EFFECT OF SOME INTENSE SWEETENERS ON RHEOLOGICAL, TEXTURAL AND SENSORY PROPERTIES OF CHOCOLATE

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ABSTRACT

EFFECT OF SOME INTENSE SWEETENERS ON RHEOLOGICAL, TEXTURAL AND SENSORY PROPERTIES OF CHOCOLATE

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Development of a high-quality low-calorie chocolate needs the use of the most appropriate ingredients that could substitute sugar without negatively affecting several product properties. Although it is possible to formulate sugar-free or sugar-reduced chocolates with an acceptable sweetness level, these chocolates often show poorer sensory properties, especially undesirable mouthfeel, which could limit their consumption. In this study, low-sucrose chocolates sweetened with sucralose and stevia by using bulking agents were investigated in relation to their rheological, textural and sensory attributes. Bitter, milk and white chocolates with different amounts of sweeteners were formulated. The Casson model best fitted to the rheological data for all formulations. In dark chocolates, partial substitution of sucrose with stevia (BCSSt) gave similar plastic viscosity and yield stress values with control samples (BCS) ($p \geq 0.05$). Hardness measurements also supported these results. BCSSt sample was again found to be very similar to control in tested sensory attributes when assessed by a consumer panel. The data indicated that it was possible to manufacture a chocolate by partial replacement of sucrose with stevia without adversely affecting its important rheological, textural properties and sensory acceptance.

Keywords: bitter chocolate, low calorie chocolate, stevia, Casson model

ÖZ

BAZI YOĞUN TATLANDIRICILARIN ÇİKOLATANIN REOLOJİK, DOKUSAL VE DUYUSAL ÖZELLİKLERİNE ETKİSİ

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Yüksek kaliteliye sahip düşük kalorili çikolata üretmek için, kullanılması gereken şeker ikamesi ürünlerinin son ürünü olumsuz yönde etkilemeyecek, şekilde seçilmesi gerekmektedir. Tatlılık seviyesi kabul edilebilir olan şekeri azaltılmış ya da şekersiz çikolata reçeteleri üretmek mümkün olsa da genellikle bu çikolatalar zayıf duyusal özelliklere sahip olmaktadır. Bu çalışmada, sırasıyla sükraloz ve stevia ile tatlandırılmış, şekeri azaltılmış olarak tasarlanan çikolataların reolojileri, yapısal ve duyusal özellikleri incelenmiştir. Farklı tatlandırıcı miktarlarına sahip bitter, sütlü ve beyaz çikolata reçeteleri geliştirilmiştir. Reolojik açıdan incelendiğinde, çikolataların en iyi Casson modeline sahip olduğu gözlemlenmiştir. Kısmi olarak stevia ile tatlandırılan ve sükroz içeren bitter çikolatanın, sadece sükroz içeren bitter çikolata ile aynı plastik viskoziteye sahip olduğu gözlemlenmiştir ($p \leq 0.05$). Sertlik ölçümleri ile de bu gözlem desteklenmiştir. Panel testi ile yapılan duyusal analizlerde de kısmi olarak stevia ile tatlandırılan ve sükroz içeren bitter çikolatanın sadece sükroz içeren bitter çikolata ile yakın puanlar aldığı gözlemlenmiştir. Çalışmanın sonucunda, kısmi olarak stevia ile tatlandırılan ve sükroz içeren bitter çikolatanın, reolojik, yapısal ve duyusal özellikleri olumsuz yönde etkilenmeden endüstriyel olarak üretilmesinin mümkün olduğu çıkarılabilir.

Anahtar Kelimeler: Çikolata, stevia, düşük kalorili çikolata, Casson Model, reoloji

To My Beloved Family

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LIST OF ABBREVIATIONS

BCS	Bitter Chocolate with Sucrose
BCSp	Bitter Chocolate with Sucralose
BCSt	Bitter Chocolate with Stevia
BCSSp	Bitter Chocolate with Sucrose and Sucralose
BCSSt	Bitter Chocolate with Sucrose and Stevia
MCS	Milk Chocolate with Sucrose
MCSp	Milk Chocolate with Sucralose
MCSt	Milk Chocolate with Stevia
WCS	White Chocolate with Sucrose
WCSp	White Chocolate with Sucralose
WCSt	White Chocolate with Stevia
BCP 10:0.5	Bitter Chocolate with 10 g sucrose and 0.5 g Sucralose
BCP 12:0.5	Bitter Chocolate with 12 g sucrose and 0.5 g Sucralose
BCP 14:0.5	Bitter Chocolate with 14 g sucrose and 0.5 g Sucralose
BCP 15:0.5	Bitter Chocolate with 15 g sucrose and 0.5 g Sucralose
BCT 10:0.5	Bitter Chocolate with 10 g sucrose and 0.5 g stevia
BCT 12:0.5	Bitter Chocolate with 12 g sucrose and 0.5 g stevia
BCT 14:0.5	Bitter Chocolate with 14 g sucrose and 0.5 g stevia
BCT 15:0.5	Bitter Chocolate with 15 g sucrose and 0.5 g stevia

CHAPTER 1

INTRODUCTION

1.1 Chocolate

Even most people describe chocolate as a food that elevates mood and gives positive emotions and pleasure (Macht, Dettmer, 2006), the scientific definition of chocolate is semi-solid suspensions of non-fat particles of sweeteners, cocoa solids and milk solids in a continuous fat phase of cocoa butter (Afoakwa, 2010, p.1).

Cocoa solid which is the main ingredient of chocolate is derived from cocoa beans obtained from *Theobroma cocoa* tree and the use of cocoa beans has more than 1000 years history. Cocoa is known as one of the major blocks of Mayan agriculture and religion. Also, cocoa was presented like valuable gift to deceased dignitaries at their funeral ceremonies.

In 1500s, the evidences showed that chocolate was treated as a medicine when brought to Europe. Chocolate mixed with various species like vanilla, Roses of Alexandria, cinnamon was advised to the patients according to their complaints until 1600s. The first sweet chocolate recipe was prepared with the addition of honey, cinnamon and cane sugar in Oaxaca, Mexico by monks. After the discovery of this sweet taste, chocolate industry started to grow and develop. When the dates showed 1900s, all inventions that made chocolate as modern as today was completed (www.callebaut.com. Last visited: October, 2015). New technologies are being developed day by day to produce high quality chocolates.

The consumption and the preference of the chocolate change depending on the country, gender and age. A person consumes about 100 gr of chocolate per year in Asia, whereas England is the top of the consumption list with 11 kg chocolate per year (www.callebaut.com. Last visited: October, 2015).

There are three common types of chocolate that are produced in the industry; bitter, milk and white chocolate. Bitter chocolate or dark chocolate mainly consists of cocoa mass, cocoa butter and sugar. Cocoa powder is also added to the chocolate dough to increase the total cocoa solids without changing the overall fat percentage and to increase intense of the cocoa flavor. According to the Institute of Turkish Standards (ITS), there are two types of bitter chocolate; bitter chocolate and couverture bitter chocolate. Milk chocolate contains also cocoa mass, cocoa butter and sugar, in addition with milk solids and milk fats replacing some of the cocoa butter. Five different types of milk chocolate are present on ITS which differs by dry cocoa solid, milk fat and dry milk solid contents . The chocolate types and their compositions are shown in Table 1. White chocolate on the other hand does not contain cocoa solids. There are only milk powder, sugar and cocoa butter as ingredients. Usually deodorized cocoa butter is preferred to prevent the off taste of pressed cocoa butter. Chocolate should contain 20% of cocoa butter, 3.5% milk fat and 14% dry milk solids to be named as white chocolate as reported in ITS.

Table 1.1 Properties of Chocolate Types

Chocolate Type	Cocoa Butter (% w/w, min)	Non-fat Cocoa Solids (% w/w, min)	Total Dry Cocoa Solid (min)	Milk Fat (% w/w, min)	Dry Milk Solid (% w/w, min)
Bitter	18	14	35	-	-
Couverture Bitter	31	2.5	35	-	-
Milk	Depends on milk fat	2.5	25	3.5	14
Couverture Milk	Depends on milk fat	2.5	25	-	-
Extra milky	Depends on milk fat	2.5	20	5	20
Skim Milked	Depends on milk fat	2.5	25	1 (max)	4
Creamy	Depends on milk fat	2.5	25	5.5	14
White	20	-	-	3.5	14

1.2 Ingredients Used in Chocolate Industry

The quality and the flavor of the end chocolate is mostly determined by the quality, quantity and type of ingredients used in the production. With the properly selected process parameters such as conching temperature and duration, high quality chocolate can be produced. In other words, the higher quality of the raw materials in production, the higher quality of the end chocolate produced.

1.2.1 Cocoa

Cocoa is the most important and most used ingredient in the production of chocolate. Besides cocoa, cocoa mass and cocoa butter are also obtained from cocoa and cocoa beans. The type of cocoa, the climate and the soil conditions of cocoa beans cultivated naturally effect the cocoa flavor, and also cocoa mass and butter. In further steps of the production, the majority flavor of the chocolate is developed by the roasting and conching steps.

To produce cocoa mass also known as cocoa liquor, firstly fermented and dried cocoa beans are selected and roasted, then by using winnowing, the shell is removed the roasted nibs are ground using pin mills and ball mills. Cocoa butter is produced by pressing of the cocoa mass.

1.2.2 Sweeteners

1.2.2.1 Sugar

Sucrose, which is extracted from either sugar cane, or sugar beet, is used for chocolate processing in its crystalline form as the main sugar source. Providing sweetness is the main function of sugar in chocolate. Normally industrial granulated sugar is used and depending on the particle size of the sugar its addition sequence to the processing differs. If a single stage refining process is used, sugar is milled before adding to the mixer and further reduction occurs in the process. If a two-stage refining process is used, granulated sugar is added directly to the chocolate mixer, and reduction in particle size is achieved through this two-staged process, which consists of pre-finer followed by a second stage five-roll refiner. Particle size distribution is an important attribute of final product's rheology, since the amount of very small particles plays important role. This distribution affects not only rheology, but also the yield value of the chocolate samples particularly (Afoakwa, 2010).

1.2.2.2 Sucralose

Sucralose is a non-digestible artificial sweetener that has no nutritive value. The main property of sucralose is that when it is taken to the body, it cannot be broken down (Merck, 2006). This is why it is called as non-caloric sweetener (www.foodinsight.org, last visited: May, 2015). The sweetness of sucralose is almost 600 times higher than sucrose (Grotz & Munro, 2009). Also, sucralose can remain unchanged under heat treatments. Therefore, it can be used in processes that require high temperatures (Food Sanitation Council Notice, No.5, 1999).

