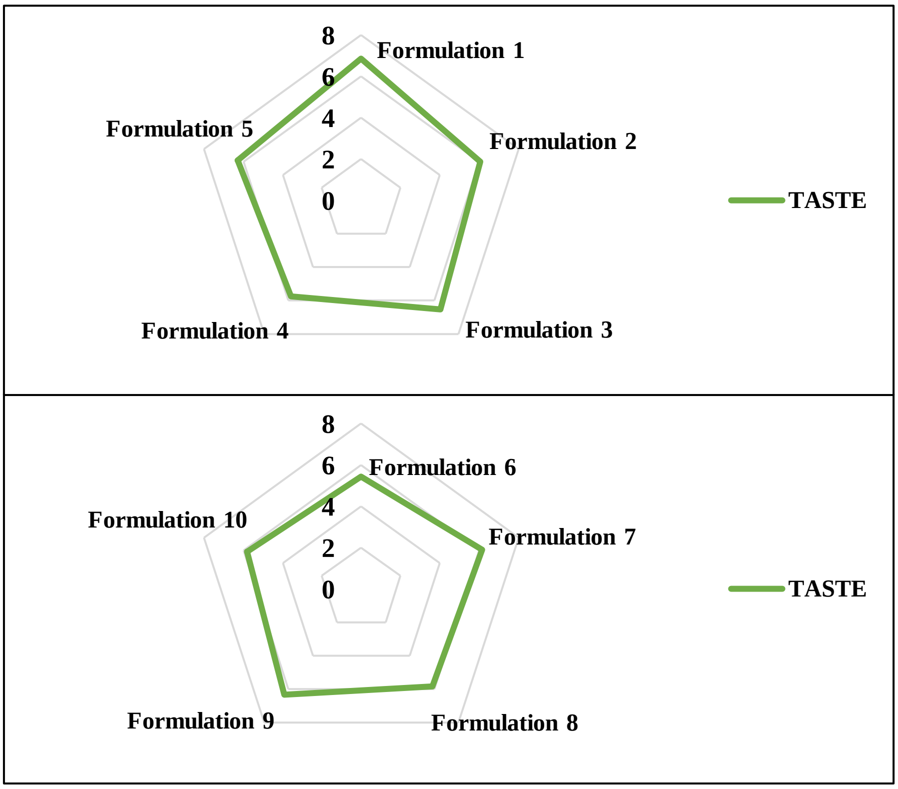


Figure 4.14. Taste Attributes of Low Sugar Apple Marmalade

- **Color of Low Sugar Apple Marmalade Samples**

The color is a major quality property for sensorial perception. First of all, the color and appearance of a product are remarkable for the consumers' quality perception. Thus, it clearly provides information about the safety and health of the products and can be predicted as non-sensorial characteristics for the acceptability of products (Granato and Masson, 2010; Antonio-Gutiérrez et al., 2019). The sensory color profiles of the low sugar apple marmalade formulations ranged from 5.76 to 7.52, as depicted in Table 4.8. The formulation 1 as a control sample yielded the highest score for color parameters, as shown in Figure 4.15. Statistically significant differences between formulations were determined. The formulation 3 having the second highest score remained very similar to the control sample, in terms of color attributes of the samples. Figure 4.15 demonstrated that the addition of 50 percent sucralose resulted in the lowest formulation scores for formulations 5 and 10. The least acceptance might be associated with the highly light color of the formulations containing a lower amount of sucrose. When there is a higher sucrose content for formulations, the amount of caramelized sucrose increases by increasing its concentration. This leads to a darker color and higher scores in the samples. Also, the formulation 10 contributed to the lowest value of the color parameters in all formulations.

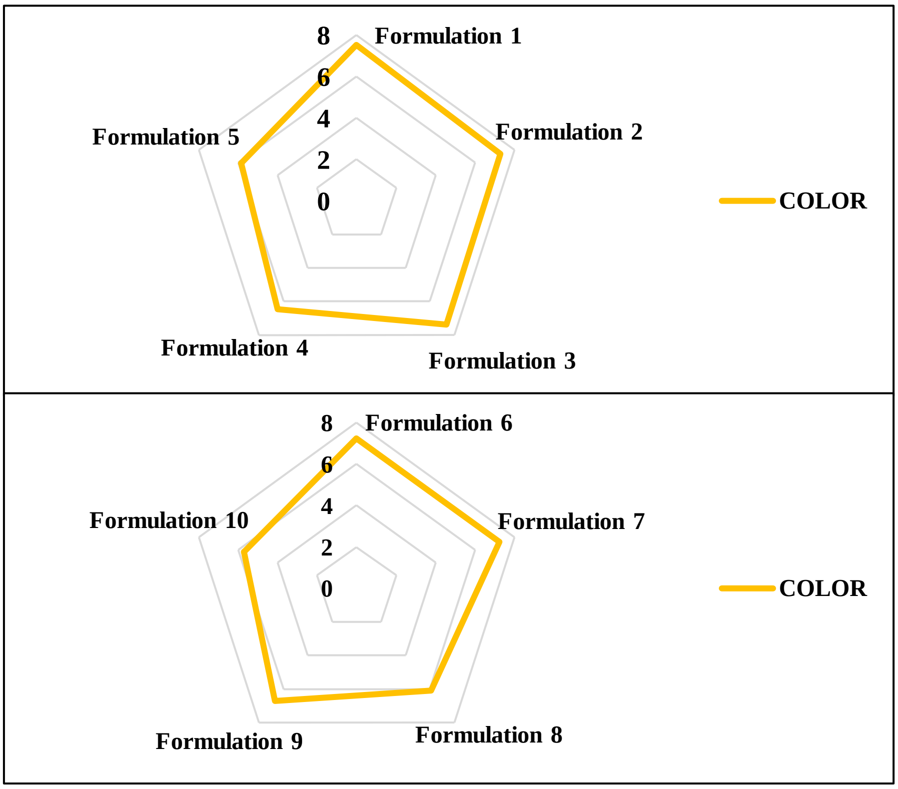


Figure 4.15. Color Attributes of Low Sugar Apple Marmalade

- **Texture of Low Sugar Apple Marmalade Samples**

The term "food texture" is strongly related to the sensorial experience. The processing and preparation of food products and the interactions of their ingredients are effective in the generation or alteration of the texture of food. The perception of texture affects the liking and buying behavior of the consumer in a product preference (Jarén, López, and Arazuri, 2016). The sensory texture data of the product formulations were found to range from 5.48 to 7.43, as depicted in Table 4.8. The samples made by using 500g sucrose were the most preferred ones in terms of texture attributes. While the formulation 1 had the highest scores of texture results, the formulation 6 had the lowest scores, as depicted in Figure 4.16. This is because the formulation 6 contains the highest amount of sucrose. Thus, its structure is highly firm and stiff, compared to the formulation

1. Nevertheless, the formulation 1 had a more spreadable consistency than the formulation 6. For the formulations containing sucrose only, there were significant differences in the formulations 1 and 6. Except for these formulations, when the texture parameters of the marmalade samples with different formulations were compared, it was determined that the addition of sweeteners to the formulations resulted in no statistically significant difference. Having the second highest score, the formulation 9 showed similar texture behaviors with the control sample (Formulation 1). In the formulations containing stevioside sweeteners, the formulation 3 yielded a higher degree than others. It was observed that the formulations 4 and 10 were very similar to each other. According to the Figure 4.16, a pentagon-shaped deformation arising from formulation 4 was observed.

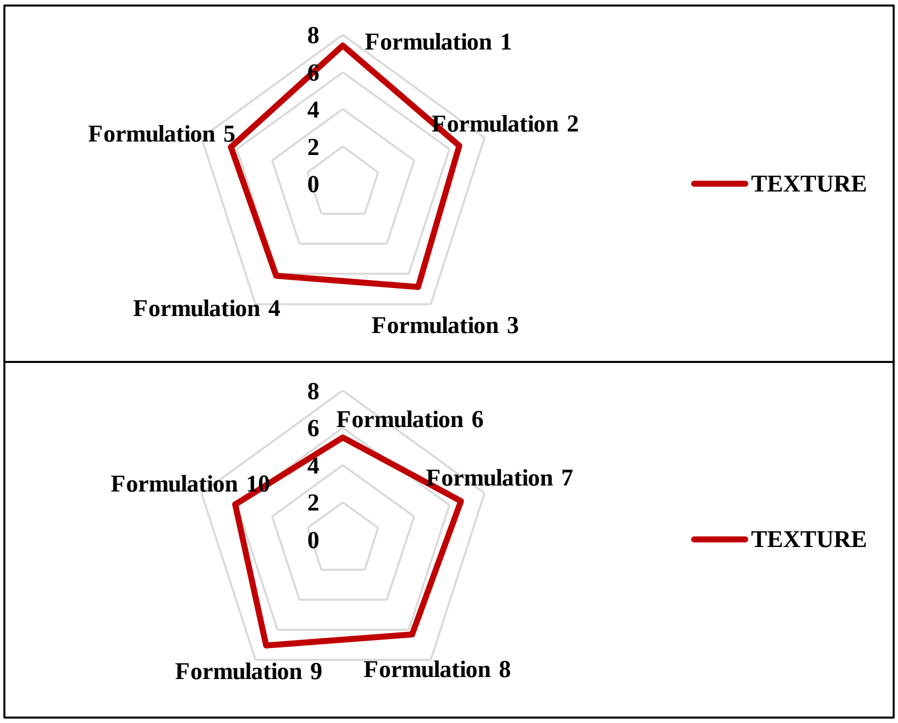


Figure 4.16. Texture Attributes of Low Sugar Apple Marmalade

- **Overall Acceptability of Low Sugar Apple Marmalade Samples**

The sensory characteristics are strongly related to the overall food acceptability. The characteristics including taste, flavor, shape/size, color, odor and texture are considered as the parameters that affect the quality acceptability of the product by the consumers. The acceptability is a subjective measurement depending mainly on the sensory attributes and is influenced by some factors such as the environmental conditions, physiological and cultural status of the panelists (Murray and Baxter, 2003; Varzakas and Tzia, 2015).

As is seen in Figure 4.17, when the overall acceptability in all formulations was scored, it was found that the most favorable formulation was the apple marmalade sample prepared with 500 g sucrose (Formulation 1). The effect of sweeteners additive on the overall acceptability of the formulation 3 had a very similar result to the formulation 1 by scoring the second-best. No statistically significant difference was observed in the overall acceptance of different formulations containing stevioside and sucralose sweeteners, as depicted in Table 4.5. The results of the low sugar apple marmalade agree with the low sugar mixed fruit jam containing various sweeteners prepared by Souza et al. (2013). It was found that the fruit jam made with sucrose and those with low-sugar did not significantly differ from each other in terms of all of the attributes in their acceptance test. In another survey, Saveski and Stamatovska (2015) evaluated the highest degree in the raspberry jams containing different sweeteners by applying a 9-poins hedonic test. The jam containing sorbitol provided the best acceptance, compared to the formulations containing fructose and agave syrup. Similarly, Basu, Shivhare, and Singh (2013) reported that the mango jam made by using sorbitol level (70) with 75% sorbitol substitution showed the best acceptability, compared to those containing sucrose. Rubio-Arraez et al. (2015) supported the marmalade made with healthy sweeteners. Their marmalades were scored the best with respect to those made using sucrose only in terms of overall preference and buying intention. In contrast, the formulations 1 and 6 were significantly different from each other. Figure 4.17 showed that the formulation 6 provided the lowest score of overall acceptability in all formulations. This can be due to the fact that the formulation 6 had a firmer structure and an extremely higher sweetness. For the marmalade samples reformulated with sucralose sweeteners, the formulation 9 was scored as the highest, compared to others. The formulation 9 had the highest score for all the sensory attributes with respect to those formulations made with sucralose and consequently, this evaluation contributed to a high score in the overall acceptability of the formulation. Also, the formulations 4 and 10 have similar scores with slight differences. The formulation 4 showed a similar trend with texture in the radial chart and the proper pentagon shape was deformed because of the lower score. At the same time, it was observed that the formulations 4 and 10, having almost the same score were very similar to each other.

