EFFECTS OF SOME SOY PRODUCTS ON RHEOLOGICAL, FUNCTIONAL AND SENSORY PROPERTIES OF MILK CHOCOLATE

BAZI SOYA ÜRÜNLERİNİN SÜTLÜ ÇİKOLATANIN REOLOJİK, FONKSİYONEL VE DUYUSAL ÖZELLİKLERİ ÜZERİNE ETKİLERİ

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İZLEM CANSU MECLİS

ABSTRACT

EFFECTS OF SOME SOY PRODUCTS ON RHEOLOGİCAL, FUNCTIONAL AND SENSORY PROPERTIES OF MILK CHOCOLATE

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Today, different types of diets and food products are of great interest. Soy chocolate, which is one of these products, is produced in various countries and there is not much research on the effect of the soy products on chocolate rheology and functionality. Rheology defines the flow properties of molten chocolate which is important parameter for producers and antioxidant capacity attracts the interest of consumer. In this study, two different soy products (soy milk powder and soy protein isolate) and two different milk products (whole and skimmed milk powder) were used to produce laboratory-scale milk chocolates with different conching times (2, 4, 6 hours). Rheological and functional properties of chocolates were investigated. Soymilk powder was produced as laboratory-scale and proximate and chemical analysis was performed. Physical properties of powder ingredients used in chocolate production were determined and functional properties of soy products were defined. Soymilk powder was found to have 44.43 % protein, 18.14 % fat and 6.06 % ash content. According to the chemical analysis, inactivation of 99.1 % for LOX-1, 100 % for LOX-3 and 98.5 % for trypsin inhibitors was achieved by heat treatment of 98 °C for 20 minutes.

Casson, Herschel-Bulkley and Bingham models were used to define the rheology of molten chocolates. According to the results, rheological behaviors of the chocolates were compatible with all models (R^2> 0.99). Apparent viscosities of chocolates were determined at constant shear rate (50 s^{-1}) and SoMC and WMC samples had lower viscosity values due to their fat content other than cocoa butter. According to the Casson model, these samples were found to have apparent viscosity values of 2.199 and 2.174 Pa.s respectively, after 6 hours of conching. The highest apparent viscosity value (4.430 Pa.s) was obtained by Herschel-Bulkley model in SPC2 samples, while the lowest apparent viscosity (1.854 Pa.s) was obtained by Casson model in WMC4 samples. According to the particle size distribution analysis, the 2 hour conched chocolates had a larger particle size than the 6 hour conched samples. All chocolates had a lower particle size at the end of the conching process, even though no linear decrease was observed with the increase of the conching time. This reduction in size is thought to occur in powder products added to chocolates. D_{90} values were obtained as 35.70 µm for SMC6 samples, 25.55 µm for WMC6 samples, 24.30 µm for SoMC6 samples and 60.35 µm SPC6 samples. Although SMC and SPC samples showed a significant reduction in particle size by concing, the desired particle size (<30 µm) was not obtained by 6 hours conching process. For this reason, it is recommended to extend the conching times for SMC and SPC chocolates in terms of particle size. The specific surface area data increased with increasing time of the conching. WMC6 samples had 1401 m^2/kg specific surface area, which was the highest value among the samples, while SPC2 samples had the lowest value with 746 m^2/kg. The yield stress, particle size and texture of chocolates are known to be related. It was observed that the chocolates snap more easily and texture becomes mellow with decreasing particle size.

Polymorphic structure was determined by X-ray diffractometer and sensory analysis was performed in order to evaluate the quality of chocolates. The analysis results showed that the polymorphic structure of all chocolates were in the desired β crystal form. Sensory analyzes showed that there was no statistically significant difference (P>0.05) in terms of after taste, appearance, flavour, odor and texture of the chocolates. This shows that soy chocolates are as much appreciated as milk chocolates.