Sucralose is obtained by substitution of three hydroxyl groups with chlorine groups. Primary alcohol group is selected and chlorination is partially applied to acetylated sugar with excess chlorinating agent. Then, acetyl groups are removed from molecules to get desired the product (Bert Fraser-Reid, 2012). Chemical structure of sucralose is sown in Figure 1 (http://pubchem.ncbi.nlm.nih.gov/compound/Sucralose, last visited: October, 2015). As seen, sucralose is a disaccharide consisting of a galactose and fructose residue where 3 OH groups are chlorinated. Currently the commercial brand name of sucralose is Splenda (www.splenda.com, last visited: May, 2015)

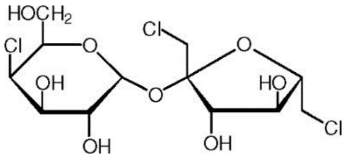

Figure 1. Chemical structure of sucralose

McNeil Nutritionals, LLC (McNeil), a Johnson & Johnson company, markets the sweetener as Splenda® which contains the non-nutritive sweetener sucralose, and maltodextrin that adds texture and volume (Grotz & Munro, 2009).

1.2.2.3 Stevia

Stevia is a natural sweetener that is extracted from the leaves of the Stevia rebaudiana (http://www.intheraw.com/products/stevia-in-the-raw. Last visited: October, 2015). Steviol glycosides are the main structure that makes stevia 300 times sweeter than sucrose (Geuns, 2003) and it is also non-digestible (Goyal, Samsher, & Goyal, 2010). Stevia in the Raw® sweetener includes mixture of stevia and bulking agents (dextrose or maltodextrin) (http://www.intheraw.com/products/stevia-in-the-raw. Last visited: October, 2015). Chemical structure of sucralose is sown in Figure 2 (http://www.inchem.org/. last visited: October, 2015)

When taken to the body, it is metabolized to steviosides, then broken down to glucose and steviol. This emerged glucose is used by the bacteria located on the colon and cannot be absorbed into the bloodstream. Also, steviol cannot be digested further, and finally it is excreted (Koyama, E., et al., 2003).

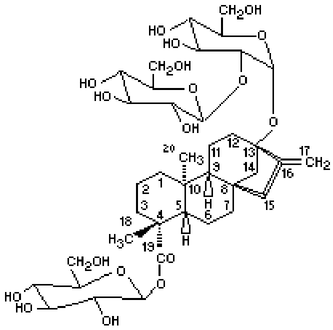

Figure 2. Chemical structure of stevia

1.2.3 Milk

In order to produce milk chocolate and white chocolate, different forms of milk are used in combination with cocoa and sugar. When developing a formulation for a milk and white chocolate, it is crucial to understand the characteristic and composition of each milk products so as to meet the legal requirements.

1.2.3.1 Whole milk powder or full cream milk powder

Spray drying and roller drying are the common ways of producing milk powder, which is also known as dehydrated whole milk. The main principle of the process is evaporating water in the milk by using either heated air in a spray tower, or on heated rolls. Both processes results in powder but even the compositions are same; flavor characteristics and process performance differ. The composition is 26-27% milk fat, 70% non-fat milk solids and 3.5% water approximately (Afoakwa, 2010, p.52). For maintaining creamy and milky notes, whole milk powder is preferred due to its flavor influence. Roller dried powder has minor caramelization due to heated rollers in its process, and makes it more preferable. In addition to this, as compared to spray dried powder, roller drying powder has higher levels of free fat content. In spray dried powder, milk fat bounds to powder particles. The free fat content is important because it dominates the mixing and refining stages of chocolate processing, and eventually rheology of chocolate is affected. The moisture content of the powders also controls process parameters and rheology (Beckett, 2010).

Spray drying is a trending process due to its less investment as compared to roller drying. In the light of information above, it is important to choose wisely which milk powder is used in production, considering flavor, fat contribution to rheology at the same fat content, and cost (Beckett, 2010).

1.2.3.2 Skimmed milk powder

Before drying process, nearly all-fat content is removed from the liquid form of the milk, and this results in very low fat content powder, which is less than 1%. Skimmed milk introduces milky flavor to the formulation. When formulating new recipes, it can be used to increase milk solid levels while not affecting the overall fat content. In addition, combining with milk fat instead of whole milk powder gives a softer final product (Afoakwa, 2010).

1.2.3.3 Milk fat or butter oil

This is the natural fat in milk, which is nearly liquid at room temperature; and its solid fat content is about 11% (Afoakwa, 2010). Manufactured form of milk fat is either butter or cream. Milk fat in combination with cocoa butter is used to give softer texture to the final product. Milk fat addition is also used for avoiding bloom formation.

1.2.4 Emulsifiers

1.2.4.1 Lecithin E322

Soya lecithin is a common emulsifier used in chocolate. Even small amounts have powerful effect on the rheology on chocolate. Lecithin addition results in reduction in both viscosity and the yield value. It means that less cocoa butter is needed so it is cost-saving (Afoakwa, 2010).

As particle size (fineness) of chocolate decreases, the amount of required lecithin increases to improve rheology. As the fat content of chocolate increases, the ability of lecithin to rule rheology decreases. Last not but least, feature of the lecithin is its effect on the yield value (Afoakwa, 2010). Above certain concentrations, the impact on yield value is reversed. For every formulation, there is an optimum level with respect to the fat, moisture content and particle size distribution (www.solae.com. Last visited October, 2015).

1.2.4.2 PGPR E476

PGPR (polyglycerol polyricinoleate) is mainly used as emulsifiers in chocolate processing. It is not used as sole emulsifier; it is used as combination with lecithin or ammonium phosphatide. The effect of PGPR on viscosity is insignificant; however it has compelling effect on yield value (Schantz and Rohm, 2005).

1.2.5 Inulin

Inulin is a kind of polysaccharide that occurs naturally in most plants that is used to store the energy in roots or rhizomes (Roberfroid M., 2005). Inulin is a sub-group of dietary fibers known as fructans and has heterogeneous collection of fructose polymers. Glucosyl and fructosyl components are linked by β (2,1) bonds that make inulin non-digestible by enzymes present in the human alimentary system (Kalyani Nair et al, 2010). Moreover, inulin has no taste and has little impact on sensory characteristic of the products in which added. When considered the nutritional benefits, inulin is a source of soluble fibers and can be classified as prebiotics. With these properties, inulin can be used to replace sugar or fat in the reduced calorie products and the products suitable for diabetics.

1.2.6 Maltodextrin

Maltodextrin is a polysaccharide that is produced from starch by partial hydrolysis. Maltodextrin is an easily digestible and absorbable food additive. D-glucose units connected by chains of variable length with range from three to seventeen glucose units long are the structure of the maltodextrin (Kennedy et al, 1995). Maltodextrin is classified by using dextrose equivalent (DE) number from 3 to 20. The DE is the measurement of the amount of reducing sugar present in a product. The higher the DE value, the shorter the glucose chains; the higher the sweetness, the higher the solubility and the lower heat resistance (Hashizume and Okuma, 2009). In addition maltodextrin has no flavor, it improves the mouthfeel of the products added in. By taking this advantage, maltodextrin is used as a filler in sugar-substituted products.

1.3 Compositional Effects on Rheology

1.3.1 The Effect of Fats

The main fat source of chocolate comes from cocoa butter, which makes chocolate a unique food. The actual fat content depends on the type and the application. For instance, the ice cream coating chocolate has higher amount of fat than tablet chocolate. It can be considered that a high quality chocolate means high fat content with lower particle size (Afoakwa, 2010).

Plastic viscosity is proportionally more affected than yield value when there is a change in fat content. According to Beckett (2010), this phenomenon is explained due to extra fat combining with only free moving fat whose function is to help particles while flowing past each other. The higher free fat content, the less energy required to keep moving once motion. Ultimately, plastic viscosity decreases dramatically as the fat content increases.

1.3.2 The Effect of Sweeteners

Sugar is used in chocolate as it gives sweetness. Changes up to 2% of sugar affect cost; on the other hand, changes below 5% result in large flavor alterations (Beckett, 2010).

In the industry, fine crystalline sucrose is usually preferred as sweetener. The reason why monosaccharides as glucose and fructose are rarely used is to have difficulties to dry them. Additional moisture increases the interaction between sugar particles which causes increase in viscosity.

Moreover, there is an increasing trend in reduced-calorie and sugar-free chocolates for the people who are cautious on their diets (Parpinello et al. 2001). Chocolates that contain sugar alcohols are suitable for diabetics (Zumbe & Grosso, 1993; Olinger, 1994; Olinger &Pepper, 2001; Sokmen & Gunes, 2006). The most common sugar alcohols used in chocolate industry are sorbitol, mannitol, xylitol and lactitol. Replacement of sugar with its substitutes brings rheological and textural changes that affect the quality of end product. According to the research of Sokmen and Gunes (2006), maltitol containing chocolate as sweetener had similar rheological properties of chocolate contained sucralose.

The point, however, that should be considered when planned to produce chocolate containing sugar alcohols is the EU legislation that limits the consumption of sugar alcohols to maximum 20 g per day due to its laxative effects (Krüger, 1999).

1.3.3 The Effect of Milk and Products

Milk contains about 5% lactose, 5% milk fat, 3.5% protein and 0.7% minerals. Milk fat is liquid at room temperature and used in chocolate to soften the texture, yet, milk fat has risk to be oxidized due to its chemistry and effects the sensory attributes and shelf life of the end products.

Milk proteins, 80% caseins and 20% whey proteins, are used to give chocolate a creaminess taste. Besides, caseins create fraction like surfactants and reduce the viscosity of chocolate, while whey proteins have opposite effects by increasing the viscosity (Haylock & Dodds, 1999).

1.3.4 The Effect of Emulsifiers

Chocolate consists of continuous fat phase with sugar that makes it have both hydrophilic and lipophilic structures and never being dissolved naturally each other. It is the reason to use surface-active agents to be coat surfaces with fat. It also helps to reduce fat amount in the production. Lecithin, gums, soluble polysaccharides are generally preferred as natural surfactants in the chocolate industry by considering the desired end product specifications (Schantz & Rohm, 2005).