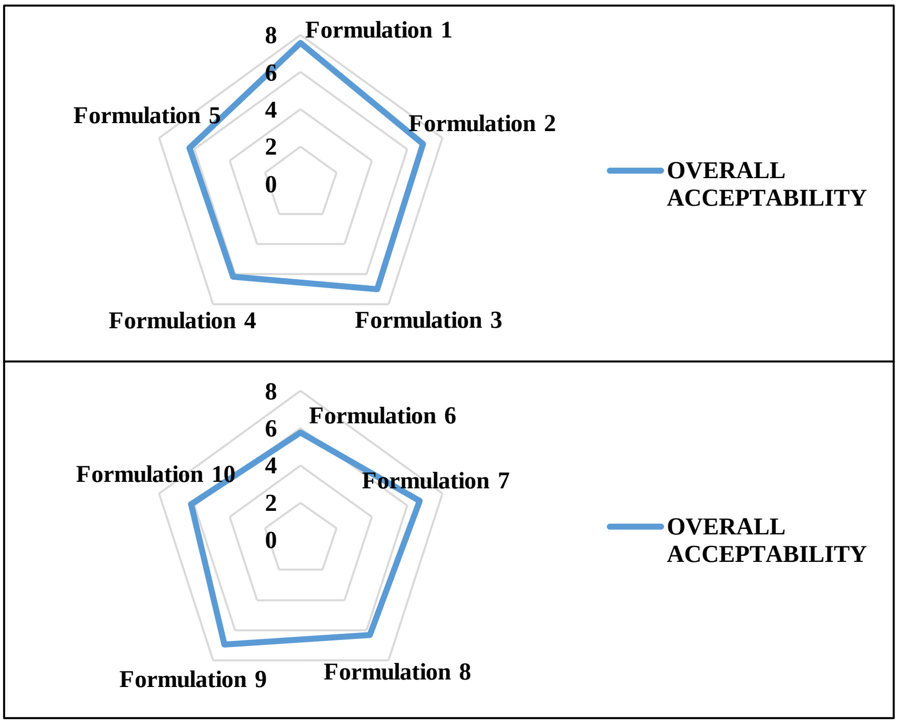


Figure 4.17. Overall Acceptability of Low Sugar Apple Marmalade

4.6. Microstructural Properties of Low Sugar Apple Marmalade

The morphological differences of low sugar apple marmalade formulations which were prepared by using stevioside and sucralose sweeteners were compared with using scanning electron microscopy (SEM). SEM images examined at 500x magnifications were shown in Figures 4.18 and 4.19. Micrograph of the freeze-dried marmalade samples containing 500g sucrose (formulation 1) showed smooth surface with partial networks due to pectin, acid, sucrose gel mixture (Figure 4.18a). While the content of the marmalade was changed by substitution of 25% sucrose with stevioside (Formulation 2), the pectin network structure slightly disappeared and became more homogenous (Figure 4.18b). By increasing the stevia concentration, i.e., replacing 50% of sucrose with stevioside, the surface roughness increased. On the other hand, the addition of sucralose sweeteners (formulation 4), a rough surface occurred with pores. As increasing sweeteners concentrations, the formation of porous structure increased (formulation 5). Compared to the formulation 1, both sweeteners increased the surface roughness but the increase in the concentration of sucralose led to more surface deformation than stevioside.

As the amount of sucrose increases, it is thought that a better pectin network is formed. Therefore, a smoother surface appearance is obtained. In Figure 4.19a, the formation of the network structure was observed more clearly, compared to Figure 4.18a. The SEM images of low sugar apple marmalade in the figures were in agreement with the results of the apple jam which was reported by Tan et al. (2014). The authors prepared apple jam by using both 15 g sucrose and cross-linked acetylated starch (CAS). SEM micrograph of sucrose containing apple jam showed a smoother surface. Further, porous structures were obtained by addition of a varied amount of CAS in the apple jam. When stevioside concentration was increased, i.e., 25% and 50% sucrose was substituted with stevioside sweeteners, the images showed the formation of porous structure due to the loss of the mesh structure of pectin (Figure 4.19b, c). On the other hand, the increase in the amount of sucrose from 500 g to 600 g contributed to the pectin network formation in the presence of sucralose (Figure 4.19d, e). It was observed that the formulation 10 remained very similar to the formulation 6 in terms of surface homogeneity and formation of the pectin network (Figure 4.19e).

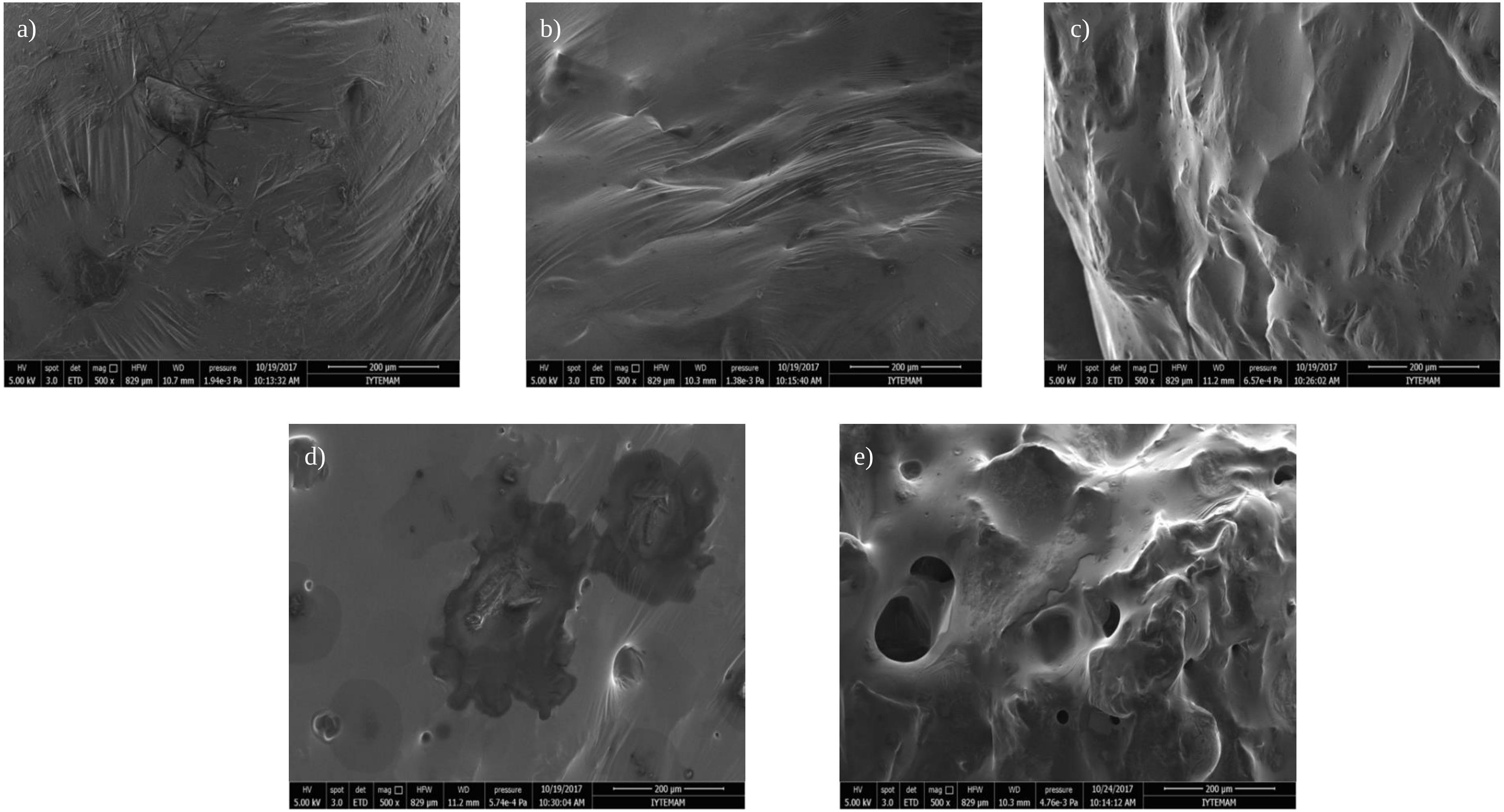


Figure 4.18. SEM Micrographs of Low Sugar Apple Marmalade Formulations (500g) at 500x Magnifications a) Formulation 1 b) Formulation 2 c) Formulation 3 d) Formulation 4 e) Formulation 5

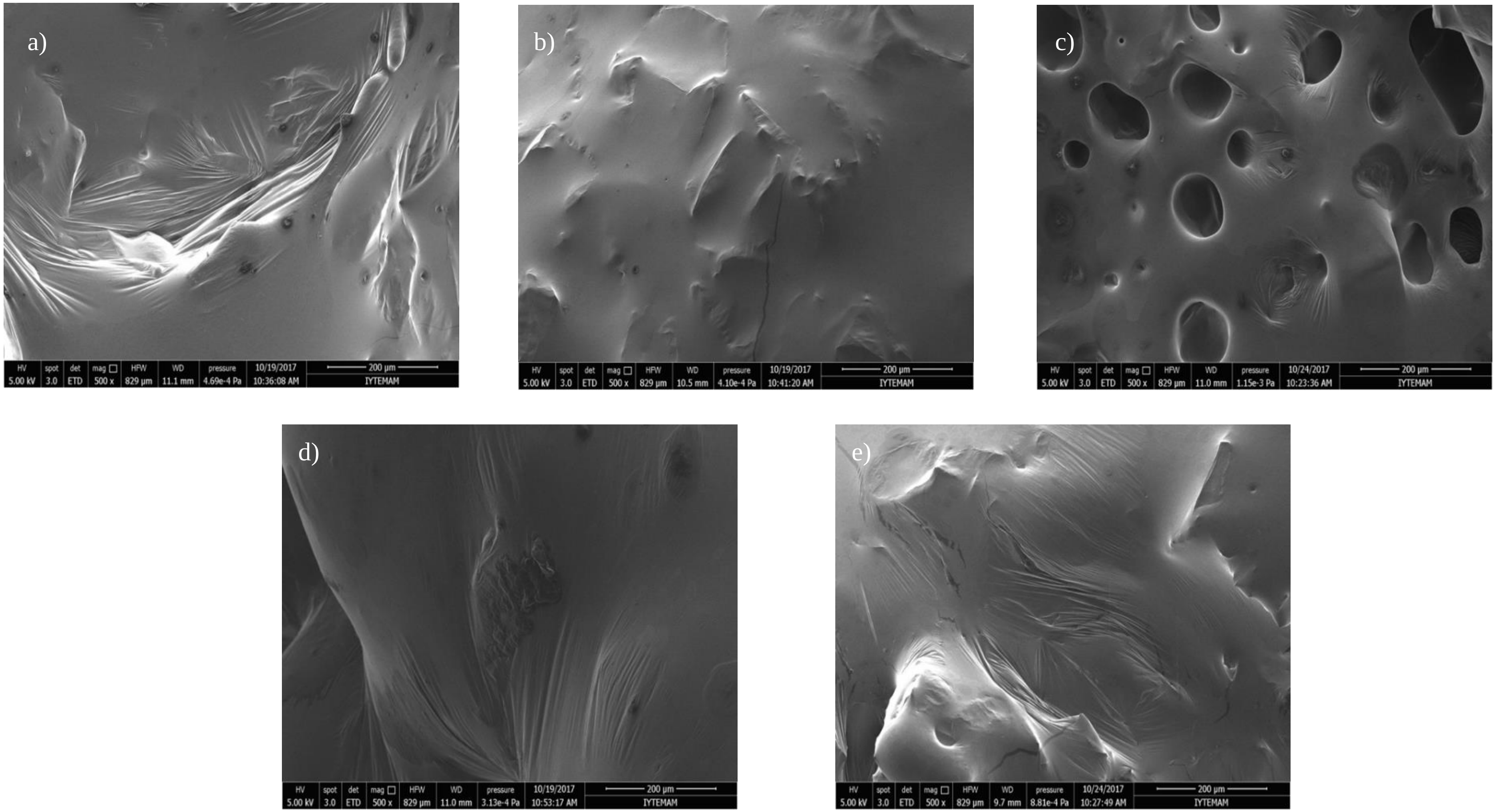


Figure 4.19. SEM Micrographs of Low Sugar Apple Marmalade Formulations (600g) at 500x Magnifications a) Formulation 6 b) Formulation 7 c) Formulation 8 d) Formulation 9 e) Formulation 10

CHAPTER 5

CONCLUSIONS

In this study, low sugar apple marmalade formulations were produced by using two types of sweeteners (stevioside and sucralose) at different concentrations. It was aimed to reveal the best marmalade formulation containing sweeteners similar to the control samples with respect to their physicochemical, textural, rheological and sensorial properties.

The addition of the sweeteners to the formulations had a significant effect on the most of the physicochemical, textural, rheological and sensorial properties of the marmalade samples. Changes in the properties were mostly related to the gel structure of the marmalade samples arising from the interactions between sucrose-acid-pectin. Increasing the amount of sucrose contributed to the increase in the hardness of the gel. As a healthy alternative, the addition of the sweeteners led to a softer and moister structure in the marmalade formulations.

Formulation changes with sweeteners concentrations were significantly effective on the physicochemical properties of the marmalade samples, except for the total ash content. As increasing the sweetener concentrations, water activity and titratable acidity significantly increased, whereas total soluble solid (TSS) and total solid content significantly decreased. This resulted in the soft structure and low gel strength in the apple marmalade products. Similarly, both lightness (L*) and yellowness (b*) values increased with increasing sweetener substitutions, greenness (a*) values showed a reverse trend in the color properties of the formulated samples and consequently, the samples turned into more lighter, yellow and green.

Rheological measurements were carried out by the following 4 steps procedure. The shear stress values plotted vs shear rate data in stepped flow test were fitted very well to a common rheological model, i.e., Herschel-Bulkley equation. The rheological model parameters depended linearly on the applied loading stress in the examined torque interval. The flow behavior index increased by the increase of the sweeteners substitutions, whereas the consistency index reduced with increasing the sweeteners percentages from 25% to 50%. All marmalade formulations exhibited Non-Newtonian (shear thinning) behaviors. In oscillation stress sweep test, dynamic viscoelastic properties of the low sugar marmalade samples were determined within the linear viscoelastic region. It was found that the elastic modulus (G′) dominantly contributed to the viscoelastic properties of the apple marmalades throughout the stress range. This is an indication of the solid-like behavior exhibited by all marmalade formulations due to higher elastic modulus values.