Functionality of chocolates was evaluated in terms of total phenolic content and total antioxidant capacity. The antioxidant capacities were determined by ABTS, DPPH and CUPRAC methods. These methods were carried out by the QUENCHER procedure, which did not require any extraction step. The results were significantly higher than the literature data. According to Total Phenolic Content (TPC) analysis, SPC4 had the lowest value with 80.10 mg GAE/g dry sample and, and SoMC2 had the highest value with 129.33 mg GAE/g dry sample. Among the antioxidant capacity methods, the highest results were obtained by ABTS method as 6.79 mM Trolox/g dry sample for SoMC4 samples while the lowest results were obtained by DPPH method as 4.96 mM Trolox/g dry sample for SPC2 samples. It was observed that SoMC samples had the highest antioxidant capacity and SMC samples had the highest total phenolic content. This shows that soy-ingredients has an increasing effect on the amount of total phenolics and antioxidant capacity but the phenolics in soy are sensitive to the process. It is thought that high antioxidant content is caused by isoflovans in soy. SPC samples showed the lowest antioxidant activity and total phenolic content. This may be due to the fact that the proteins in soy mask the phenolic substances and antioxidant character.

In conclusion, although soy protein isolate provides high protein contribution in soy-chocolate, soymilk powder is recommended to be used in chocolate in terms of rheology and functionality of the product.

Keywords: milk chocolate, soymilk, rheology, particle size distribution, functional properties

ÖZET

BAZI SOYA ÜRÜNLERİNİN SÜTLÜ ÇİKOLATANIN REOLOJİK, FONKSİYONEL VE DUYUSAL ÖZELLİKLERİ ÜZERİNE ETKİLERİ

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Günümüzde farklı tüketim biçimleri ve gıda ürünleri çok ilgi çekmektedir. Bu tür ürünlerden olan soyalı çikolata çeşitli ülkelerde üretilmekte olup, soyanın çikolata reolojisi ve fonksiyonelliği üzerindeki etkisi üzerine çok fazla araştırma bulunmamaktadır. Reoloji, erimiş çikolatanın akış özelliklerini tanımladığından, antioksidan kapasite de tüketicilerin ilgisini çektiğinden çikolata üreticileri için önemli parametrelerdir. Bu sebeple çalışmamızda iki farklı soya ürünü (soya sütü tozu ve soya protein izolatı) ve iki farklı süt ürünü (tam yağlı ve yağsız süt tozu) kullanılan çikolatalar laboratuvar ortamında farklı konçlama süreleri ile (2, 4, 6 saat) üretilmiş, çikolataların reolojik ve antioksidan analizleri yürütülmüştür. Ayrıca çalışmada kullanılan soya sütü tozu laboratuvar ortamında üretilip besinsel ve kimyasal analizleri gerçekleştirilmiştir. Çikolata üretiminde kullanılan toz ürünlerin fiziksel özellikleri belirlenerek, soya ürünlerinin fonksiyonel özellikleri tanımlanmıştır. Üretilen soya sütü tozunun % 44,43 protein, % 18,14 yağ ve % 6,06 kül içeriğine sahip olduğu bulunmuştur. Yapılan kimyasal analizlere göre 20 dakika 98 °C sıcaklık uygulaması ile LOX-1 için % 99,1, LOX-3 için % 100 ve tripsin inhibitörleri için % 98,5 inaktivasyon sağlanmıştır.