Lecithin is the byproduct of soya oil production and a mixture of phosphoglycerides. Lecithin enables the lubrication between sugar, cocoa particles and fat crystals that facilities the flow of the chocolate mass more liquid. The phenomena behind is that all hydrophilic particles, sugar and cocoa particles are covered with a lecithin mono molecular layer that gives lubrication with hydrophobic fats (www.solae.com). Last visited October, 2015). 0.1% to 0.3% addition of the lecithin decreases the viscosity of the chocolate. Yield values starts to increase more than 0.5% by addition of lecithin while plastic viscosity continues to fall (Chevalley, 1999; 2000; Schantz & Rohm, 2005). The limit for addition of the lecithin is up to 1% maximum.

Polyglycerol polyricinoleate, PGPR is used to adjust the yield value of the chocolate. Even 0.2% addition of PGPR can reduce the yield value by 50% and makes the chocolate behave like Newtonian liquid (Rector, 2000; Schantz & Rohm, 2005). The same rheological property can be obtained by adding more cocoa butter. However, the more cocoa butter addition, the higher the cost. Instead, PGPR is usually preferred due to its economics. The EU legislation limits the addition of PGPR in cocoa based confectionary at 0.5% maximum level (Rector, 2000).

Usually in chocolate industry, the combination of PGPR and lecithin is used to adjust the rheological properties of chocolate. Yield value decreases and viscosity slightly increases by adding PGPR with 0.5% of lecithin (Rector, 2000).

1.4 Quality of Chocolate

1.4.1 Rheological Measurements

Rheology describes the relationship between the force and the deformation as a function of time. The word rheology comes from *rheo*, from the Greek word for flow, and *–ology*, meaning study of. Chocolate has a complex structure since it contains solid particles that are sugar, non-fat cocoa solids and milk solids in a fat continuous phase of cocoa butter. The flow of chocolate is non-Newtonian liquid exhibiting non-ideal plastic behavior; the mean is that when the yield value has been overcome first, shear-thinning exists. While shear rate increases, three dimensional structure of material aligns in the stream lines which is formerly collapsed and became asymmetric particles. This incident causes a decrease in viscosity, and at certain point, it is independent of shear rate at high shear stress (Afoakwa, 2010).

In case of chocolate rheology has two major aspects; plastic viscosity and yield value. The plastic velocity is the energy required to keep the motion of the chocolate after start to flow. It is the measure of the internal friction of a fluid. Yield value is the force needed to start the flow, it is shear stress at which not only deformation occurs but stationary flow begins (Beckett, 2010). The rheological properties of the chocolate are important to determine the process parameters such as pumping or for the quality of the final products and exact weight control during molding, enrobing or dipping applications (Afoakwa, 2010).

Even the Herschel–Bulkley model and the Casson model are used to describe the non-ideal plastic behaviors, the Casson model is accepted by International Confectionery Association (IOCCC) for chocolate rheology with recommendation of that yield values be measured at low shear rates and viscosities at high shear rates because only a single equation using a small set of parameters is not enough to describe chocolate flow sufficiently. Rotational viscometers with concentric cylinders (bob and cup geometry) is suggested with using the parameters of the stress and viscosity at shear rates between 2 and 50 s^{-1} using up and down curves, preceded by a pre-shear at 5 s^{-1} of more than 5 minutes (Servais et al., 2004).

1.4.2 Sensory Evaluation of Chocolate

When it is of chocolate, it is inevitable to talk about sensory evaluation. The evaluation is applied for appearance, taste, mouth feel, flavor and aftertaste either subjectively or objectively. If the taster just says his opinions whether likes or dislikes, it becomes subjective evaluation. It can be considered as objective measurements when there are scoring systems determined by the trained panelists or instrumental analyses such as rheology or textural studies. The main point is that there should be consistency of instrumental data and sensory attributes.

Sensory analyses can be done in three ways; analytical and affective methods. The aim is to rate the differences or similarities between the products for sensory attributes usually applied with 10-20 assessors in analytical methods. If the large number of panelists selected based on the target group of the products are involved to investigate the preference or acceptance of the products, then it is called the affective method. In descriptive method, some special techniques as Flavour Profile®, Quantitative Descriptive Analysis®, Texture Profile Analysis® and Sensory Spectrum® and words that describe the products are used together (Lawless and Heymann, 1998).

1.5 Objective of the Study

Development of a high-quality low-calorie chocolate needs the use of the most appropriate ingredients that could substitute sugar without negatively affecting several product properties. The developed chocolate should be feasible for manufacturing and meet the sensorial expectations for consumers. Therefore, the main objective was to investigate the effect of some intense sweeteners on rheological, textural and sensory properties of chocolate to develop high-quality low-calorie chocolate.

Sucralose and stevia were chosen as sweeteners in this study. Formulations with sucrose as control were also investigated. The reason was to observe the effect both artificial and natural sweeteners to properties of developed chocolate.

CHAPTER 2

MATERIALS AND METHOD

2.1. Materials

For chocolate production, raw materials which are cocoa liquor, cocoa butter and whole milk powder were supplied from ETİ Food Industry and Co. Inc. (Eskişehir, Turkey). Icing sugar (Bağdat Baharat, Ankara, Turkey) was bought from local markets. As sweeteners, sucralose (Splenda®, USA, having 5% sucralose) and stevia extract (Stevia in The Raw®, USA, having 5% stevia extract) were used. Lecithin (Cargill) was used as emulsifying agent. Inulin (Smart Kimya Ltd., Cigili, Turkey) was used to develop taste and texture of chocolate containing sweeteners. Maltodextrin (Sunar Co. Inc., Adana, Turkey) was used as a filler to stretch the taste of sweetness of chocolate containing sweeteners and as bulking agent to replace sugar.

2.2 Methods

2.2.1 Chocolate Preparation

2.2.1.1 Mixing of raw materials

Firstly cocoa butter was melted in a double boiler (Fondue Maker, Tchibo Inc, Germany) and then cocoa liquor was added. After melting of both, sucrose, sucralose or stevia was added to the mixture. Lastly, lecithin was added and the mixture was stirred. For milk chocolate, whole milk powder was added at this stage. For white chocolate, only cocoa butter was melted in the double boiler. Whole milk powder was also added at this stage.

Factors and the corresponding levels studied and the chocolate formulations are given in Table 2.1 and Table 2.2 respectively at the end of the chapter.

2.2.1.2 Refining and conching of chocolate mixture

The mixture was transferred to ball mill vessel for refining and conching steps simultaneously. The ball mill (Retsch, PM 100, Germany) was adjusted to manual settings with 250 rpm for 60 minutes.

2.2.1.3 Tempering of chocolate

Performing tempering on a marble is usually considered an old and classical way. It is a common convention that is conducted at R & D laboratories of chocolate manufacturing companies when special tempering equipment is not available. In this study, tempering was conducted using a marble. Following conching and refining, the chocolate mixture was heated to 45±2 °C using the double boiler and afterwards 2/3 portion of the chocolate was cooled gradually to 27±1 °C on to the marble surface. Finally, remained portion of the chocolate in the boiler that was at 45±2 °C was added to the one on the surface and temperature was increased to 30±1 °C while continuously spreading the chocolate on the marble surface.

2.2.1.1.4 Molding of chocolate

Chocolates were molded for texture analysis. Tempered chocolate was poured to rectangular molding materials with dimensions of 75 mm length, 25 width and 10 mm height.

2.2.3 Sensory Evaluation of Chocolate Samples

Sweetener levels that were used on chocolate formulations were based on literature and the standards that are used worldwide. Stevia and sucralose concentrations on the formulations were kept at 0.5% by considering the studies of Abhishek et. al (2010) and Medeiros de Melo et. al (2009) respectively. These were also the levels that are determined by Turkish Food Codex for artificial sweeteners on confectionery products.

In the literature, sucrose-sweetener combination formulations have also been studied. To decide the concentrations for the combinations of sucrose and sweeteners, sensory analysis was conducted on 30 untrained panelists. Acceptance test was used to determine the combination concentrations to see which sample was the most accepted as near as control chocolate prepared with sucrose. All sensory experiments was conducted on bitter chocolate formulations to exclude the effect of other ingredients (i.e. milk powder).

For these tests, chocolates with sucrose-sucralose ratios of 10:0.5 (10 g sucrose/0.5 g Sucralose), 12:0.5. 14:0.5 and 15:0.5 in 100 g weight basis were prepared and tasted by the panelists. The highest score resulted in the bitter chocolate with sucrose-sucralose ratio of 14:0.5. Therefore, this sweetener ratio was used for the rest of the study as the ‘combination’ concentration (Table 2b). Similarly, same procedure was repeated for bitter chocolates prepared with same sucrose and stevia ratios. The most preferred one was again the dark chocolate with sucrose-stevia ratio of 14:0.5.

Following the selection of the most appropriate ratio, to decide on the most acceptable chocolate formulation that are given on Table 2.2, another acceptance test was conducted.

Panelists have filled in the form that is given on Appendix B. The results were analysed by analysis of variance (ANOVA).

2.2.4 Rheological Characterization of Chocolate

Rheological properties of the molten chocolates were studied using a rheometer (Anton Paar, MCR51 model, Austria) with concentric cylinder system (cup and bob). All measurements were done at 40°C. Samples were pre-sheared at 5 s^{-1} at 40 °C before starting the measurement cycle. Shear stress was measured as a function of increasing shear rate from 4 s^{-1} to 350 s^{-1}. The data were fitted to both Casson and Herschel-Bulkey models. Throughout the experiments, shear rate versus shear stress data were collected for measurements of linear viscosity, Casson and Herschel-Bulkey model parameters of the replicates. Since Casson model gave better results than Herschel-Bulkey, only parameters of Casson model were displayed and discussed in the results.

2.2.5 Physical Analyses of Chocolate

2.2.5.1 Color

Colour of the chocolate bars was measured with a colorimeter (Colorflex, Broomfield, Colorado, USA). Colour was expressed in terms of the CIELAB system L*, a* and b*: L*, luminance ranging from 0 (black) to 100 (white); and a* (green to red) and b* (blue to yellow). After measuring the L*, a* and b* values, total colour difference (ΔE*) was calculated based on equation given below:

$$\Delta E^{*} = \sqrt{(L^{*}_{sample} - L^{*}_{ref})^{2} + (a^{*}_{sample} - a^{*}_{ref})^{2} + (b^{*}_{sample} - b^{*}_{ref})^{2}} \qquad \textbf{(2.1)}$$

where L_{*ref}, a_{*ref}, b_{*ref} indicated the L*, a* and b* values of the control chocolate samples (sucrose containing) for bitter, milk and white chocolates respectively. Mean values from 3 replicate measurements and standard errors were reported on the results.