Textural properties of low sugar apple marmalade products were determined by using Texture Profile Analysis (TPA). The parameters, i.e., hardness, adhesiveness, gumminess and chewiness, significantly affected by the increase of the sucrose content. The addition of artificial sweeteners to the formulations containing only 600 g sucrose had significant effect on these parameters. On the other hand, it was observed that there was no significant difference between the formulations prepared with using sweeteners ($p>0.05$). However, the hardness of the samples decreased with increasing sweeteners substitutions due to low amount of sucrose content. This resulted in the softer and moisture structure in the end product.

In sensory analysis, with respect to overall acceptability, the formulation 1 containing only 500 g sucrose and the formulation 3 prepared by replacing of 50% of sucrose with stevioside sweeteners were the most preferred samples with the highest score. Thus, the formulations prepared by using sweeteners were as acceptable as those prepared with only sucrose.

According to SEM micrographs, it was observed that the surface changed depending on the increase of substitutions and sucrose content. But these images are not enough to explain about the microstructural changes of the low sugar apple marmalade samples.

As a future study, low sugar apple marmalade products can be produced using a combination of stevioside and sucralose sweeteners. Besides, as further research, time-dependent rheological behaviors of these products can be investigated in detail.

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APPENDIX A

EXPERIMENTAL DATA OF LOW SUGAR APPLE MARMALADE

Table A.1. Rheological Data for Step1

Formulation	Oscillation Stress Sweep Test (Step 1)			
	G′ (Pa)		G″ (Pa)	
	Mean + Std dv	**Range**	**Mean + Std dv**	**Range**
1	11518.77 ± 6599.74	-2.61-16226.67	3327.49 ± 1682.14	6.20-4541.67
2	8805.34 ± 5576.32	-2.03-13106.67	2564.35 ± 1445.71	4.34-3658.33
3	8085.75 ± 5100.28	-4.49-12076.67	2129.68 ± 1159.71	1.27-2999.67
4	7404.70 ± 4641.87	-1.59-10999.67	2066.97 ± 1159.32	3.64-3169.33
5	9066.74 ± 5839.40	-10.31-13696.67	2388.72 ± 1337.96	1.64-3395.67
6	10243.96 ± 5907.83	-1.43-14460.00	2958.23 ± 1511.66	4.91-3952.00
7	9774.83 ± 5690.24	-1.51-13853.33	2992.43 ± 1486.52	6.69-3973.67
8	8289.98 ± 5401.70	-3.47-12550.00	2372.98 ± 1358.32	1.21-3414.67
9	9214.53 ± 5486.37	-1.49-13210.00	2567.14 ± 1358.75	4.61-3514.33
10	13113.19 ± 7104.18	-2.16-17933.33	4043.71 ± 1822.16	6.74-5075.33

Table A.2. Rheological Data for Step 3

Formulations	Oscillation Time Sweep Test (Step 3)	
	G′ (Pa)	
	Mean	Range
1	13024.21 ± 298.51	12486.67-13490.00
2	12287.49 ± 314.23	11709.33-12786.67
3	11403.82 ± 672.00	10215.00-12500.00
4	12733.49 ± 324.77	12170.00-13250.00
5	12965.84 ± 516.90	12056.67-13770.00
6	13756.11 ± 297.68	13223.33-14223.33
7	11763.04 ± 432.64	11001.33-12416.67
8	12588.25 ± 477.15	11776.67-13353.33
9	15187.50 ± 371.28	14529.67-15758.67
10	15509.60 ± 478.73	14673.33-16230.00

Table A.3. Rheological Data for Step 4

Formulations	Oscillation Stress Sweep Test (Step 4)			
	G′ (Pa)		G″ (Pa)	
	Mean + Std dv	Range	Mean + Std dv	Range
1	13466.41 ± 236.75	12873.33-13660.00	3585.68 ± 50.48	3418.33-3654.00
2	12155.13 ± 294.58	11413.33-12373.33	3139.39 ± 132.22	2872.00-3329.67
3	10737.69 ± 159.82	10293.33-10876.67	2555.07 ± 154.28	2320.33-2876.00
4	14752.05 ± 197.30	14266.67-14920.00	3709.28 ± 78.28	3446.67-3807.00
5	12740.26 ± 300.46	11946.67-12963.33	3044.23 ± 186.85	2664.67-3364.67
6	13826.15 ± 191.14	13320.00-13960.00	3647.96 ± 95.32	3457.00-3795.67
7	13919.49 ± 226.22	13343.33-14106.67	3891.25 ± 119.92	3617.00-4059.67
8	12557.69 ± 260.12	11880.00-12766.67	3276.35 ± 103.85	3033.67-3443.00
9	13042.82 ± 153.30	12666.67-13173.33	3292.68 ± 36.65	3224.00-3379.33
10	13354.36 ± 127.09	13070.00-13486.67	3850.07 ± 55.98	3728.33-3930.33

APPENDIX B

MODELING OF LOW SUGAR APPLE MARMALADE USING DIFFERENT RHEOLOGICAL MODELS

1) Herschel-Bulkley (HB) Model
2) Casson Model
3) Power Law (PL) Model

1) Herschel-Bulkley Model

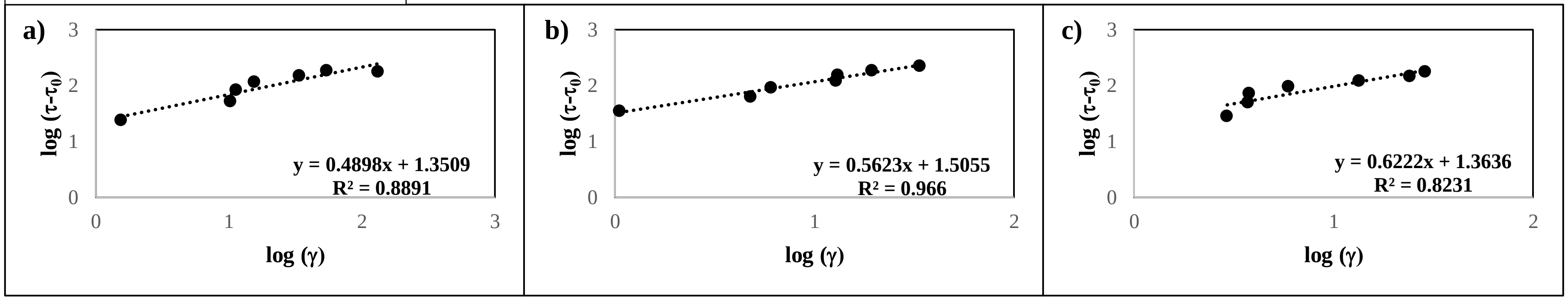

Figure B.1. Modeling of Rheological Data of Low Sugar Apple Marmalade Formulation 1 a) First Measurement b) Second Measurement c) Third Measurement

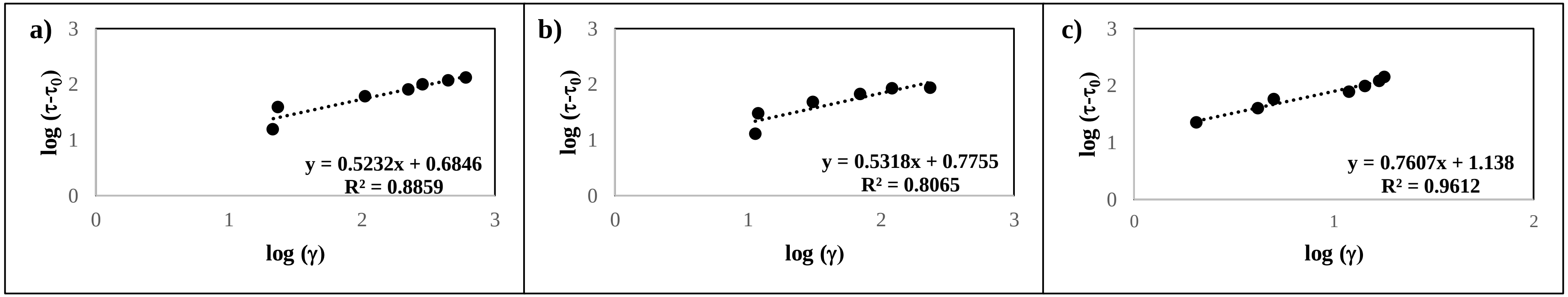

Figure B.2. Modeling of Rheological Data Low Sugar Apple Marmalade Formulation 2 a) First Measurement b) Second Measurement c) Third Measurement

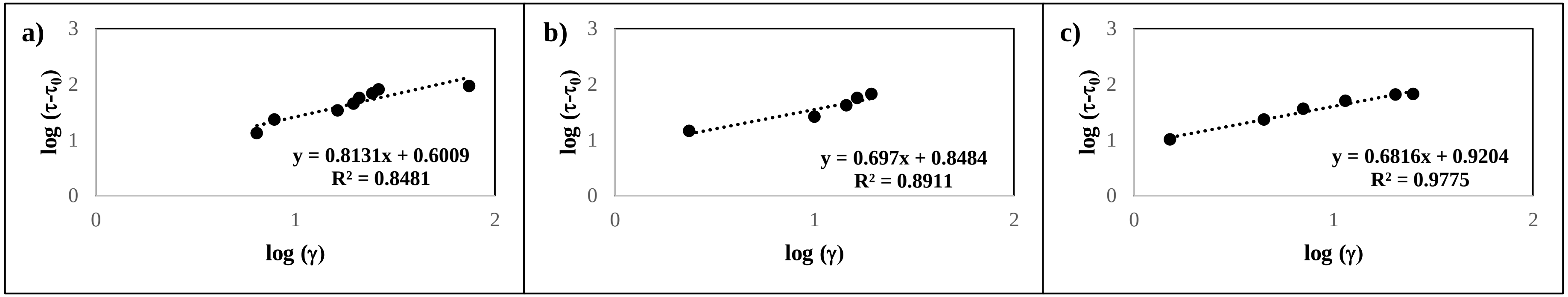

Figure B.3. Modeling of Rheological Data of Low Sugar Apple Marmalade Formulation 3 a) First Measurement b) Second Measurement c) Third Measurement

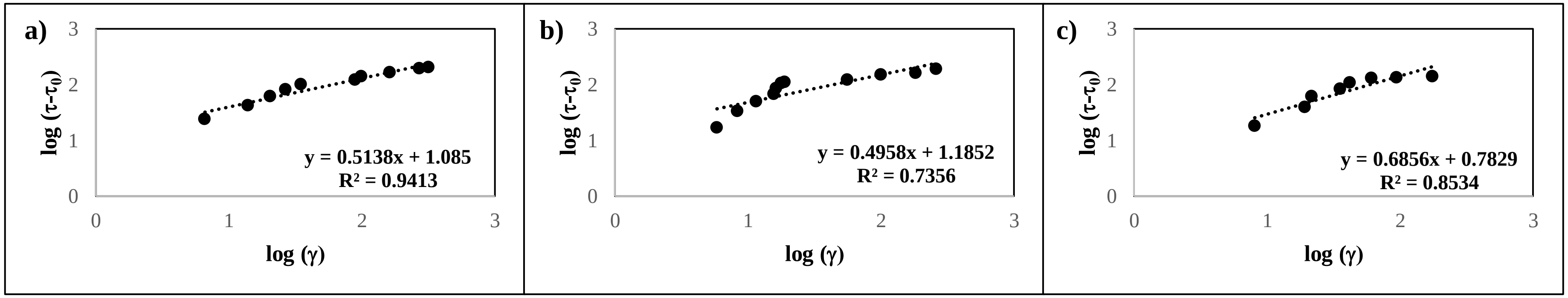

Figure B.4. Modeling of Rheological Data of Low Sugar Apple Marmalade Formulation 4 a) First Measurement b) Second Measurement c) Third Measurement

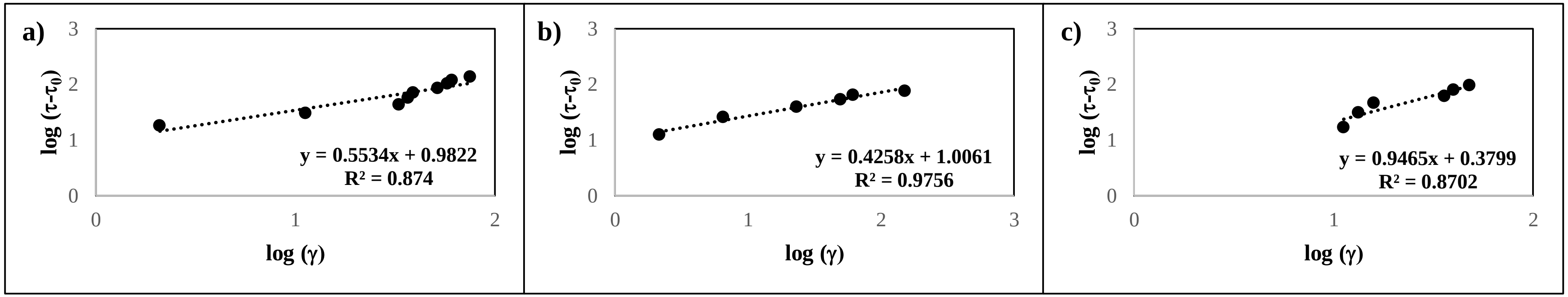

Figure B.5. Modeling of Rheological Data of Low Sugar Apple Marmalade Formulation 5 a) First Measurement b) Second Measurement c) Third Measurement