Çikolataların reolojik özelliklerini tanımlamak için erimiş çikolata reolojisini tanımlamada en çok kullanılan Casson, Herschel-Bulkley ve Bingham modelleri uygulanmış ve model sabitleri belirlenmiştir. Sonuçlara göre çikolataların reolojik davranışları tüm modellere uymuştur (R^2>0.99). Sabit kayma hızında (50 s^{-1}) belirlenen görünür viskozitelere göre kakao yağı dışında yağ içeren SoMC ve WMC örneklerinin daha düşük viskozite değerlerine sahip olduğu görülmüştür. Bu örneklerin 6 saat konçlanandıktan sonra Casson modeline göre sırasıyla 2,199 ve 2,174 Pa.s görünür viskozite değerlerine sahip olduğu bulunmuştur. En yüksek viskozite değeri (4,430 Pa.s) SPC2 örneklerinde Herschel-Bulkley modeli ile elde edilirken, en düşük viskozite (1,854 Pa.s) WMC4 örneklerinde Casson modeli ile elde edilmiştir. Çikolataların reolojik ve duyusal özelliklerine etki eden partikül boyut dağılımı analizleri gerçekleştirilmiştir. D_{90} değerlerine göre 2 saat konçlanan çikolataların partikül boyutunun 6 saat konçlanan çikolatalara göre daha büyük olduğu görülmüştür. Konçlama süresinin artmasıyla doğrusal bir azalma gözlenmese bile süre sonunda tüm çikolatalar 2 saat konçlamaya göre daha düşük partikül boyutuna sahip olmuşlardır. SMC6 örneklerinin 35,70, WMC6 örneklerinin 25,55, SoMC6 örnkelerinin 24,30 ve SPC6 örneklerinin 60,35 µm partikül boyutuna sahip olduğu gözlenmiştir. Bu boyut azalmasının çikolatalara eklenen toz ürünlerde gerçekleştiği düşünülmektedir. SMC ve SPC örneklerinde konçlama ile partikül boyutu belirgin bir şekilde azalmasına rağmen 6 saat konçlama ile istenen boyuta inilememiş, diğer ürünlerde ise istenen partikül boyutu (<30 µm) elde edilmiştir. Bu sebeple partikül boyut dağılımı açısından SMC ve SPC çikolatalarının konçlama sürelerinin uzatılması önerilmektedir. Çikolataların akma gerilmesi, partikül boyutu, tekstürü birbiriyle ilişkili olarak bilinmektedir. Bu parametreler birbiriyle ilişkilendirildiğinde partikül boyutunun azalması ile tekstürünün daha yumuşak bir hale geldiği ve daha kolay kırılabildiği gözlenmiştir. Spesifik yüzey alanı verileri ise konçlama süresinin artması ile artmış, WMC6 örnekleri 1401 m^2/kg ile en yüksek değere sahipken, SPC2 örneklerinin 746 m^2/kg ile en düşük değere sahip olduğu gözlenmiştir.

Üretilen çikolataların kalitesini değerlendirmek açısından X-ray difraktometresi ile polimorfik yapısı belirlenmiş ve duyusal analiz gerçekleştirilmiştir. Analiz sonuçları, tüm çikolataların polimorfik yapısının istenen β kristal yapısında olduğunu göstermiştir. Duyusal analizler çikolataların ağızda kalan tat, görünüş, tat, koku ve

doku açısından istatistiksel olarak birbirinden önemli bir farkının olmadığını ($P>0.05$) göstermiştir. Bu durum, soyalı çikolataların sütlü çikolatalar kadar beğenildiğini göstermektedir.

Çikolataların fonksiyonelliği toplam fenolik madde miktarı ve toplam antioksidan kapasitesi açısından değerlendirilmiş, antioksidan kapasiteleri, ABTS, DPPH ve CUPRAC metotları ile belirlenmiştir. Bu yöntemler, ekstraksiyon aşamasına ihtiyaç duymayan QUENCHER prosedürü ile gerçekleştirilmiştir. Sonuçlar literatür verilerinden oldukça yüksek bulunmuştur. Toplam fenolik madde (TPC) analizlerine göre 80,10 mg GAE/g kuru örnek ile SPC6 tüm örnekler arasında en düşük, 129,33 mg GAE/g kuru örnek ile SoMC2 ise en yüksek değere sahiptir. Antioksidan kapasite yöntemleri arasından en yüksek sonuçlar SoMC4 örnekleri için 6,79 mM Trolox/g kuru örnek olarak ABTS yöntemi ile elde edilirkenen düşük sonuçar SPC2 örnekleri için 4,96 mM Trolox/g kuru örnek olarak DPPH yöntemi ile elde edilmiştir. SoMC örneklerinin en yüksek antioksidan kapasiteye, SMC örneklerinin ise en yüksek toplam fenolik madde miktarına sahip olduğu gözlenmiştir. Bu durum, soyanın toplam fenolik ve antioksidan madde miktarını artırdığı ancak fenolik maddelerin proses koşullarına duyarlı olduğunu göstermiştir. Yüksek antioksidan içeriğinin soyadaki izoflovanlardan kaynaklandığı düşünülmüştür. SPC örnekleri ise en düşük antioksidan aktivite ve toplam fenolik madde miktarı değerlerini vermiştir. Bu durumun soyadaki proteinlerin, fenolik maddeleri ve antioksidan karakteri maskelemiş olmasından kaynaklanabileceği düşünülmektedir.