2.2.5.2 Texture Profile Analysis

Hardness and fracturability measurements of chocolates were done by using a Texture Analyser (Stable Micro Systems TA HD plus, Surrey, UK) with a load cell of 20 N. Hardness was reported as the maximum penetrating force (g) required for the needle to penetrate through a sample (80 x 10 mm, depth 10mm). One point bend probe was used by setting instrument to compression force mode with trigger force 5.0 g, pre-test speed of 1.0 mm/s, test speed of 2.0 mm/s, post-test speed of 10.0 mm/s and rupture distance of 10 mm. Mean values from 3 replicate measurements and standard errors were calculated.

The fracturability is the maximum load (g) necessary to fracture a bar (80 × 10 × 10 mm) of tempered chocolate. The probe descended at 10 mm/min until the chocolate bar was broken. For every chocolate, 3 bars were subjected to the one point bend test. Mean values from 3 replicate measurements and standard errors were calculated. Texture analysis experiments were conducted for bitter chocolate samples only.

2.2.5.3 Moisture Content Measurements

To better interpret the texture results, moisture content of bitter chocolate samples were measured using a Time Domain NMR instrument (Bruker Inc, Germany). Free induction decay sequence with an echo time of 3ms was used for the measurements. Mean values from 3 replicate measurements and standard errors were calculated.

2.2.6 Experimental Design and Statistical Analysis

The overview of this study can be summarized in Table 2.1 and 2.2. The factors are chocolate types which are bitter, milk and white chocolates and sweetener types which are sucrose, sucralose and stevia. By combination of these factors, the responses of color, rheology, texture and sensory scores were evaluated. The complete set of formulations are also explicitly given in Table 2.2.

Analysis of variance (ANOVA) was performed to determine whether there was a significant difference on the quality attributes between chocolate formulations. If significant difference was obtained, means were compared by the Tukey test using MINITAB (Version 16) software. For ANOVA to be meaningful, normality test was applied prior to analysis. If normality test was satisfied, constant variance assumption was checked. If either of the test failed, transformation was applied if necessary to satisfy the tests. Square root, logarithmic and Box-Cox transformations were evaluated if necessary.

Table 2.1. Experimental Design Table

Factors	**Responses**
Chocolate types	*Color*
Bitter Chocolate	L,a,b & ΔE values
Milk Chocolate	*Rheology*
White Chocolate	Casson Model Fitting
Sweeteners	*Texture*
Sucralose	Hardness
Splenda	Fracturability
Stevia	*Sensory Evaluation*
	Acceptance Score

Table 2.2. Composition of Chocolate Formulations

	Ingredients (%w/w)								
Formulation	Cocoa Liquor (%)	Cocoa Butter (%)	Milk Powder (%)	Sugar (%)	Sucralose (%)	Stevia (%)	Inulin (%)	Lecithin (%)	Maltodextrin (%)
BCS	40	24.5	-	30	-	-	-	0.5	5
BCSt	40	24	-	-	-	0.5	15	0.5	20
BCSc	40	24	-	-	0.5	-	15	0.5	20
BCSSt	40	25	-	14	-	0.5	15	0.5	5
BCSSc	40	25	-	14	0.5	-	15	0.5	5
MCS	36	27.5	18	18	-	-	-	0.5	-
MCSt	36	25	18	-	-	0.5	15	0.5	15
MCSc	36	25	18	-	0.5	-	15	0.5	15
WCS	-	49.5	20	30	-	-	-	0.5	-
WCSt	-	49	20	-	-	0.5	15	0.5	15
WCSc	-	49	20	-	0.5	-	15	0.5	15

BC: Bitter chocolate. MC: Milk chocolate. WC: White chocolate. S: Sucrose. St: Stevia.
Sc: Sucralose.
SSt: Sucrose and Stevia.
SSc: Sucrose and Sucralo

CHAPTER 3

RESULTS AND DISCUSSIONS

3.1 Effects of Sweeteners on Sensory Properties of Different Chocolate Formulation

As explained in Chapter 2, sensory evaluation was conducted by untrained panelists in two steps. The aim of the first step was to determine the concentration for partial replacement of sucrose with two selected sweeteners; sucralose and stevia, separately. Firstly, bitter chocolates with sucrose-sucralose ratios of 10:0.5 (10 g sucrose/0.5 g Sucralose), 12:0.5, 14:0.5 and 15:0.5 were prepared and tasted by the panelists. The highest score resulted in the bitter chocolate with sucrose-sucralose ratio of 14:0.5 ($p \leq 0.5$) (Figure 3.1). Therefore, this sweetener ratio was used for overall sensory evaluation. Similarly, same procedure was repeated for bitter chocolates prepared with same sucrose-stevia ratios. The most preferred one was again the bitter chocolate with sucrose-stevia ratio of 14:0.5 ($p \leq 0.5$) (Figure 3.2).

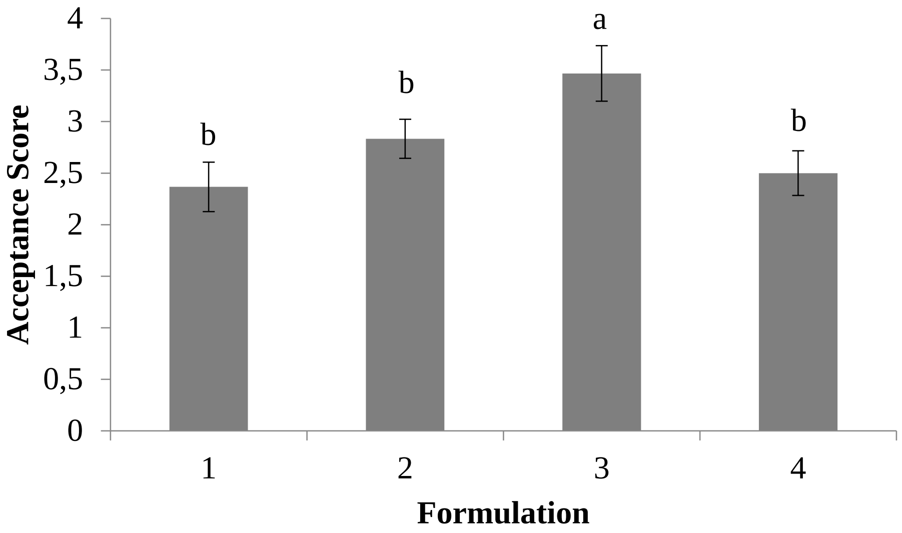

Figure 3.1 Preference of bitter chocolate with composition of sucrose-sucralose **1**. 10:0.5; **2**. 12:0.5; **3**. 14:0.5; **4**. 15:0.5. Bars indicate standard error of the replicates.

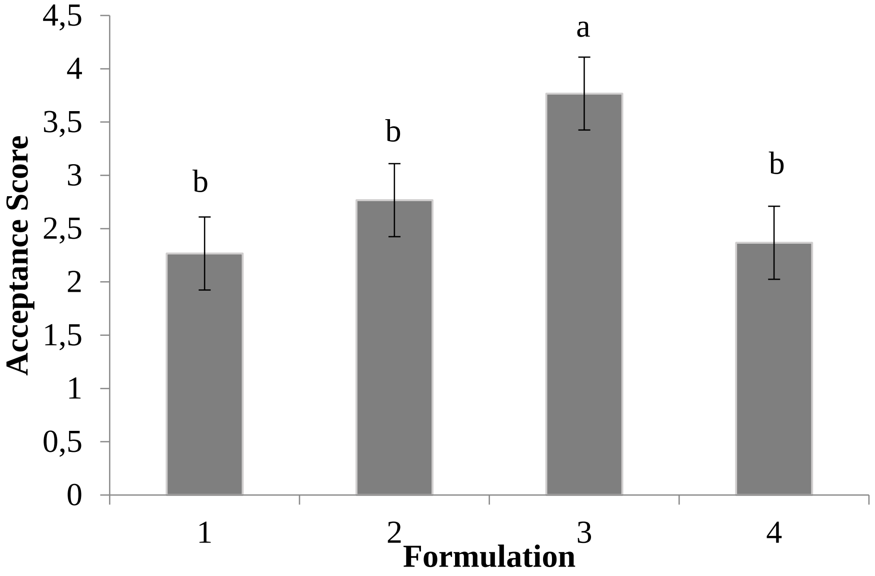

Figure 3.2 Preference of bitter chocolate with composition of sucrose-Stevia 1. 10:0.5; 2. 12:0.5; 3. 14:0.5; 4. 15:0.5. Bars indicate the standard error of the replicates.

The second sensory test aimed to find the most acceptable bitter chocolate formulation on Table 2.2. Acceptance test scores are given on Figure 3.3. There was no significant difference between the sucrose (BCS) and sucrose-stevia combination (BCSSt) ($p \geq 0.05$) but they were significantly different from BCSt and BCSp ($p \leq 0.05$). BCSSp and BCSSt samples received similar preferences ($p \geq 0.05$).

Melo et al. (2009) investigated partial sucrose replacement with stevia and sucralose and showed that partial sucrose substitution by sucralose was more acceptable than stevia substituted bitter chocolate. On the other hand, in another study of Melo et al. (2010), chocolate containing only stevia had the highest mean acceptance score. According to Bolini-Cardello et al (2007) stevia leaf extract had more effective aftertaste in bitter chocolate. In other words, an increased bitter aftertaste was perceived with bitter chocolate with stevia. These results was contradictory to our findings where BCS and BCSSt samples were similar ($p \geq 0.05$) and BCSSt and BCSSp received similar scores ($p \geq 0.05$). It is hypothesized that, presence of partial sucrose might have masked the bitter taste of stevia and could have increased its potential for preference. Flavor of the cocoa mass used or tempering conditions could also have influenced the taste of the chocolate resulting in a different trend when compared with the studies in the literature.