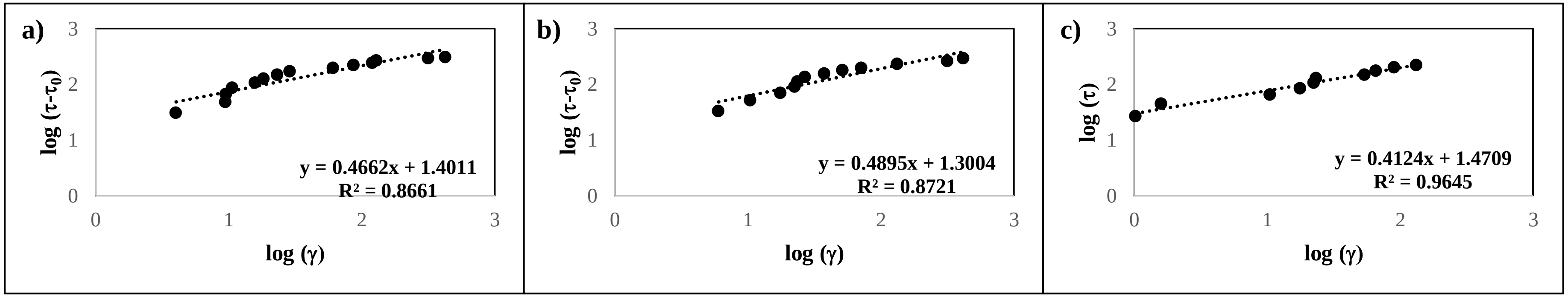

Figure B.6. Modeling of Rheological Data of Low Sugar Apple Marmalade Formulation 6 a) First Measurement b) Second Measurement c) Third Measurement

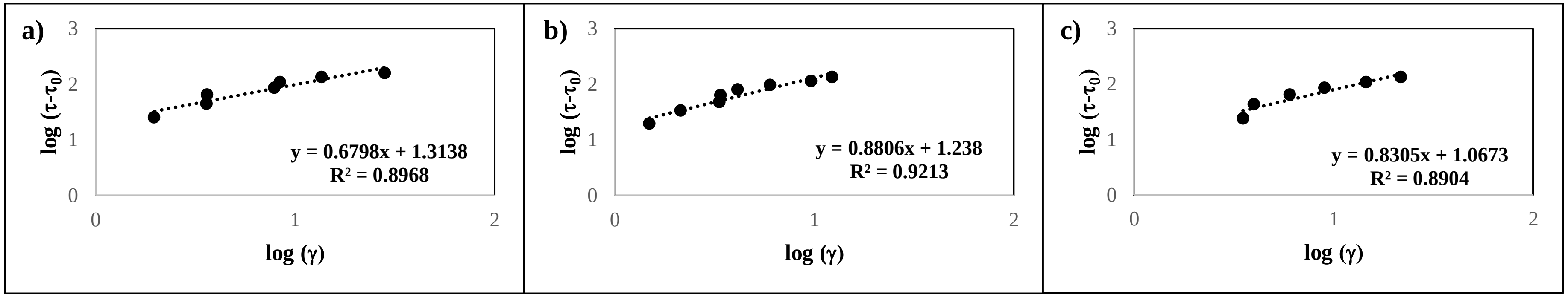

Figure B.7. Modeling of Rheological Data of Low Sugar Apple Marmalade Formulation 7 a) First Measurement b) Second Measurement c) Third Measurement

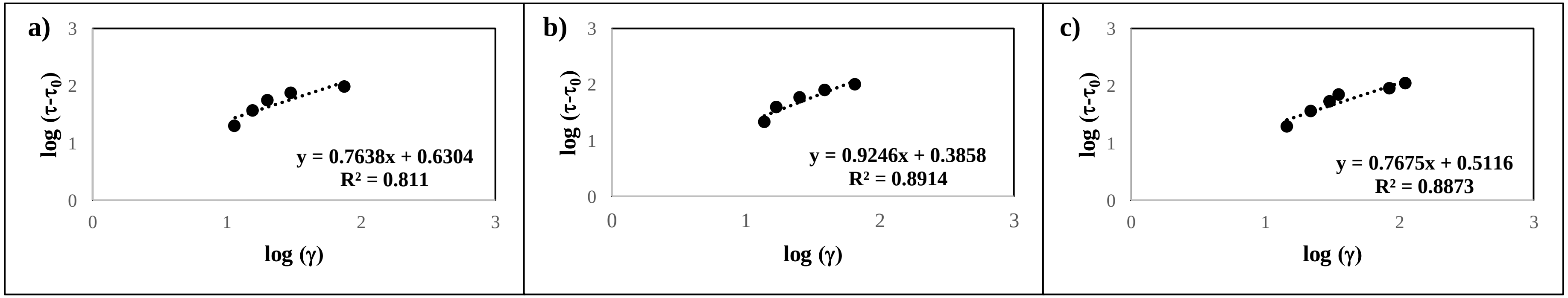

Figure B.8. Modeling of Rheological Data of Low Sugar Apple Marmalade Formulation 8 a) First Measurement b) Second Measurement c) Third Measurement

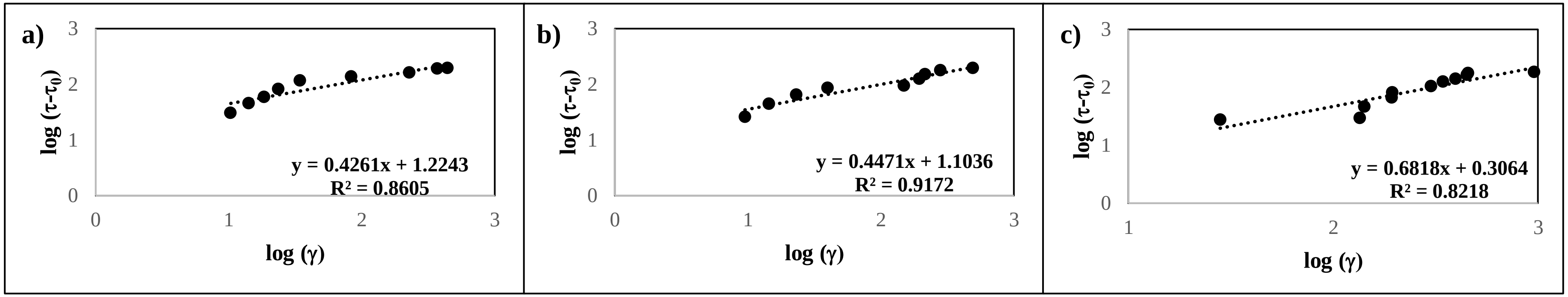

Figure B.9. Modeling of Rheological Data of Low Sugar Apple Marmalade Formulation 9 a) First Measurement b) Second Measurement c) Third Measurement

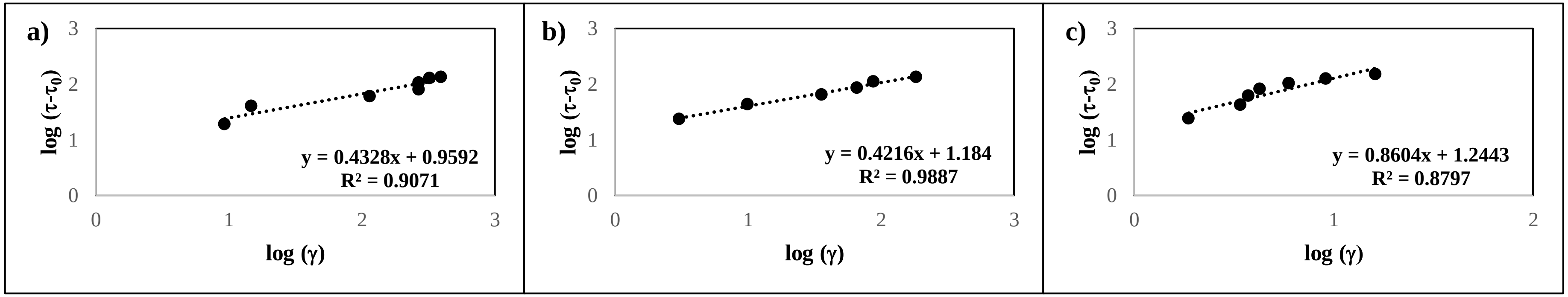

Figure B.10. Modeling of Rheological Data of Low Sugar Apple Marmalade Formulation 10 a) First Measurement b) Second Measurement c) Third Measurement

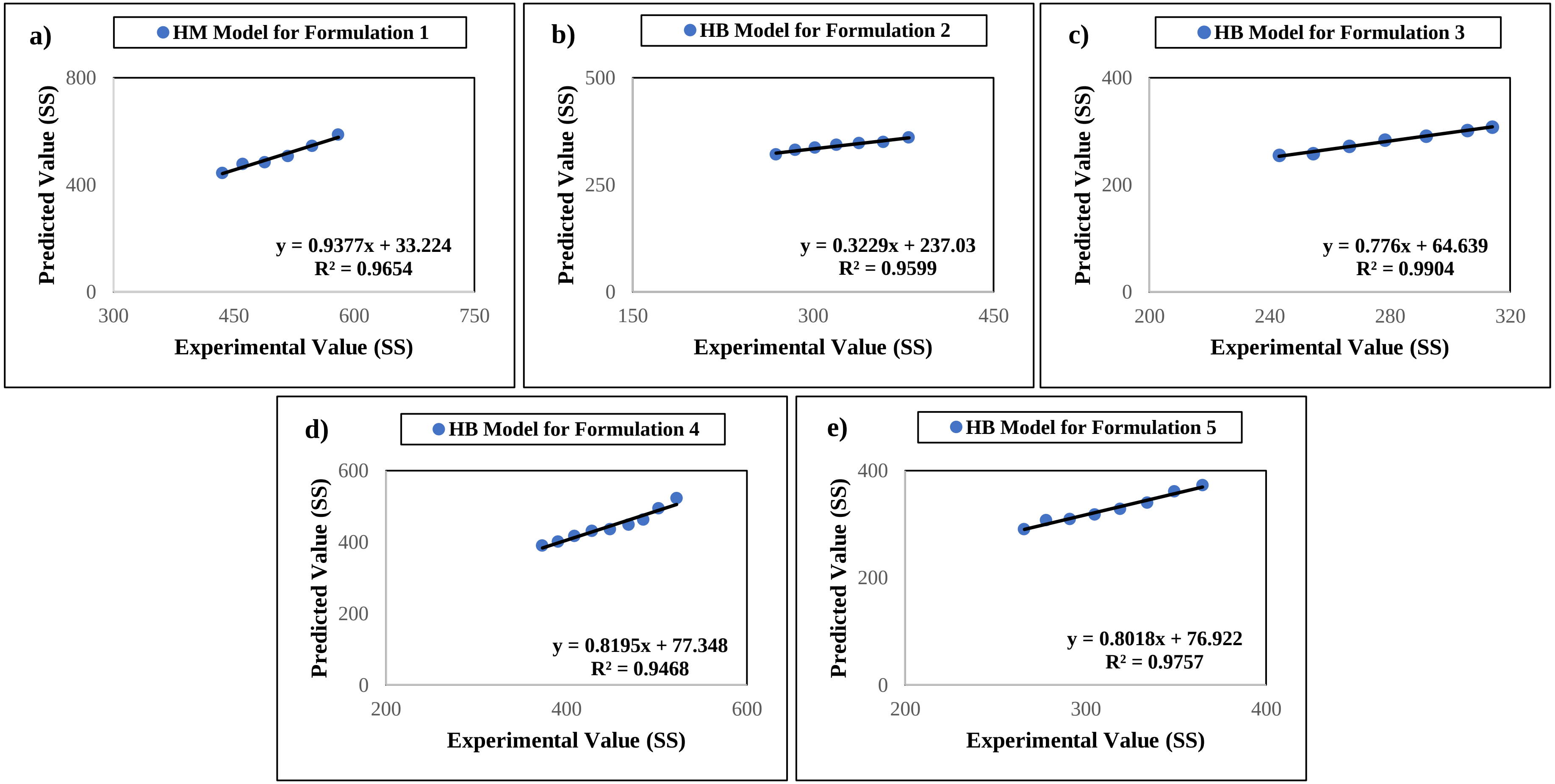

Figure B.11. Experimental vs Predicted Values in terms of Herschel Bulkley Model for Low Sugar Apple Marmalade Formulations (500g) a) Formulation 1 b) Formulation 2 c) Formulation 3 d) Formulation 4 e) Formulation 5

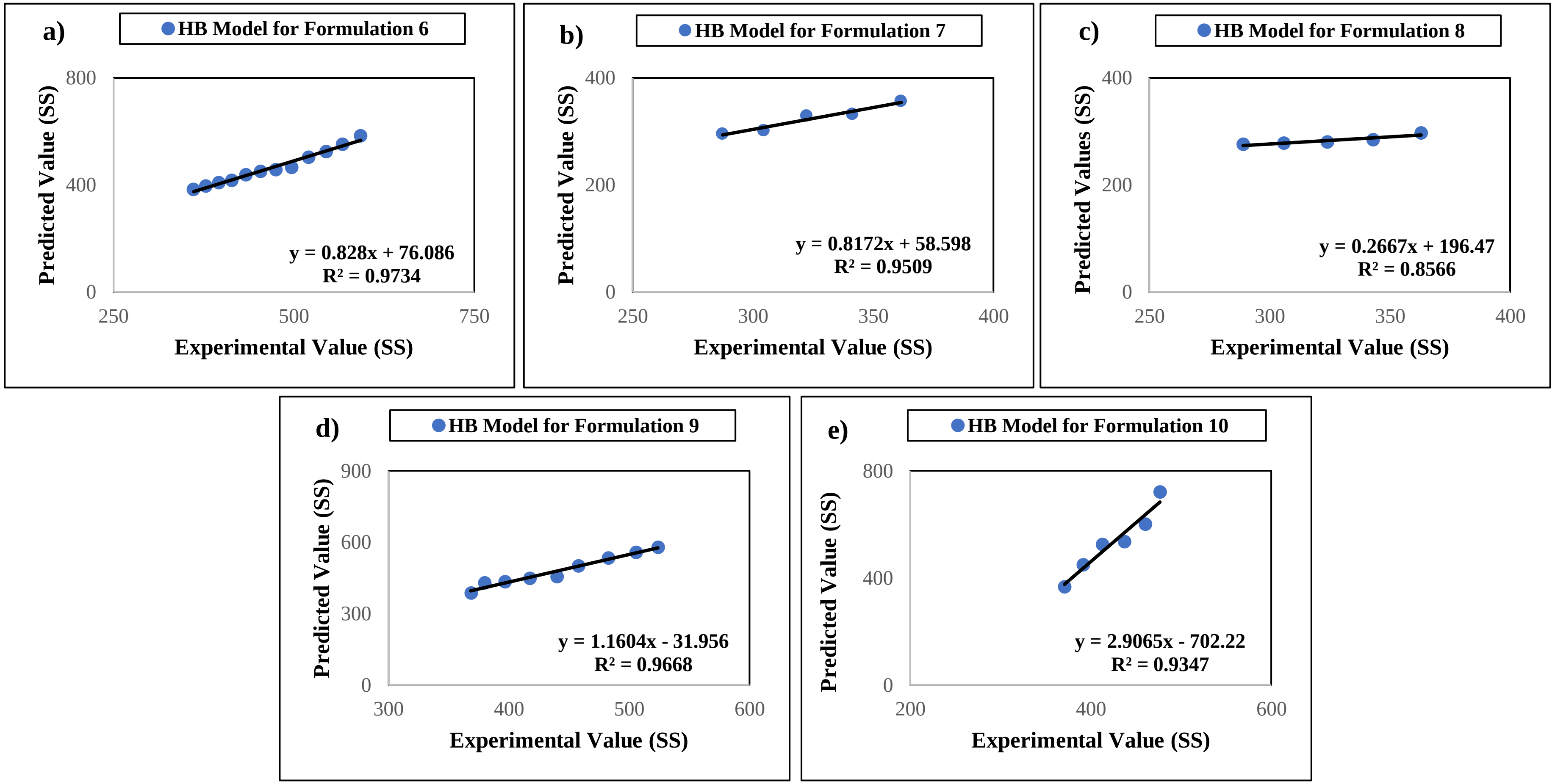

Figure B.3. Experimental vs Predicted Value in terms of Herschel-Bulkley Model for Low Sugar Apple Marmalade Formulations (600g) a) Formulation 6 b) Formulation 7 c) Formulation 8 d) Formulation 9 e) Formulation 10

2) Casson Model

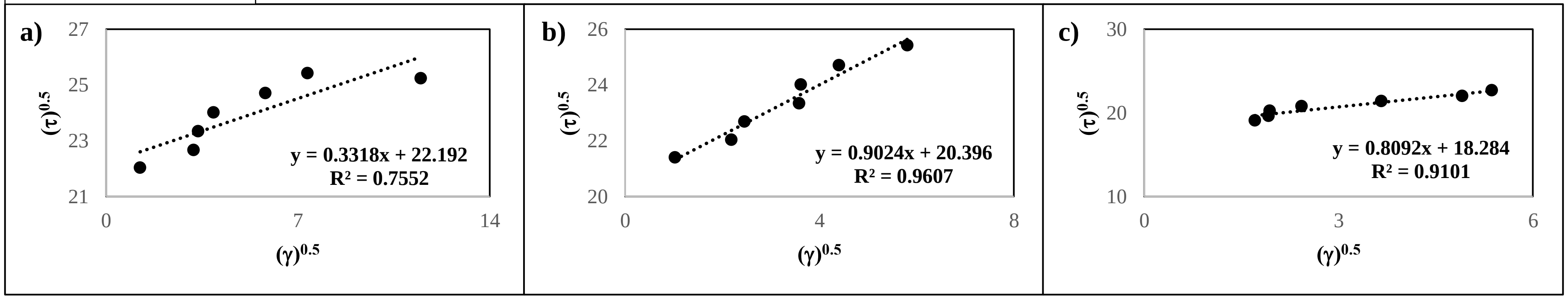

Figure B.4. Modeling of Rheological Data of Low Sugar Apple Marmalade Formulation 1 a) First Measurement b) Second Measurement c) Third Measurement

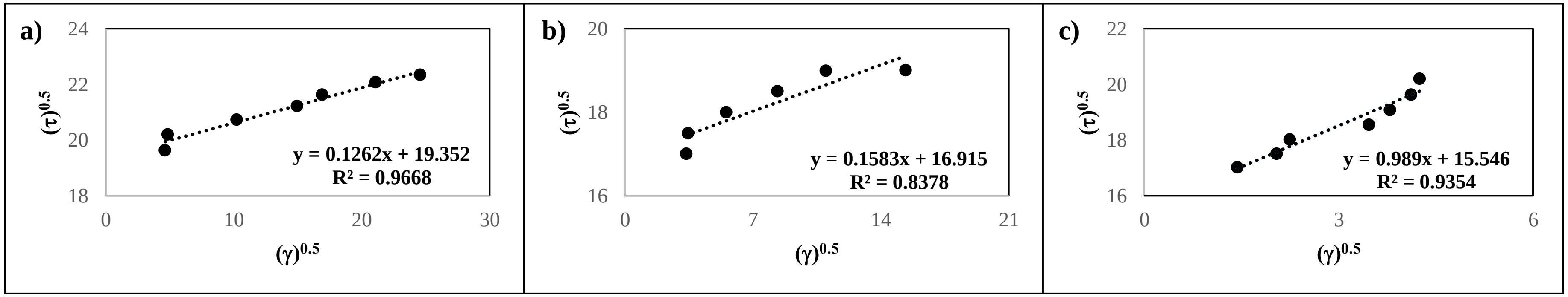

Figure B.5. Modeling of Rheological Data of Low Sugar Apple Marmalade Formulation 2 a) First Measurement b) Second Measurement c) Third Measurement

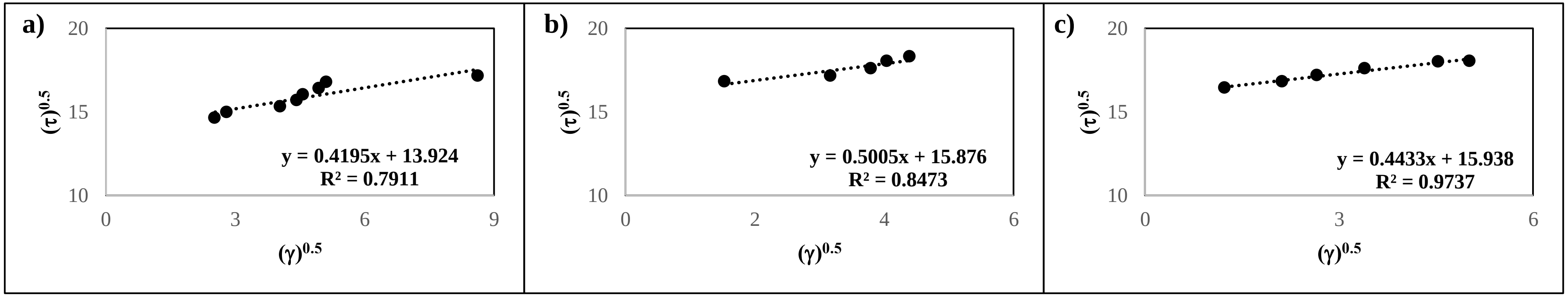

Figure B.6. Modeling of Rheological Data of Low Sugar Apple Marmalade Formulation 3 a) First Measurement b) Second Measurement c) Third Measurement

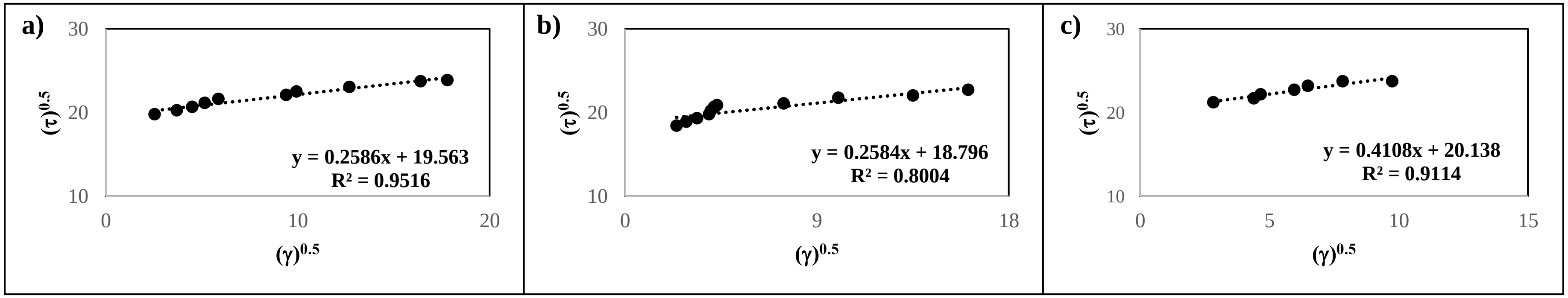

Figure B.7. Modeling of Rheological Data of Low Sugar Apple Marmalade Formulation 4 a) First Measurement b) Second Measurement c) Third Measurement

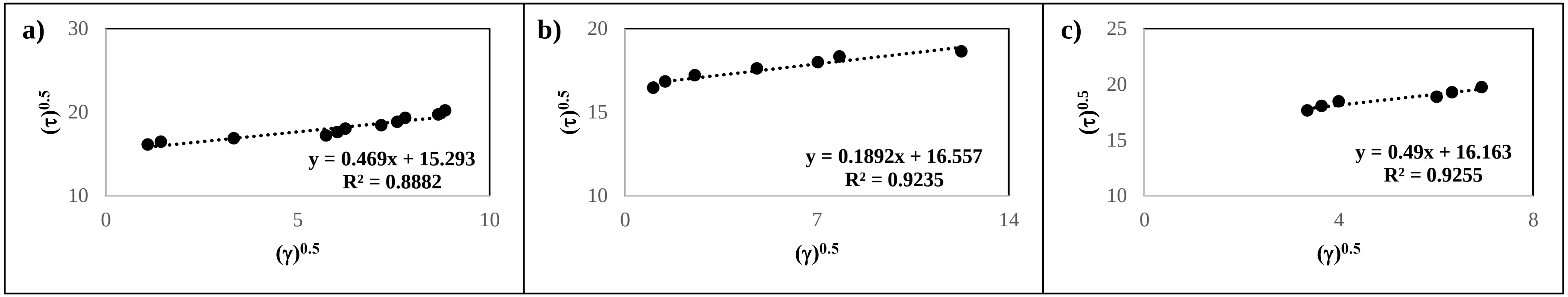

Figure B.8. Modeling of Rheological Data of Low Sugar Apple Marmalade Formulation 5 a) First Measurement b) Second Measurement c) Third Measurement

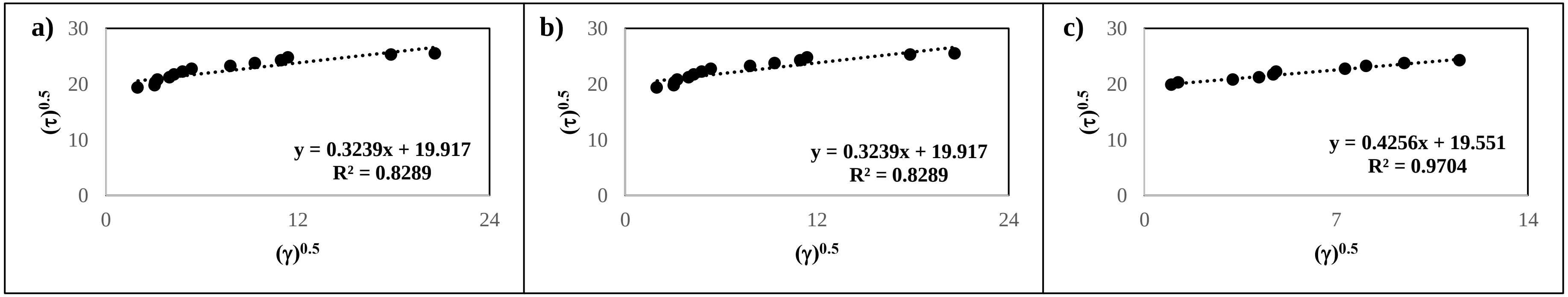

Figure B.9. Modeling of Rheological Data of Low Sugar Apple Marmalade Formulation 6 a) First Measurement b) Second Measurement c) Third Measurement