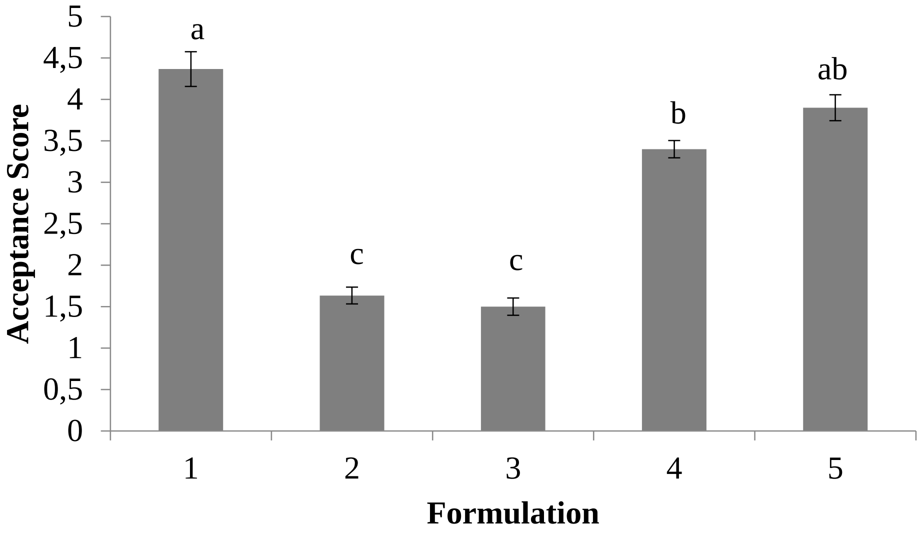

Figure 3.3 Preference of bitter chocolate with different sweeteners 1. BCS; 2. BCSp; 3. BCSt; 4. BCSSp; 5. BCSSt. Bars indicate standard error of the replicates.

3.2. Effects of Sweeteners on Rheological Properties of Different Chocolate Formulations

Rheological properties of molten chocolate are important in manufacturing and product quality (Afoakwa et al., 2007a, 2007b; Aidoo et al., 2014; Taylor et al., 2008). For chocolate, Casson and Herschel – Bulkley models are among the widely used rheological models (Afoakwa, 2010; Aidoo, et al., 2014; Briggs & Wang, 2004; Farzanmehr & Abbasi, 2009; Keogh et al., 2003; Sokmen & Gunes, 2006). Casson model is mainly accepted by International Confectionery Association (IOCCC) for quantifying chocolate rheology (Bouzas & Brown, 1995; Sokmen & Gunes, 2006).

Molten chocolate is known to exhibit an apparent non-Newtonian flow behavior (Shah et al. 2010) and same behavior was observed in this study.

In this study, the rheological properties of the molten chocolate samples were characterized using the Casson model as shown in Equation (3.1):

$$\tau^{0.5} = \tau_0^{0.5} + \eta_{pl}^{0.5} * \gamma^{0.5} \tag{3.1}$$

where τ denotes shear stress, τ_0 is yield stress, η_{pl} is plastic viscosity and γ is shear rate. A typical chocolate flow curve showing the measurement of shear stress as a function of increasing shear rate was given in Figure 3.4 which also showed that BCSSt samples exhibited very similar flow to control sample (BCS). Statistical results showed that the Casson model was providing a perfect fit to describe the flow behavior of chocolate with $R^2 \geq 0.99$ regardless of the sweetener type used. Casson viscosity and yield stress values of all samples were displayed in Table 3.1.

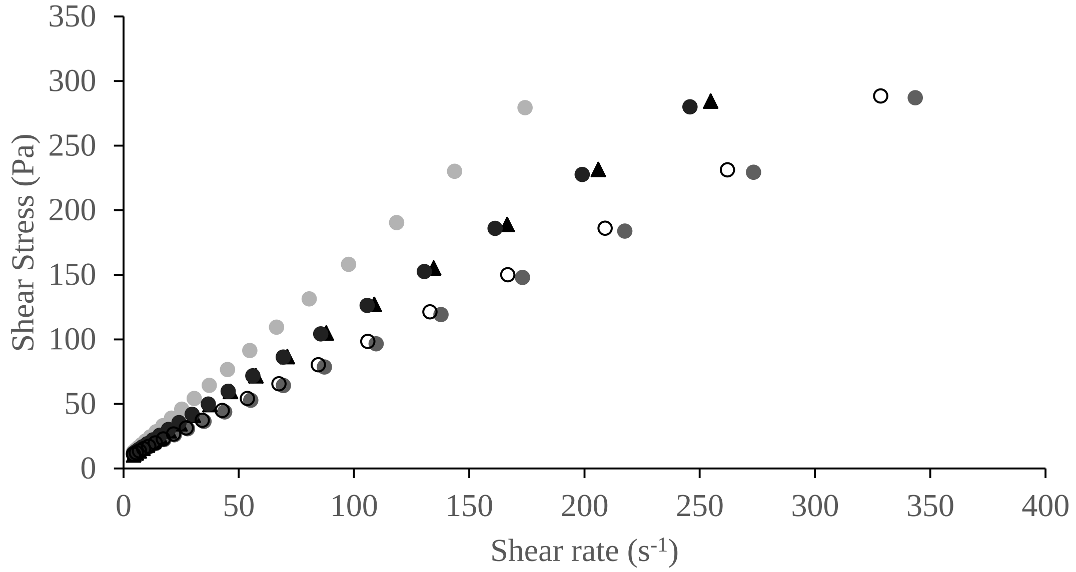

Figure 3.4. Flow Curves for BCS (Gray Circle), BCSSt (Light Gray Circle), BCSc
(Triangle), BCSSt (Empty Circle) and DCSSc (Black Circle)

Plastic viscosity is the amount of energy required to keep a non-Newtonian liquid moving once motion has been initiated (Afoakwa *et al*. 2007b). It is associated with filling of rough surfaces, pumping characteristics, coating properties and sensory properties (Aidoo *et al*. 2015). Among all the samples, the highest plastic viscosity was obtained in MCS sample as 3.13 Pa.s. Control samples of white (WCS) and bitter chocolates (BCS) exhibited similar values in Casson viscosity. As shown in Table 2.2, sucrose content of MCS was different than WCS and BCS. The difference in sucrose content between MCS and the other chocolate types could be considerable factor on the plastic viscosity due to its influence on particle-particle interactions. It was also obvious that the presence of milk powder affected the particle-particle interactions and thus the viscosity values. WCSt and WCSc samples gave the lowest plastic viscosity results between 0.44 and 0.55 Pa.s and it was explained with their less aggregate packing network structure. Since white chocolate had higher fat content, most probably due to interaction with sweeteners, bulking agents, milk fat and cocoa butter and resulting lubricating action, particle–particle interactions reduced, increasing mobility resulting in a decrease on the viscosity (Glicerina *et al*. 2013; Vernier 1998).

It was observed that effect of sweetener type was not significant on Casson viscosity except for bitter chocolate formulations ($p \leq 0.05$). In dark chocolates, BCSt gave significantly higher plastic viscosity results than BCSc samples. It was stated in the study of Shah *et al*. (2010), Beckett (2000) and Afoakwa *et al*. (2007a,b) that high viscosity in chocolate had a persisting pasty mouth-feel and thereby related to composition, particle size distribution and processing strategy. Presence of milk powder in milk chocolate samples; presence of higher amount of cocoa butter in white chocolate samples might have isolated the effect of sweetener type.

While control samples of milk and white chocolates containing only sucrose (MCS and WCS, respectively) had the highest viscosity results among their formulations, Casson viscosity of other control sample (BCS) was the lowest among all dark chocolate formulations ($p \leq 0.05$). This was explained with the maltodextrin percentage in the formulations. As can be seen in Table 2.1 whenever sucrose was removed from the formulation maltodextrin concentration was adjusted accordingly. Since maltodextrin had higher percentage in dark chocolate than other control chocolates, it resulted in higher solid volume fraction in dark chocolate due to its bulking agent function thereby increased plastic viscosity. On the other hand, amount of maltodextrin in milk and white chocolates remained lower. In the study of Shah *et al*. (2010), it was noted that higher plastic viscosity could be identified with higher solid volume fraction. Moreover, particle size could be correlated with viscosity in such a way that higher particle size lead smaller surface area that was in contact with continuous fat phase in chocolate and thereby internal friction and viscosity showed a decrease (Sokmen and Gunes 2006).

Table 3.1. Flow Parameters of All Chocolate Formulations

Formulation	Casson viscosity (Pa.s)	Casson yield stress (Pa)	R^2
Bitter Chocolate			
BCS	0.69 ± 0.07^{c}	1.61 ± 0.15^{a}	0.9997
BCSt	1.40 ± 0.10^{a}	0.78 ± 0.06^{c}	0.9994
BCSc	0.97 ± 0.09^{b}	1.07 ± 0.10^{bc}	0.9996
BCSSt	0.73 ± 0.06^{b}	1.54 ± 0.14^{a}	0.9998
BCSSc	0.96 ± 0.10^{b}	1.41 ± 0.12^{ab}	0.9996
Milk Chocolate			
MCS	3.13 ± 0.27^{a}	0.46 ± 0.04^{a}	0.9971
MCSt	1.12 ± 0.10^{b}	0.40 ± 0.03^{a}	0.9996
MCSc	1.11 ± 0.09^{b}	0.51 ± 0.05^{a}	0.9989
White chocolate			
WCS	0.71 ± 0.06^{a}	4.76 ± 0.48^{a}	0.9905
WCSt	0.55 ± 0.05^{ab}	0.17 ± 0.02^{b}	0.9994
WCSc	0.44 ± 0.03^{b}	0.25 ± 0.03^{b}	0.9995

*Lettering was conducted based on 5% significant level.
**Each chocolate type was treated on its own while conducting ANOVA. So lettering
should be considered for each chocolate.

Yield stress is a material property which characterizes the minimum shear stress required to induce the flow (Shah *et al.* 2010). It expresses the low shear rate properties of chocolate and is influenced by the specific surface area, fraction of the particles, emulsifiers, and moisture, particle-particle interactions (Afoakwa *et al.* 2007a; Aidoo *et al.* 2014; Aidoo *et al.* 2015). The Casson yield stress value was higher for WCS which was one of the control samples made with sucrose (4.76 Pa). This showed that amount of energy required to start flow was the highest in WCS samples. Among milk chocolate samples, no significant difference were observed on the yield stress for MCS, MCSSc or MCSSt. Moreover, sweetener type (Sucralose and Stevia) was not significantly different for yield stress among the same chocolate types (p ≤ 0.05).