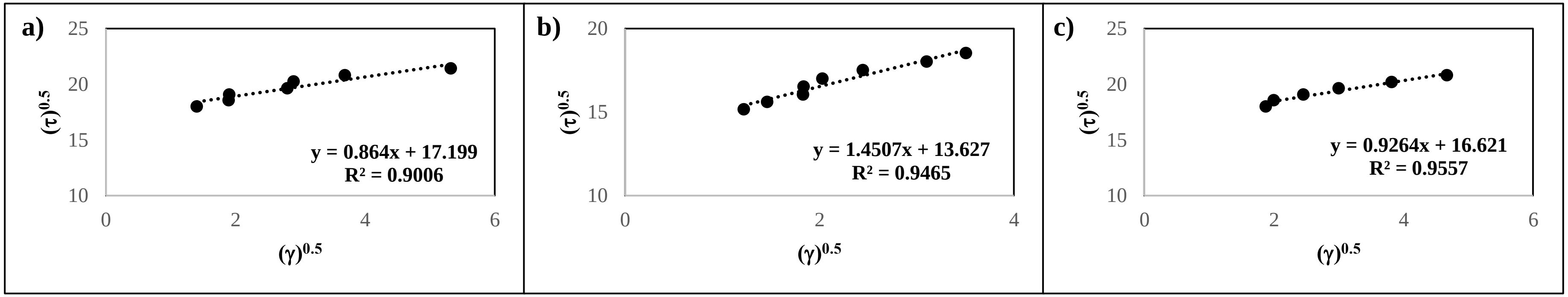

Figure B.10. Modeling of Rheological Data of Low Sugar Apple Marmalade Formulation 7 a) First Measurement b) Second Measurement c) Third Measurement

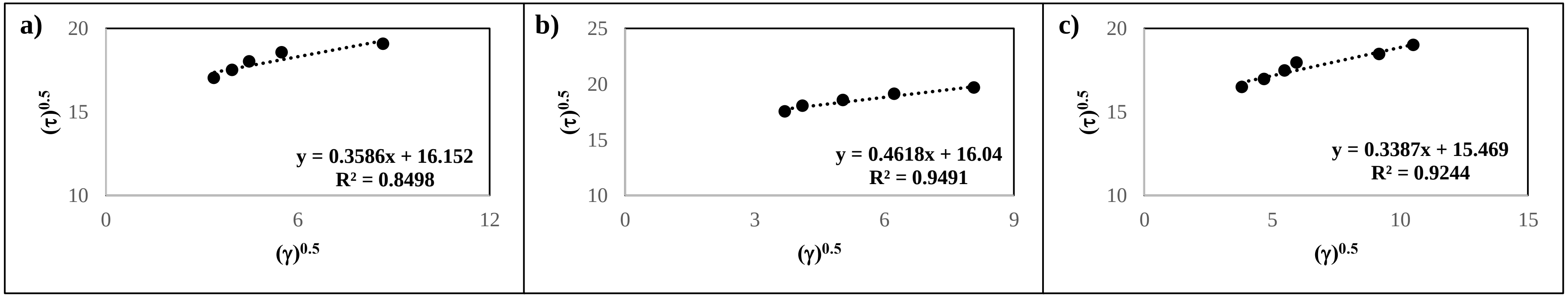

Figure B.20. Modeling of Rheological Data of Low Sugar Apple Marmalade Formulation 8 a) First Measurement b) Second Measurement c) Third Measurement

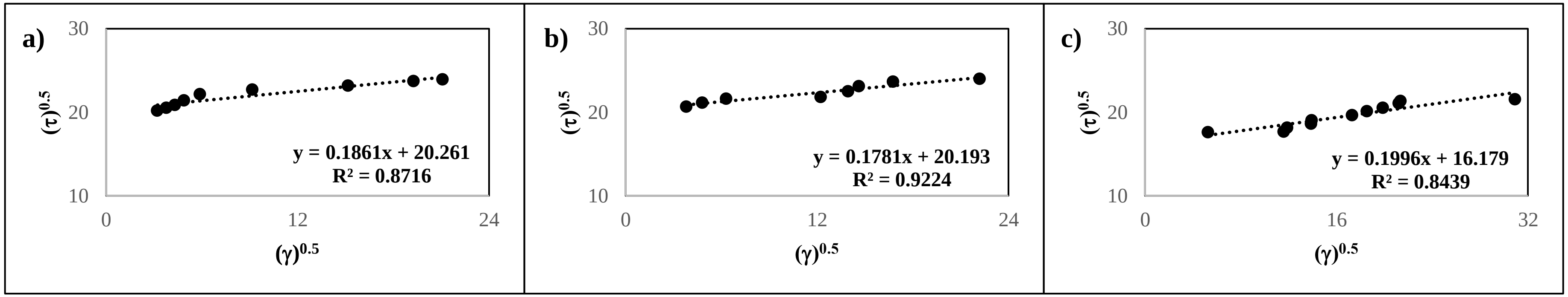

Figure B.21. Modeling of Rheological Data of Low Sugar Apple Marmalade Formulation 9 a) First Measurement b) Second Measurement c) Third Measurement

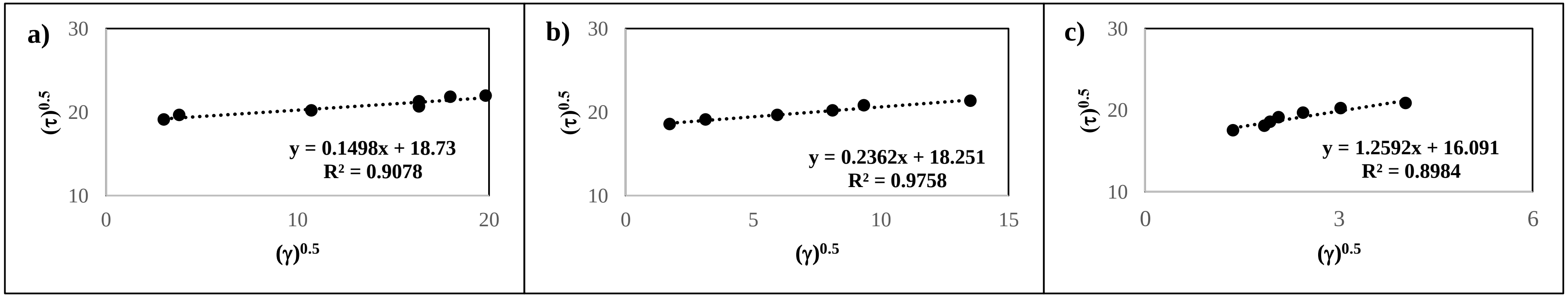

Figure B.11. Modeling of Rheological Data of Low Sugar Apple Marmalade Formulation 10 a) First Measurement b) Second Measurement c) Third Measurement

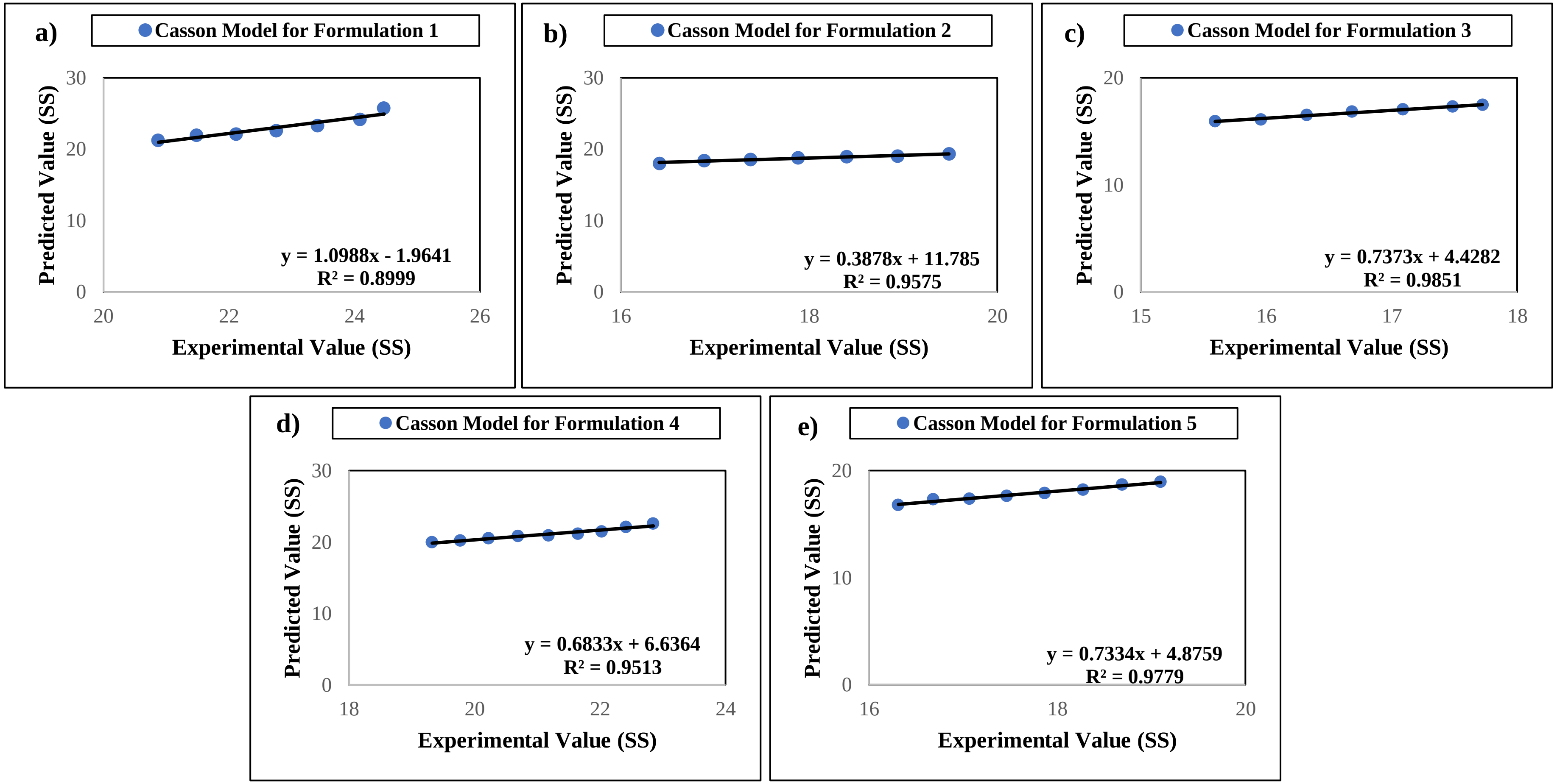

Figure B.12. Experimental vs Predicted Value in terms of Casson Model for Low Sugar Apple Marmalade Formulations (500g) a) Formulation 1 b) Formulation 2 c) Formulation 3 d) Formulation 4 e) Formulation 5

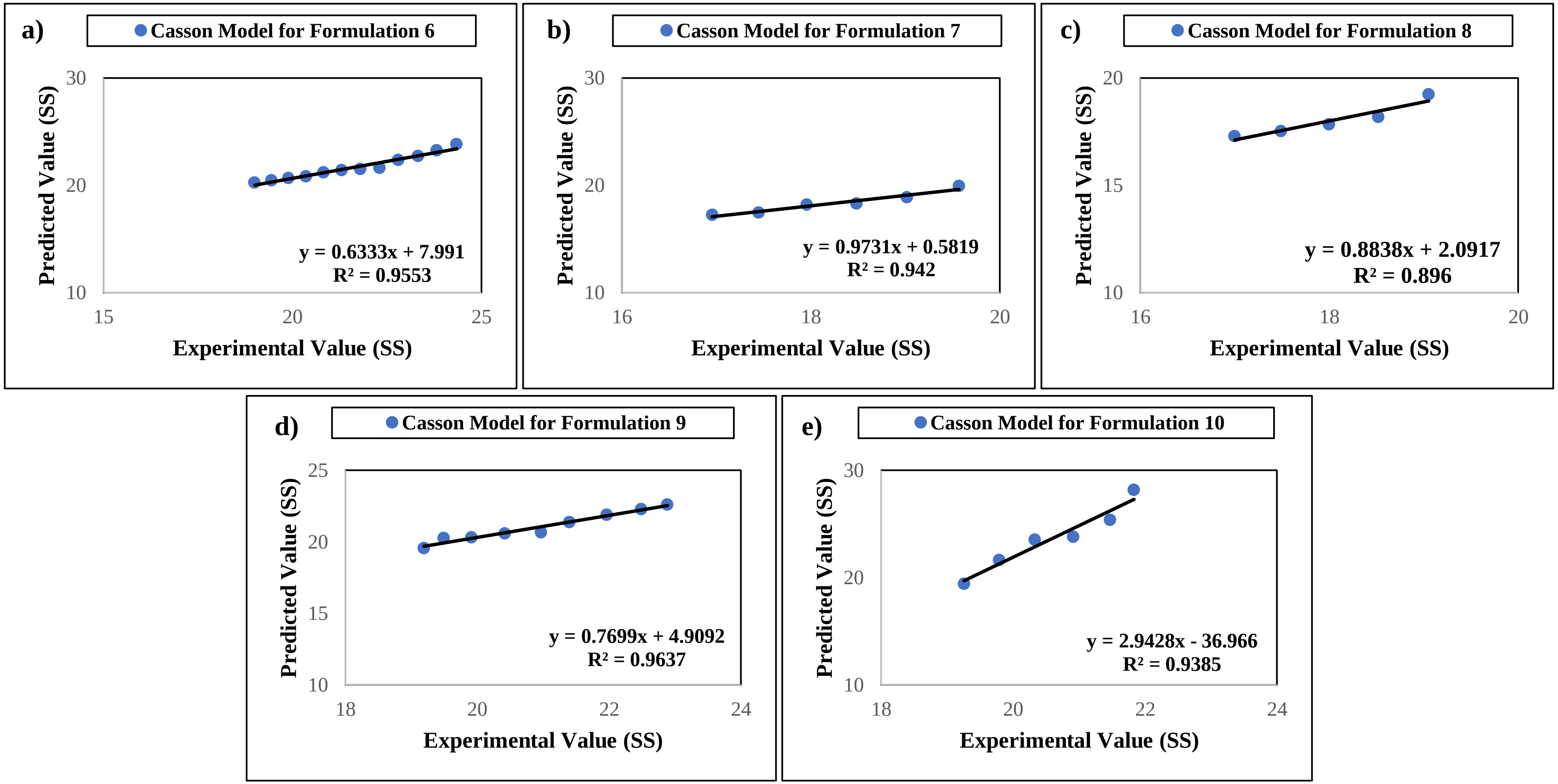

Figure B.13. Experimental vs Predicted Value in terms of Casson Model for Low Sugar Apple Marmalade Formulations (600g) a) Formulation 1 b) Formulation 2 c) Formulation 8 d) Formulation 9 e) Formulation 10