Generally, white chocolate samples showed lower results in terms of viscosity and yield stress than all other samples except for WCS. Likely, Glicerina, *et al.* (2015 a,b, 2016) found that white chocolate having the highest amount of fat had the smallest sized particles and the lowest yield stress, viscosity values than bitter and milk chocolates while milk chocolate gave intermediate results. Moreover, in the same study, it was mentioned that the presence of crystalline lactose in milk and white chocolates should be considered as a factor that could have an impact on their lower viscosity values, promoting the release of entrapped milk-fat. In rheological characteristics, particle-particle interactions are influenced by composition, thus stronger interaction could lead to higher rheological properties (Afoakwa *et al.* 2009; Glicerina *et al.* 2013; Glicerina *et al.* 2016).

Attaining similar flow properties in chocolates prepared with sugar substitutes as that of conventional chocolates is key for final product quality. In bitter chocolates, BCSSt became the most similar one to control sample BCS with regard to rheological properties.

3.2 Effects of different sweeteners on texture properties of bitter chocolate

Perception of chocolate texture has substantial importance during mastication. Textural properties like hardness control consistency, viscosity and spreadability (Afoakwa et al. 2008). Hardness designates the physical rigidity and is directly relevant to sensory perception during consumption (Shah et al. 2010). Texture of a chocolate is also affected from moisture significantly. Chocolate seizing which is known to occur upon addition of a couple cold drops of water changes the texture irreversibly due to sugar in the chocolate interacting with water rather than the emulsifier: lecithin. Moisture content of the bitter chocolates formulated in this study are given on Figure 3.5.

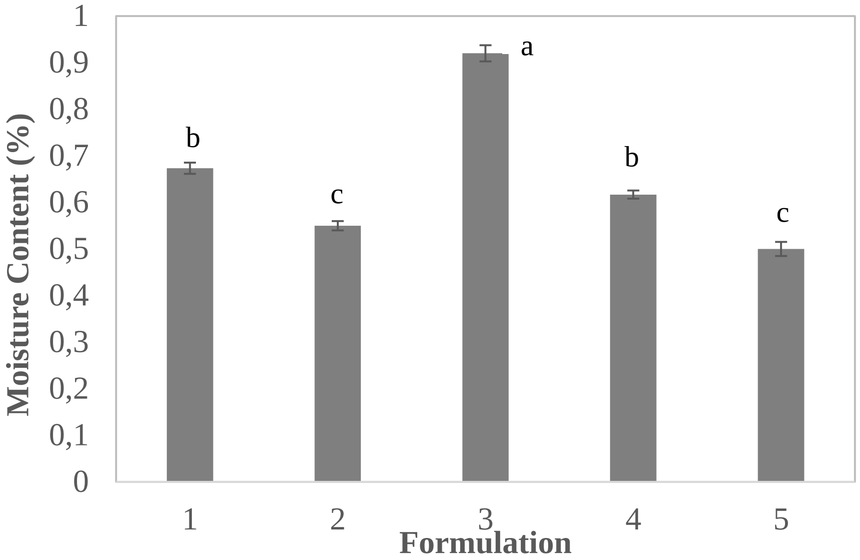

Figure 3.5 Moisture contents bitter chocolate with different sweeteners 1. BCS; 2. BCSp; 3. BCSt; 4. BCSSp; 5. BCSSt. Bars indicate standard error of the replicates.

As seen on Fig. 3.5, BCSt chocolates had the highest moisture content whereas BCSc and BCSSt samples had the lowest. Stevia containing a glucose unit could have resulted in more moisture retention on the chocolate resulting in higher moisture. On the other hand, this effect might have been compensated with the presence of sucrose on BCSSt samples resulting in lower moisture contents.

Fig. 3.6 illustrated the effects of different sweeteners on the hardness of the bitter chocolates. Results showed that partial replacement of sucrose with stevia (BCSSt) had significant effect on chocolate hardness when compared with only stevia containing samples (BCSSt) ($p \leq 0.05$). According to the Figure 3.6., BCSSt samples showed lower hardness compared to BCSt. A similar trend was also shown in moisture contents between BCSt and BCSSt samples.

Fracturability or the maximum load to fracture a bar of chocolate was displayed in Fig. 3.7. The fracturability is the consequence of a high degree of hardness and a low degree of cohesiveness. Hence it leads to combination of the force needed to compress a substance between molar teeth and the degree to which a substance is compressed between the teeth before it breaks (De Clercq et al. 2012; Szczesniak 2002). Fracturability was similar to hardness. In order to understand the correlation between hardness and fracturability, Pearson correlation was conducted between hardness, fracturability and moisture content and a significant positive correlation ($p<0.05$) was obtained with a coefficient of greater than 0.69. 70% correlation could be considered a satisfactory correlation for chocolate quality parameters since during chocolate making many uncontrollable factors could have contributed and standardization could be challenging.

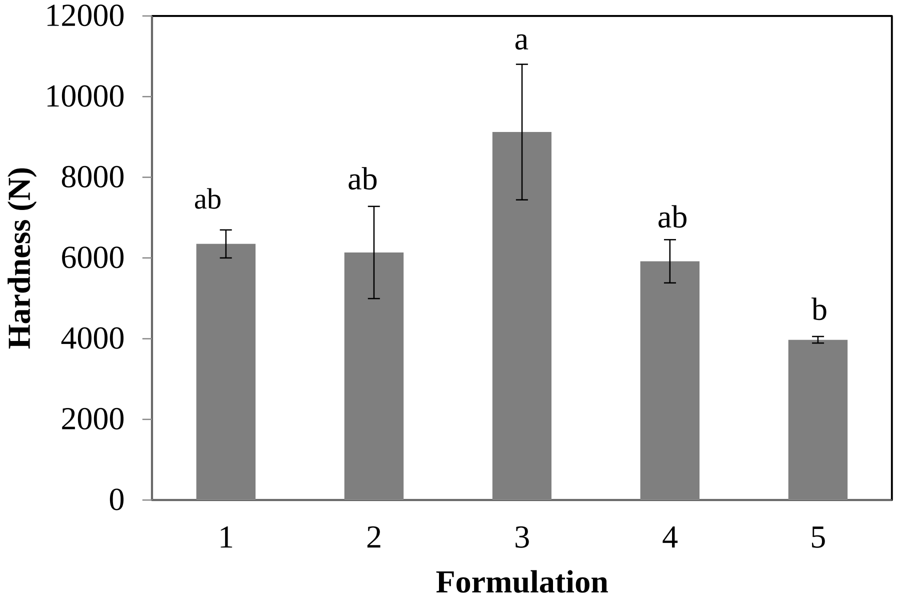

Figure 3.6 Hardness of bitter chocolate with different sweeteners 1. BCS; 2. BCSp; 3. BCSt; 4. BCSSp; 5. BCSSt Bars indicate standard error of the replicates.

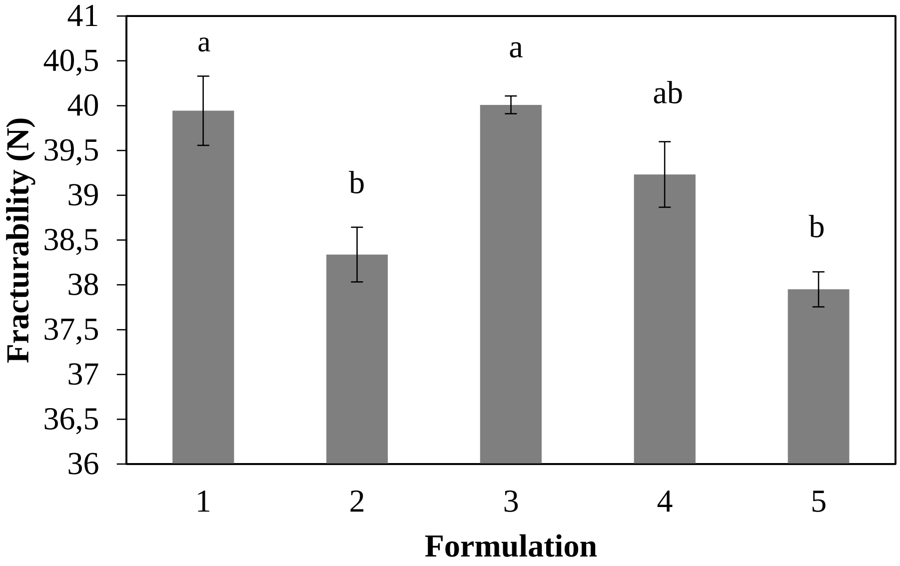

Figure 3.7 Fracturability of bitter chocolate with different sweeteners 1. BCS; 2. BCSp; 3. BCSt; 4. BCSSP; 5. BCSSt. Bars indicate standard error of the replicates.

3.3 Effects of Different Sweeteners on Color of Chocolates

Color analysis of the formulations studied are given in Table 3.2 and Table 3.3.

Table 3.3 and 2 Way ANOVA Results on Appendix showed that the effect of sweeteners was not significant on L* values ($p>0.05$) whereas chocolate type was significant ($p<0.05$). Milk chocolates were found to have an average L* value of 25.8 whereas this was 24.6 for bitter chocolate samples. However, when multiple comparison test (Tukey) was conducted, it was seen that all samples were same in terms of L values ($p>0.05$). Sweetener and chocolate type interactions were insignificant ($p>0.05$) resulting in this trend. According to the study of Aidoo et al, (2014) milk and bitter chocolate samples should have significantly different L* values. This is explained in such a way that the addition of polysaccharides gives acceleration for caramelization and Maillard reaction which could lighten the color of chocolates. ANOVA results on Appendix were in accordance with this behavior. Lightness values for white chocolate samples also did not change with sweetener type (Table 3.2, $p>0.05$).

a* values denote the redness/greenness of a sample in color determination. For white chocolate samples these were not meaningful to interpret, that is why they will not be discussed.

Significant difference was detected between a* values of bitter and milk chocolates ($p<0.05$) and milk chocolate samples (Mean ‘a’= 5.7) were found to have higher a* values compared to bitter ones (Mean ‘a’= 4.3) (Appendix). Moreover, chocolates with sucralose and stevia were found to be significantly different from chocolates with sucrose (Table 3.3 and Appendix A5). Similar results were also observed in another study (Psimouli and Oreopoulou, 2013) where the effect of alternative sweeteners on batter rheology and cake properties in were investigated. Sucrose was substituted by different sweeteners and then a* value measurements were recorded. It was found that all a* values were significantly different from each other ($p<0.05$).