3) Power Law Model

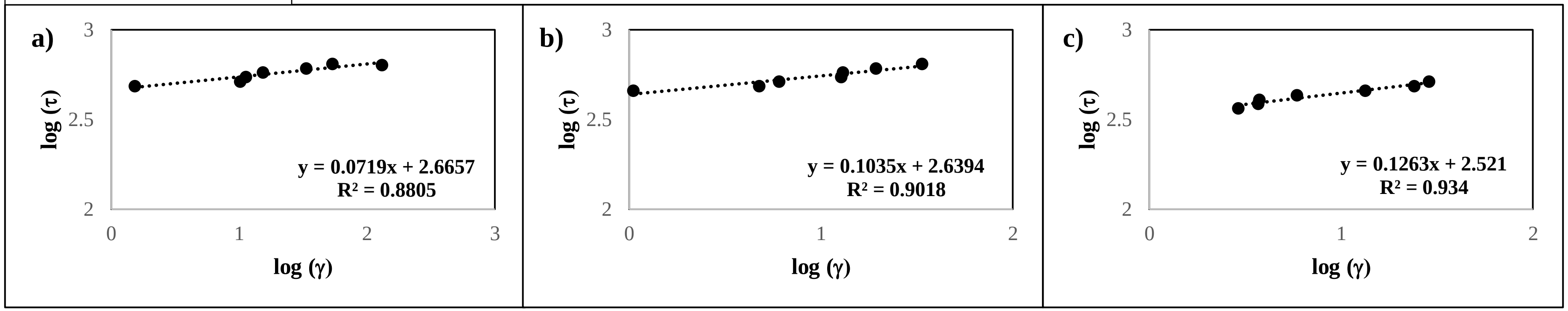

Figure B.14. Modeling of Rheological Data of Low Sugar Apple Marmalade Formulation 1 a) First Measurement b) Second Measurement c) Third Measurement

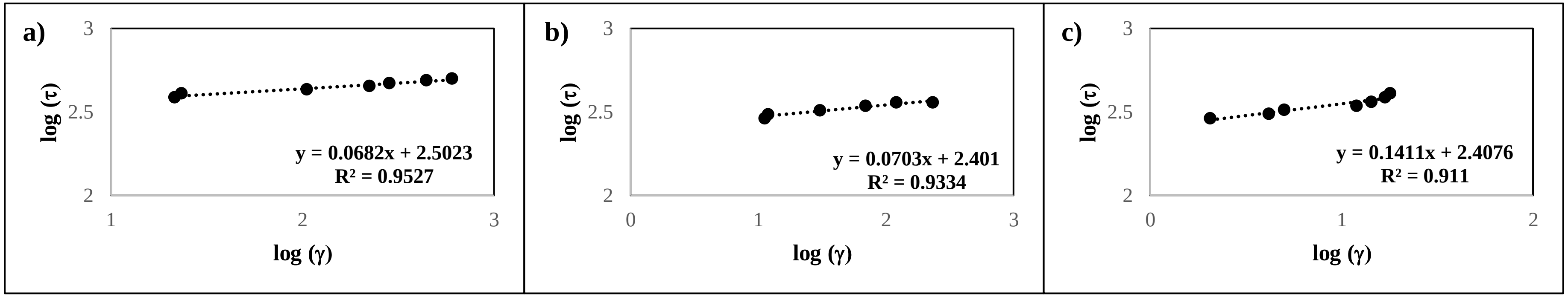

Figure B.15. Modeling of Rheological Data of Low Sugar Apple Marmalade Formulation 2 a) First Measurement b) Second Measurement c) Third Measurement

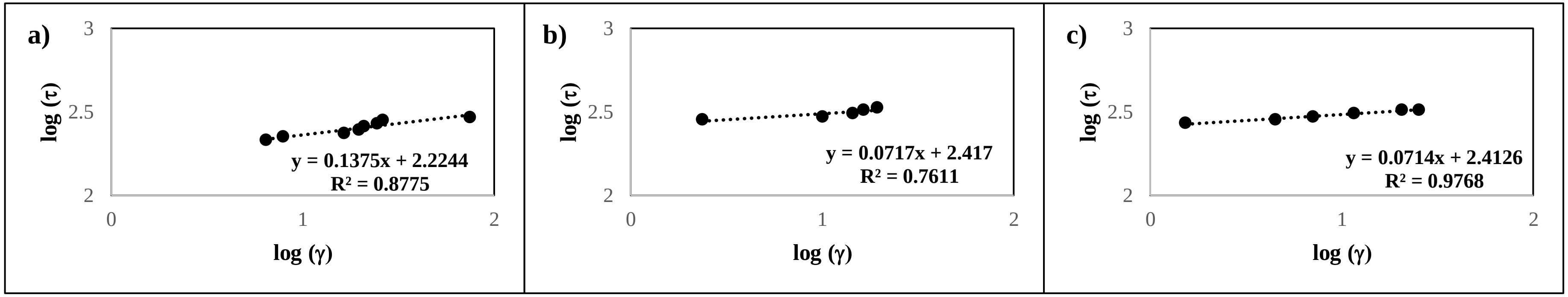

Figure B.16. Modeling of Rheological Data of Low Sugar Apple Marmalade Formulation 3 a) First Measurement b) Second Measurement c) Third Measurement

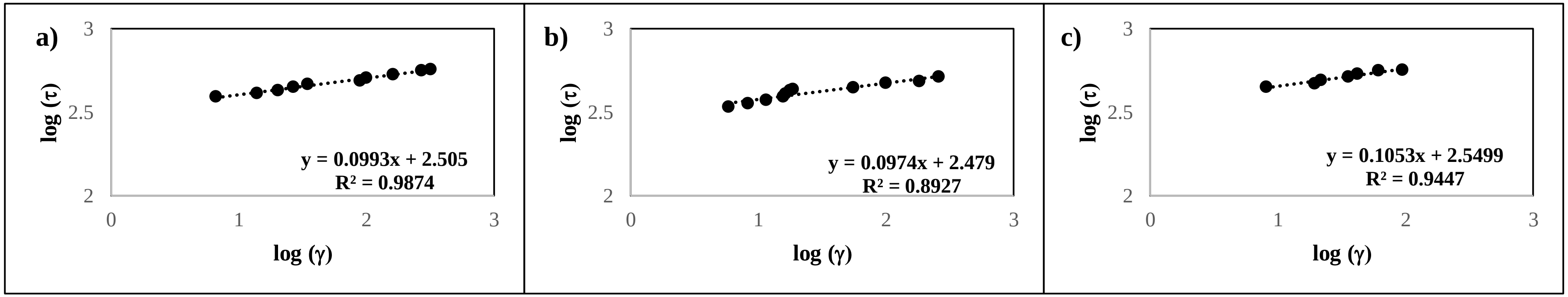

Figure B.17. Modeling of Rheological Data of Low Sugar Apple Marmalade Formulation 4 a) First Measurement b) Second Measurement c) Third Measurement

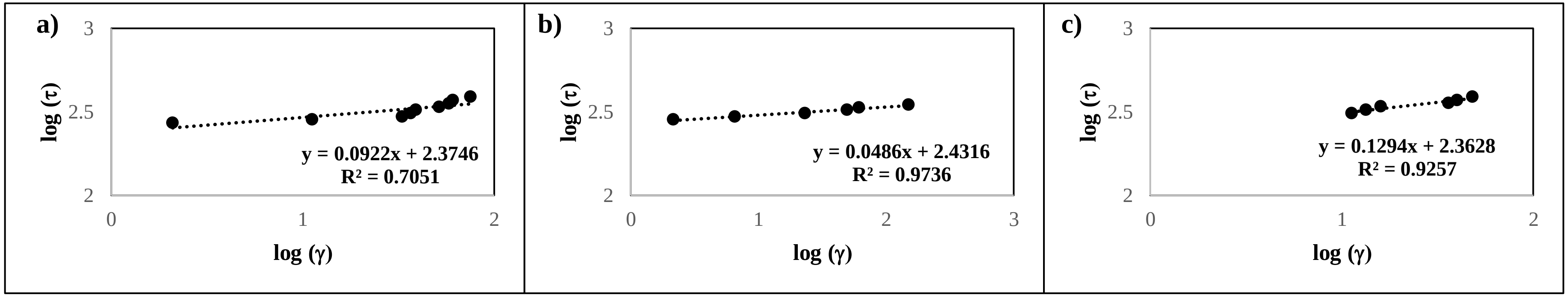

Figure B.18. Modeling of Rheological Data of Low Sugar Apple Marmalade Formulation 5 a) First Measurement b) Second Measurement c) Third Measurement

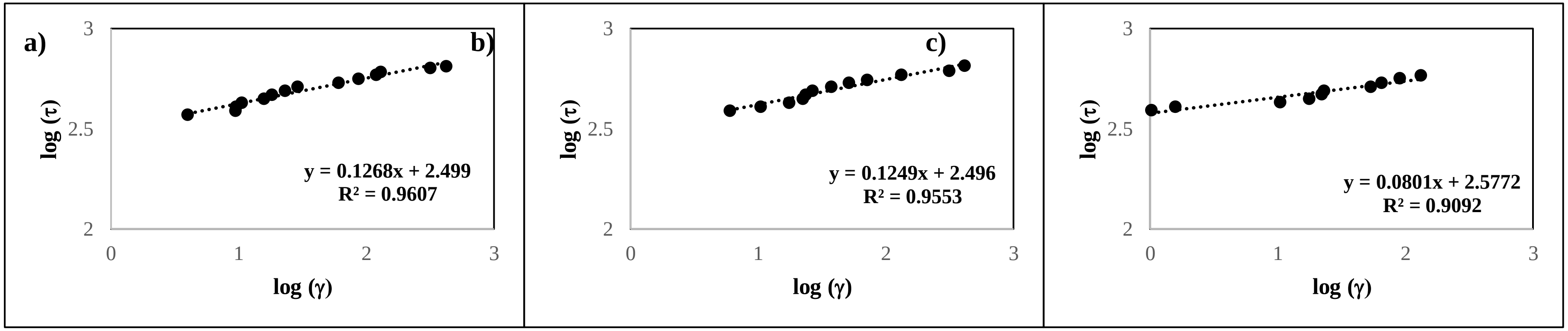

Figure B.30. Modeling of Rheological Data of Low Sugar Apple Marmalade Formulation 6 a) First Measurement b) Second Measurement c) Third Measurement

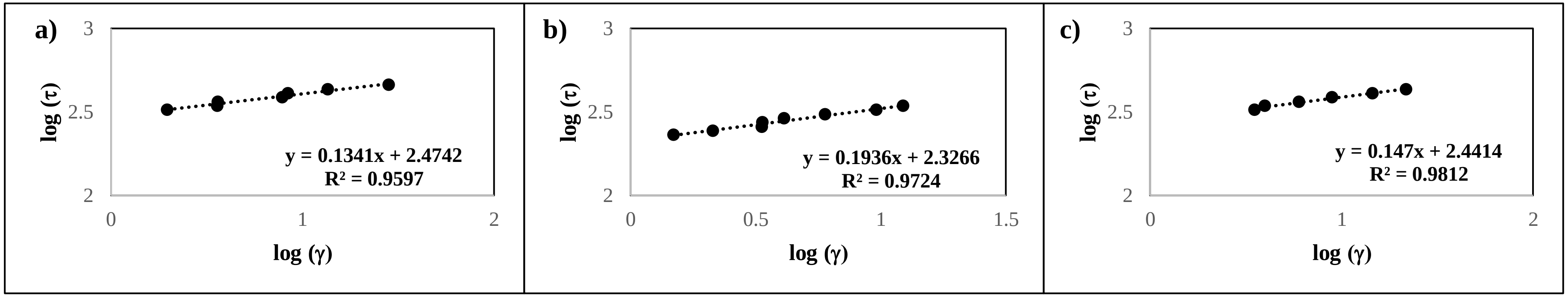

Figure B.19. Modeling of Rheological Data of Low Sugar Apple Marmalade Formulation 7 a) First Measurement b) Second Measurement c) Third Measurement