Table 3. 2 Color Analysis Results of Chocolate Formulations

Formulation	L			a			b			ΔE		
BCS	24.70^{a}	±	0.70	5.20^{a}	±	0.15	4.10	±	0.06	**		**
BCSt	24.27^{a}	±	0.58	3.73^{b}	±	0.27	3.23	±	0.12	24.33^{a}	±	0.15
BCSc	24.87^{a}	±	0.13	3.97^{ab}	±	0.12	3.33	±	0.03	24.20^{a}	±	0.04
BCSSt	24.50^{a}	±	0.26	4.47^{ab}	±	0.18	4.03	±	0.12	23.48^{b}	±	0.12
BCSSc	24.53^{a}	±	0.20	3.23^{b}	±	0.58	3.13	±	0.07	24.46^{a}	±	0.12
MCS	25.70^{a}	±	0.60	6.70^{a}	±	0.23	5.80^{a}	±	0.26	**		**
MCSt	26.33^{a}	±	0.12	5.67^{ab}	±	0.03	4.63^{b}	±	0.03	21.00^{a}	±	0.12
MCSc	25.23^{a}	±	0.66	4.87^{b}	±	0.50	4.00^{b}	±	0.35	20.01^{b}	±	0.60
WCS	79.83^{a}	±	0.24	0.367	±	0.09	27.5^{a}	±	0.15	**		**
WCSt	80.47^{a}	±	0.72	-0.43	±	0.13	22.8^{b}	±	0.70	4.93^{a}	±	0.52
WCSc	78.67^{a}	±	0.17	-0.27	±	0.09	26.5^{a}	±	0.12	1.68^{b}	±	0.14

- Lettering was conducted based on 5% significant level.
- Each chocolate type was treated on its own while conducting ANOVA. So lettering should be considered for each chocolate.
- Reference values for ΔE calculations were used as the chocolate containing sucrose on its own type.
- Normality assumption for the data set was checked and it was found out that ‘b’ values for bitter chocolate did not follow a normally distributed pattern. Since b value was associated with yellowness and yellowness could not be an attribute of bitter chocolate it was found appropriate not to conduct ANOVA for that data. Similarly redness for white chocolate gave ‘-‘results for some samples and ANOVA was not conducted for that data.

Table 3. 3 Color Analysis Results of Milk and White Chocolate (2 Way ANOVA Results)

Formulation	L			a			b		
BCS	24.70^{a}	±	0.70	5.20^{bc}	±	0.15	4.10^{bc}	±	0.06
BCSt	24.27^{a}	±	0.58	3.73^{d}	±	0.27	3.23^{c}	±	0.12
BCSc	24.87^{a}	±	0.13	3.97^{cd}	±	0.12	3.33^{c}	±	0.03
MCS	25.70^{a}	±	0.60	6.70^{a}	±	0.23	5.80^{a}	±	0.26
MCSt	26.33^{a}	±	0.12	5.67^{ab}	±	0.03	4.63^{b}	±	0.03
MCSc	25.23^{a}	±	0.66	4.87^{bcd}	±	0.50	4.00^{bc}	±	0.35

- Lettering was conducted based on 5% significant level.
- Chocolate type and sweetener type were considered as different factors. Combinations of sucrose and stevia are not included since these combinations were not tested on milk chocolates.
- Normality assumption for the data set was checked and it was confirmed for all data set.

For bitter chocolates among themselves (Table 3.2) BCSt and BCS samples differed significantly, BCSt ones showing lower a* values ($p<0.05$). For milk chocolate samples, MCS and sweetener containing ones; MCSt and MCSc differed significantly showing lower a* values ($p<0.05$) and when bitter and milk chocolate ones were compared (Table 3.3), it was seen that MCS and BCS were significantly different ($p<0.05$). Moreover, BCSt and MCSt were also different; MCSt ones having higher a* values due to milk powder as expected. Sucralose did not show a significant effect on color in contrast to stevia.

It is obvious that the presence of sweeteners would affect the a* values of chocolates. Sucrose is a non-reducing sugar whereas sucralose and stevia containing formulations included maltodextrin which were capable of involving in browning reactions. So it can be concluded that without noticing the food type use of different sweeteners substituting the sucrose makes difference in redness.

b* values show the blueness/yellowness of the sample in color. b* values for bitter chocolate samples did not follow a normally distributed pattern even with transformation (square root, logarithmic , Box-Cox) that's why they were not lettered by ANOVA on Table 3.2. Moreover, it was decided that just for bitter chocolate samples it might not be meaningful to check the yellowness. And also, white chocolate samples were treated among themselves. For bitter and milk chocolate data set, comparison of b* values was conducted since the overall data satisfied ANOVA's assumptions in contrast to bitter only samples as stated above (constant variance and normal distribution) (Table 3.3). Effect of sweetener and chocolate type on b* values were similar to a* values for milk and white chocolates and they were significant ($p<0.05$. Appendix A.5). Interaction of sweetener and chocolate type was also significant according to 2-way ANOVA results (Appendix). It was clearly observed that with the presence of milk powder in the recipe, b* values increased. MCS samples showed the highest b* values.

Total color difference ΔE was calculated by using the color values of different chocolate samples and the corresponding control samples (sucrose containing ones) as the reference. As seen on Table 3.2, the effect of sweetener was significant on white and milk chocolate formulations ($p<0.05$) and sucralose containing samples both (MCSc, WCSc) had lower values in both chocolate types. For bitter chocolates the presence of sweeteners did not show a significant effect except BCSSt samples ($p>0.05$). Partial substitution of sucrose with stevia decreased the color difference slightly.

CHAPTER 4

CONCLUSION AND RECOMMENDATIONS

The development of a high-quality low-calorie chocolate has to consider several points. The first one sensorial properties. Sugar substitution with high-intensity sweeteners should not affect color, flavor, texture, aftertaste, mouthfeel and overall acceptances negatively. Secondly process parameters should be evaluated for feasibility. To decide the processability of the chocolate, rheological characterization should be conducted. Rheological properties give an idea to arrange the parameters such as temperatures, pumping requirements, motor power etc.

In this study, it was found that all chocolate types with different sweeteners follow the Casson-model for rheology.

Only stevia containing chocolates (BCSt) had higher moisture contents and were comparably harder with respect to formulations containing sucrose and stevia (BCSSt). Moreover, BCSSt samples got same acceptance score with the control samples in the sensory experiments.

Color analysis showed that L*, a* and b* and ΔE values were affected from chocolate and sweetener type significantly ($p<0.5$). Presence of bulking agents due to sweeteners and milk powder increased the* values of the chocolates.

The study showed that using intense sweeteners it is possible to formulate, low calorie chocolates having similar rheological, sensorial and physical (texture, color) properties as does sucrose containing ones. The study could be extended by formulating chocolate with higher sweeteners concentrations and changing bulking agent concentrations to see the effect of different ingredients. Moreover, effect of those ingredients could also be studied on chocolate blooming which is an important quality problem on chocolate industry.

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APPENDIX A

ANOVA Tables

Table A.1 One Way ANOVA for hardness and fracturability and moisture contents of bitter chocolates containing different sweeteners

General Linear Model: Hardness, Fracturability versus Formulation

```
Factor       Type   Levels  Values
Formulation  fixed       5  BCS, BCSc, BCSSc, BCSSt, BCSt
```

Analysis of Variance for Hardness, using Adjusted SS for Tests

```
Source       DF    Seq SS    Adj SS    Adj MS     F      P
Formulation   4  40676938  40676938  10169235  3.73  0.042
Error        10  27256447  27256447   2725645
Total        14  67933385


S = 1650.95   R-Sq = 59.88%   R-Sq(adj) = 43.83%
```

Analysis of Variance for Fracturability, using Adjusted SS for Tests

```
Source       DF   Seq SS   Adj SS   Adj MS      F      P
Formulation   4  10.3687  10.3687   2.5922  10.19  0.001
Error        10   2.5432   2.5432   0.2543
Total        14  12.9118


S = 0.504298   R-Sq = 80.30%   R-Sq(adj) = 72.43%
```

Grouping Information Using Tukey Method and 95.0% Confidence for Hardness

```
Formulation  N    Mean  Grouping
BCSt         3  9122.4  A
```

```
BCS            3     6349.0  A B
BCSc           3     6136.9  A B
BCSSc     3  5919.0  A B
BCSSt     3  3972.2    B
```

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for Fracturability

```
Formulation  N  Mean  Grouping
BCS          3  40.0       A
BCSt         3  39.9       A
BCSSc        3  39.2       A B
BCSc         3  38.3         B
BCSSt        3  38.0         B
```

Means that do not share a letter are significantly different.

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

```
Source       DF    Seq SS    Adj SS    Adj MS       F      P
Formulation   4  0.321107  0.321107  0.080277  156.38  0.000
Error        10  0.005133  0.005133  0.000513
Total        14  0.326240


S = 0.0226569   R-Sq = 98.43%   R-Sq(adj) = 97.80%
```

Grouping Information Using Tukey Method and 95.0% Confidence

```
Formulation  N  Mean  Grouping
BCSt         3   0.9       A
BCS          3   0.7         B
BCSSc        3   0.6         B
BCSc         3   0.6           C
BCSSt        3   0.5           C
```

Means that do not share a letter are significantly different.