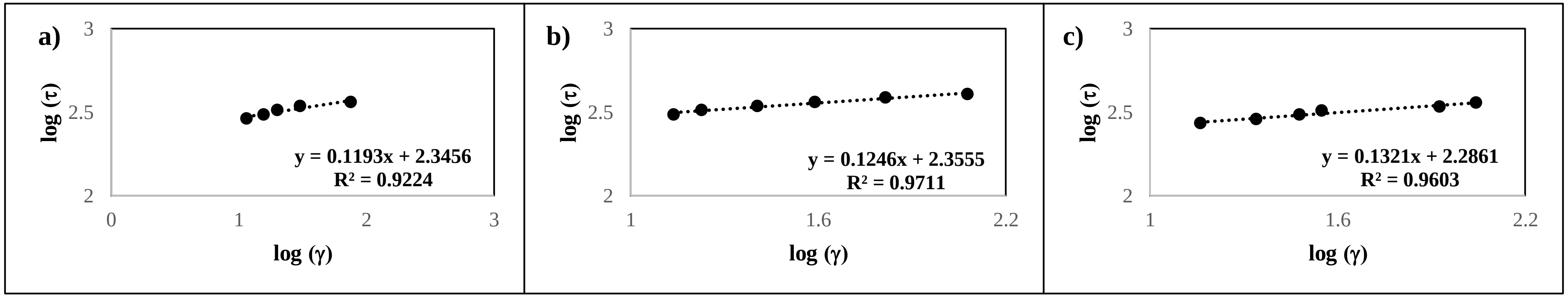

Figure B.32. Modeling of Rheological Data of Low Sugar Apple Marmalade Formulation 8 a) First Measurement b) Second Measurement c) Third Measurement

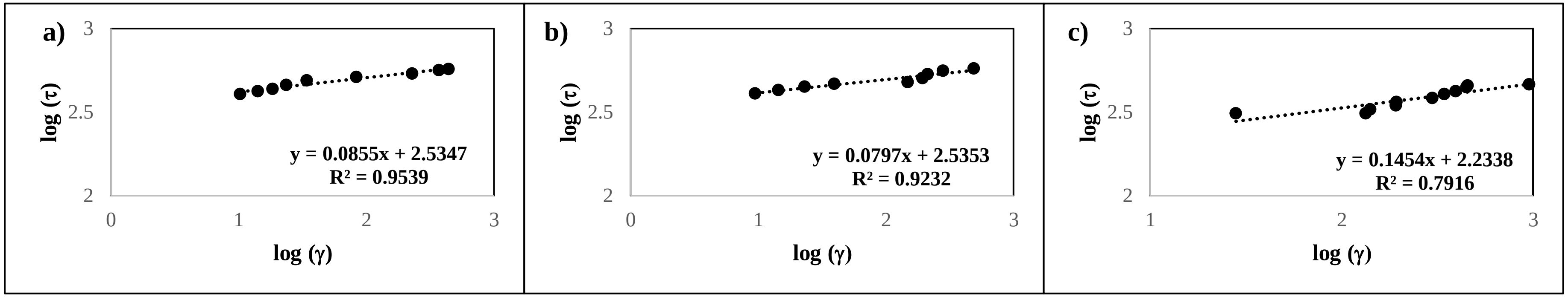

Figure B.20. Modeling of Rheological Data of Low Sugar Apple Marmalade Formulation 9 a) First Measurement b) Second Measurement c) Third Measurement

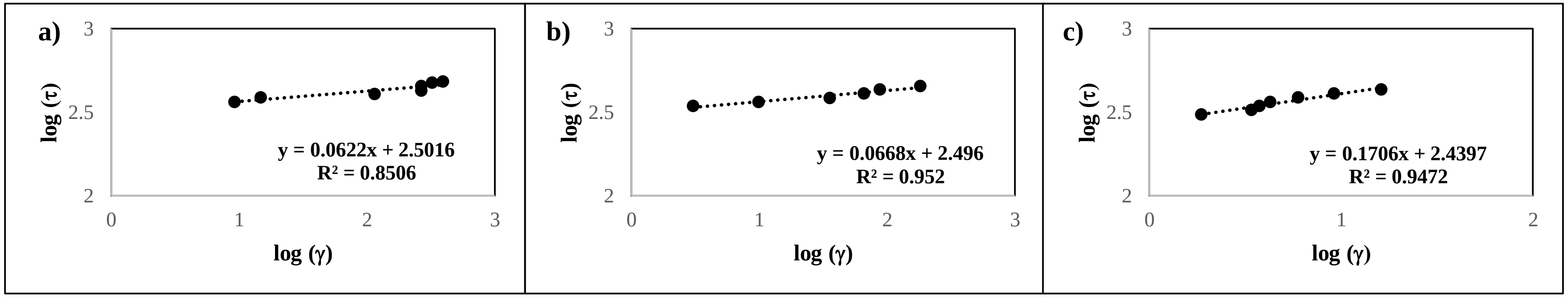

Figure B.21. Modeling of Rheological Data of Low Sugar Apple Marmalade Formulation 10 a) First Measurement b) Second Measurement c) Third Measurement

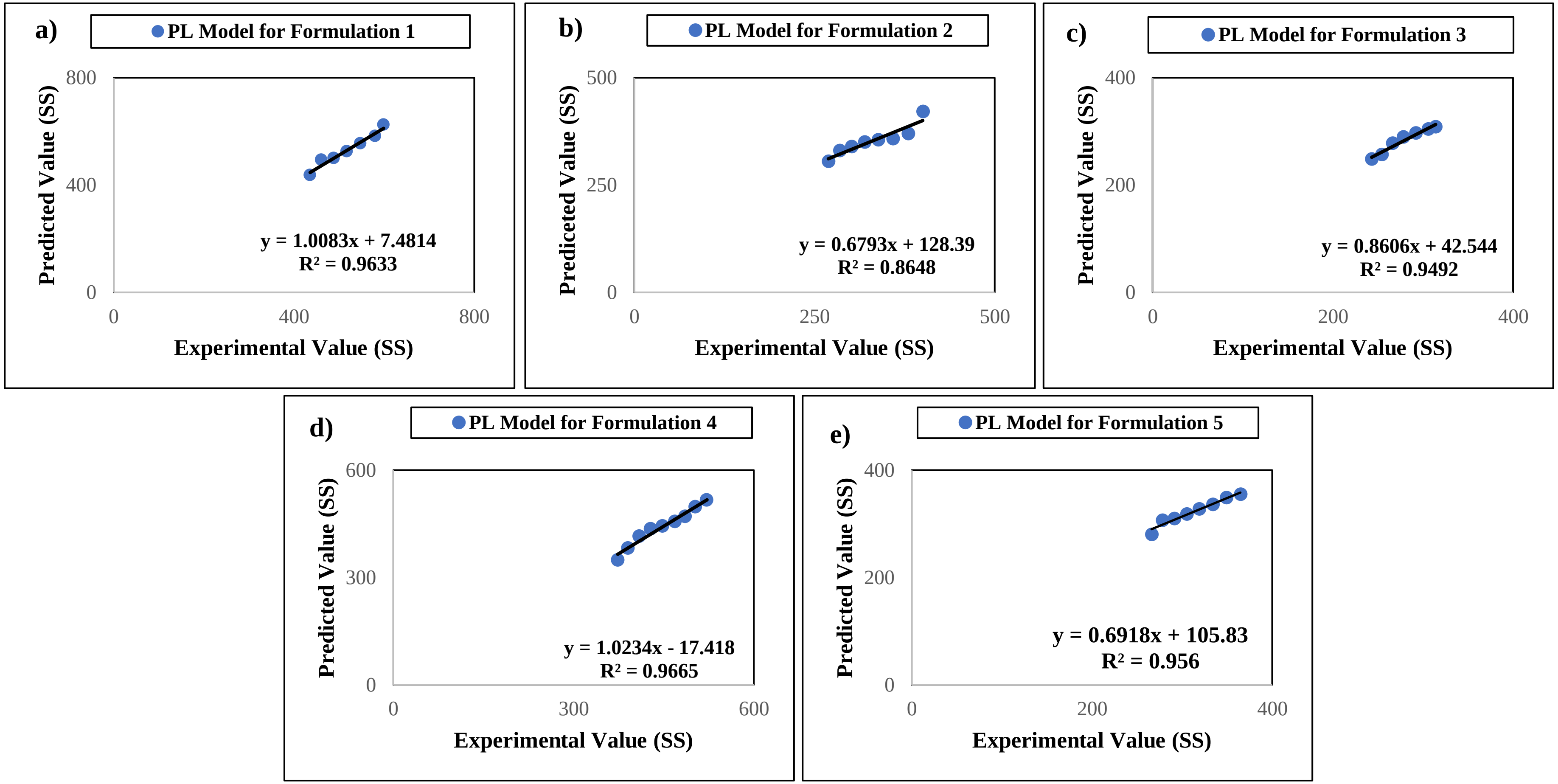

Figure B.22. Experimental vs Predicted Value in terms of Power Law Model for Low Sugar Apple Marmalade Formulations (500g) a) Formulation 1 b) Formulation 2 c) Formulation 3 d) Formulation 4 e) Formulation 5

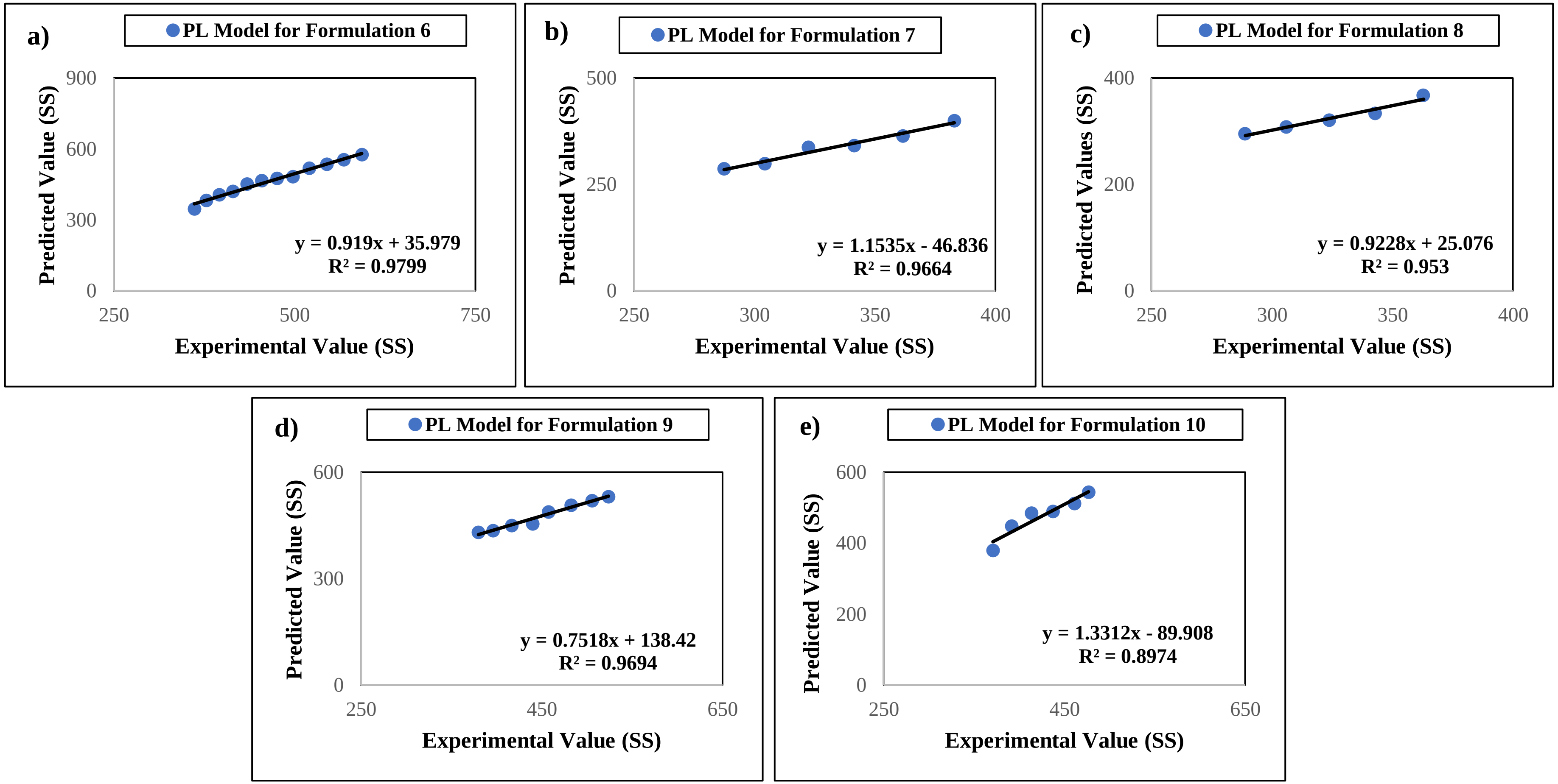

Figure B.23. Experimental vs Predicted Value in terms of Power Law Model for Low Sugar Apple Marmalade Formulations (600g) a) Formulation 6 b) Formulation 7 c) Formulation 8 d) Formulation 9 e) Formulation 10

APPENDIX C

SENSORY EVALUATION OF LOW SUGAR APPLE MARMALADE FORMULATIONS

	Appearance	Taste	Color	Texture	Overall Acceptability
127					
182					
296					
367					
541					
725					
789					
863					
935					

Figure C.1. Example of form used in the sensory analysis