Table A.2 One Way ANOVA for **L** and **a** and **ΔE** values of bitter chocolates containing different sweeteners

General Linear Model: L, a versus Formulation

```
Factor       Type   Levels  Values
Formulation  fixed       5  BCS, BCSc, BCSSc, BCSSt, BCSt
```

Analysis of Variance for ‘L’ using Adjusted SS for Tests

```
Source       DF  Seq SS   Adj SS  Adj MS     F      P
Formulation   4   0.6093  0.6093  0.1523    0.26  0.894
Error        10  5.7600  5.7600  0.5760
Total        14  6.3693


S = 0.758947   R-Sq = 9.57%   R-Sq(adj) = 0.00%
```

Analysis of Variance for ‘a’ using Adjusted SS for Tests

```
Source        DF     Seq SS   Adj SS  Adj MS     F      P
Formulation   4     6.7373      6.7373  1.6843   5.88  0.011
Error        10    2.8667      2.8667  0.2867
Total        14     9.6040

S = 0.535413   R-Sq = 70.15%   R-Sq(adj) = 58.21%
```

Grouping Information Using Tukey Method and 95.0% Confidence for ‘L’

```
Formulation   N  Mean  Grouping
BCSc          3  24.9       A
BCS           3  24.7       A
BCSSc         3  24.5       A
BCSSt         3  24.5       A
BCSt          3  24.3       A

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

Grouping Information Using Tukey Method and 95.0% Confidence for ‘a’

```
Formulation    N  Mean  Grouping
BCS            3   5.2        A
BCSSt          3   4.5        A B
```

```
BCSc           3   4.0       A B
BCSt           3   3.7         B
BCSSc          3   3.2         B
```

Means that do not share a letter are significantly different.

General Linear Model: □E versus Formulation

```
Factor       Type   Levels  Values
Formulation  fixed       4  BCSc, BCSSc, BCSSt, BCSt
```

Analysis of Variance for DE, using Adjusted SS for Tests

```
Source       DF   Seq SS   Adj SS   Adj MS      F      P
Formulation   3  1.71953  1.71953  0.57318  14.05  0.001
Error         8  0.32643  0.32643  0.04080
Total        11  2.04597


S = 0.202001   R-Sq = 84.04%   R-Sq(adj) = 78.06%
```

Grouping Information Using Tukey Method and 95.0% Confidence

```
Formulation1  N  Mean  Grouping
BCSSc         3  24.5    A
BCSt          3  24.3    A
BCSc          3  24.2    A
BCSSt         3  23.5      B
```

Means that do not share a letter are significantly different.

Table A.3 One Way ANOVA for **L** and **a**, **b** and **□E** values of milk chocolates containing different sweeteners

General Linear Model: L, a, b versus Formulation

```
Factor       Type   Levels Values
```

```
Formulation   fixed       3  MCS, MCSc, MCSt
```

Analysis of Variance for ‘L’, using Adjusted SS for Tests

```
Source       DF  Seq SS  Adj SS  Adj MS     F      P
Formulation   2  1.8289  1.8289  0.9144  1.12  0.387
Error         6  4.9133  4.9133  0.8189
Total         8  6.7422


S = 0.904925   R-Sq = 27.13%   R-Sq(adj) = 2.83%
```

Analysis of Variance for ‘a’, using Adjusted SS for Tests

```
Source       DF  Seq SS  Adj SS  Adj MS     F      P
Formulation   2  5.0689  5.0689  2.5344  8.39  0.018
Error         6  1.8133  1.8133  0.3022
Total         8  6.8822


S = 0.549747   R-Sq = 73.65%   R-Sq(adj) = 64.87%
```

Analysis of Variance for ‘b’, using Adjusted SS for Tests

```
Source       DF  Seq SS  Adj SS  Adj MS      F      P
Formulation   2  5.0022  5.0022  2.5011  13.09  0.006
Error         6  1.1467  1.1467  0.1911
Total         8  6.1489


S = 0.437163   R-Sq = 81.35%   R-Sq(adj) = 75.14%
```

Grouping Information Using Tukey Method and 95.0% Confidence for ‘L’

```
Formulation  N  Mean  Grouping
MCSt         3  26.3  A
MCS          3  25.7  A
MCSc         3  25.2  A
```

Grouping Information Using Tukey Method and 95.0% Confidence for ‘a’

```
Formulation   N  Mean  Grouping
MCS           3   6.7         A
MCSt          3   5.7         A B
MCSc          3   4.9           B
```

Grouping Information Using Tukey Method and 95.0% Confidence for ‘b’

```
Formulation3   N  Mean  Grouping
MCS            3   5.8       A
MCSt           3   4.6         B
MCSc           3   4.0        B
```

Means that do not share a letter are significantly different.

General Linear Model: ΔE versus Formulation

```
Factor       Type   Levels  Values
Formulation  fixed       2  MCSc, MCSt
```

Analysis of Variance for □E, using Adjusted SS for Tests

```
Source        DF    Seq SS  Adj SS  Adj MS     F      P
Formulation    1  1.4811  1.4811  1.4811  2.64  0.180
Error          4  2.2457  2.2457  0.5614
Total          5  3.7267


S = 0.749278   R-Sq = 39.74%   R-Sq(adj) = 24.68%
```

Grouping Information Using Tukey Method and 95.0% Confidence

```
Formulation   N  Mean  Grouping
MCSt          3  21.0       A
MCSc          3  20.0       A
```

Means that do not share a letter are significantly different.

Table A.4 2-Way ANOVA for **L** and **a**, **b** values of bitter and milk chocolates containing different sweeteners

General Linear Model: L, a, b versus Sweetener, Chocolate Type

```
Factor          Type   Levels  Values
Sweetener       fixed     3  Stevia, Sucralose, Sucrose
Chocolate Type  fixed  2  BC, MC
```

Analysis of Variance for L, using Adjusted SS for Tests

```
Source                     DF   Seq SS   Adj SS  Adj MS     F      P
Sweetener                   2   0.1900   0.1900  0.0950  0.11  0.893
Chocolate Type              1   5.8939   5.8939  5.8939  7.07  0.021
Sweetener*Chocolate Type    2   2.2144   2.2144  1.1072  1.33  0.301
Error                      12  10.0067  10.0067  0.8339
Total                      17  18.3050

S = 0.913175   R-Sq = 45.33%   R-Sq(adj) = 22.56%
```

Analysis of Variance for a, using Adjusted SS for Tests

```
Source                     DF   Seq SS  Adj SS  Adj MS      F      P
Sweetener                   2   7.9878  7.9878  3.9939  19.27  0.000
Chocolate Type              1   9.3889  9.3889  9.3889  45.31  0.000
Sweetener*Chocolate Type    2   0.8078  0.8078  0.4039   1.95  0.185
Error                      12   2.4867  2.4867  0.2072
Total                      17  20.6711


S = 0.455217   R-Sq = 87.97%   R-Sq(adj) = 82.96%
```

Analysis of Variance for b, using Adjusted SS for Tests

```
Source                     DF   Seq SS  Adj SS  Adj MS      F      P
Sweetener                   2   5.5033  5.5033  2.7517  26.21  0.000
Chocolate Type              1   7.0939  7.0939  7.0939  67.56  0.000
Sweetener*Chocolate Type    2   0.8478  0.8478  0.4239   4.04  0.046
Error                      12   1.2600  1.2600  0.1050
Total                      17  14.7050
```

```
S = 0.324037   R-Sq = 91.43%   R-Sq(adj) = 87.86%
```

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95.0% Confidence for L

```
Sweetener  N  Mean  Grouping
Stevia      6  25.3     A
Sucrose     6  25.2     A
Sucralose   6  25.1     A
```

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for L

```
Chocolate
Type      N  Mean  Grouping
MC        9   25.8      A
BC        9   24.6        B
```

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for L

```
           Chocolate
Sweetener  Type      N  Mean  Grouping
Stevia      MC        3  26.3     A
Sucrose     MC        3  25.7     A
Sucralose   MC        3  25.2     A
Sucralose   BC        3  24.9     A
Sucrose     BC        3  24.7     A
Stevia      BC        3  24.3     A
```

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for a

```
Sweetener  N  Mean  Grouping
Sucrose    6   6.0     A
Stevia     6   4.7       B
Sucralose  6  4.4        B
```

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for a

```
Chocolate
Type      N  Mean  Grouping
MC        9   5.7        A
BC        9   4.3          B
```

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for a

```
          Chocolate
Sweetener  Type      N  Mean  Grouping
Sucrose     MC       3   6.7       A
Stevia      MC       3   5.7       A B
Sucrose     BC       3   5.2         B C
Sucralose   MC       3   4.9         B C D
Sucralose   BC       3   4.0           C D
Stevia      BC       3   3.7             D
```

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for b

```
Sweetener  N    Mean  Grouping
Sucrose    6     5.0       A
Stevia     6     3.9         B
Sucralose  6     3.7         B
```

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for b

```
Chocolate
Type      N  Mean  Grouping
MC        9   4.8        A
BC        9   3.6          B
```

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for b

```
          Chocolate
Sweetener  Type       N  Mean  Grouping
Sucrose      MC        3   5.8         A
Stevia        MC        3   4.6           B
Sucrose       BC        3   4.1           B C
Sucralose    MC       3   4.0           B C
Sucralose    BC        3   3.3               C
Stevia          BC        3   3.2               C
```

Means that do not share a letter are significantly different.

Table A.5 ANOVA for **L** and **b** and **ΔE** values of white chocolates containing different sweeteners

General Linear Model: L, b versus Formulation

```
Factor        Type   Levels  Values
Formulation   fixed       3  WCS, WCSc, WCSt


Analysis of Variance for L1, using Adjusted SS for Tests

Source        DF  Seq SS  Adj SS  Adj MS     F      P
Formulation    2  5.0022  5.0022  2.5011  4.12  0.075
Error            6  3.6400  3.6400  0.6067
Total            8  8.6422


S = 0.778888   R-Sq = 57.88%   R-Sq(adj) = 43.84%


Analysis of Variance for b1, using Adjusted SS for Tests

Source          DF  Seq SS  Adj SS  Adj MS      F      P
Formulation   2  36.222    36.222   18.111  34.75  0.001
Error            6   3.127   3.127   0.521
Total            8  39.349


S = 0.721880   R-Sq = 92.05%   R-Sq(adj) = 89.41%
```

```
Grouping Information Using Tukey Method and 95.0% Confidence for L1

Formulation  N  Mean  Grouping
WCSt         3  80.5     A
WCS          3  79.8     A
WCSc         3  78.7     A

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence for b1

Formulation   N  Mean  Grouping
WCS           3  27.5     A
WCSc          3  26.5     A
WCSt          3  22.8       B

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

General Linear Model: □E versus Formulation

```
Factor       Type   Levels  Values
Formulation  fixed       2  WCSc, WCSt


Analysis of Variance for DE1, using Adjusted SS for Tests

Source        DF  Seq SS  Adj SS  Adj MS      F      P
Formulation2   1  15.870  15.870  15.870  36.08  0.004
Error          4   1.760   1.760   0.440
Total          5  17.629


S = 0.663241   R-Sq = 90.02%   R-Sq(adj) = 87.52%


Grouping Information Using Tukey Method and 95.0% Confidence

Formulation   N  Mean  Grouping
WCSt          3   4.9     A
WCSc          3   1.7     B

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

APPENDIX B

Table B.1 Sensory Evaluation Sheet

Sensory evaluation sheet

Age:

Gender:

Do you smoke? Yes….   No….

	Scale (1-5. 5 is the best. 1 is the worst)				
Principles	**123**	**452**	**854**	**128**	**957**
Preference					