Sonuç olarak çalışmada soyalı çikolata üretiminde soya protein izolatının yüksek miktarda protein katkısı sağlamasına rağmen reolojik özellikler ve fonksiyonellik açısından soya sütü tozunun kullanılması önerilmektedir.

Anahtar kelimeler: sütlü çikolata, soya sütü, reoloji, partikül boyut dağılımı, fonksiyonel özellikler

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ABBREVIATIONS AND SYMBOLS

Symbols

τ	Shear stress
τ_0	Yield stress
η_{pl}	Plastic viscosity
η_0	Apparent viscosity
γ	Shear rate
n	Flow index

Abbreviations

ABTS	2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)
BAPA	Nα-Benzoyl-DL-MF arginine 4-nitroanilide hydrocloride
CB	Cocoa Butter
CBS	Cocoa Butter Substituent
CBE	Cocoa Butter Equivalent
CBR	Cocoa Butter Replacers
CI	Carr Index
CUPRAC	Cupric Reducing Antioxidant Capacity
DPPH	2,2-diphenyl-1-picrylhydrazyl
EC	Emulsion Capacity
ES	Emulsion Stability
GAE	Gallic Acid Equivalent
HR	Hausner Ratio
LOX	Lipoxygenase
MF	Milk Fat
NSI	Nitrogen Solubility Index
OBC	Oil Binding Capacity
PDI	Protein Dispersibility Index
SMC	Skimmed Milk chocolate
SMP	Skimmed Milk Powder
SoMC	Soymilk Chocolate

SoMP	Soymilk Powder
SPC	Soymilk Powder
SPI	Soy Protein Chocolate
TAC	Soy Protein Isolate
TIA	Total Antioxidant Capacity
TPC	Tryipsin Inhibitor Activity
WHC	Water Holding Capacity
WMC	Whole Milk Chocolate
WMP	Whole Milk Powder

1. INTRODUCTION

Recently, there is an increase in food products responding to consumer's special diets and cultural or personal beliefs like organic, halal, kosher, vegetarian, vegan foods (Beckett, 2008, 2009). Researches on functional foods and new product development researches have an important role also in the confectionary market as a further trend (Afoakwa, 2010). Besides, cocoa products are recommended as a decent food medium for functional healthy ingredients (Milica Mirkovi´c, 2018). Chocolate rheological characteristics, flavour development and sensory perception designate the expected product quality which is important to meet the demand for chocolate products (Afoakwa, 2010). Quality of chocolate defines with its smell, taste, snap, shine, texture and melting smoothness in the mouth. These parameters depends on how the cocoa beans and chocolate processed (Sira, 2015). Refining and conching steps are vital steps to develop chocolate quality promoting its flavor and viscosity (Beckett, 2009).

Chocolate comprises monomeric (epicatechin, catechin) and oligomeric (procyanidin) polyphenols that has some benefits on body cells counteracting the deterioration caused by free radicals (Beckett, 2009). It may be useful to provide relevant information to consumers about certain resources and food products mostly available in the marketplace as an option to their peeference of foods (Afoakwa, 2010) due to the growing awareness about flavanol-rich products (Carla Di Mattia et al., 2014).

Soybean is a nutritious crop commonly used for food enrichment due to its rich nutritional content and good functional characteristics. The soybean products are frequently used in food sector as baking form, meat-extender or replacer, supplement of infant formulas etc. Today, there are many studies about the beneficial effects of soybean on human health. Soy proteins are known to provide hypo-cholesterolemic effect (Lovati et al., 1987). Isoflavones of soy have a protective effect on prostate cancer (Nagata et al., 2007) and bone loss (Xiao, 2008). Soymilk is a liquid extract from soybeans which is an alternative to dairy milk especially for individuals who exhibit allergic reactions to cow's milk or need

adequate proteins in their diets (Liu, 1997). Soymilk has some nutritional (isoflavones) and anti-nutritional (lipoxygenase enzymes and trypsin inhibitors) components. Heating process on soymilk focuses basically on these components. Lipoxygenases catalyze the oxidation of polyunsaturated fatty acids and also responsible the beany-flavour of soymilk (Engeseth, 1987). This enzyme need to be inhibited up to almost 99 % (Navicha, 2017). Trypsin inhibitors are known to inhibit the action of trypsin secreted from pancreas and prevent the digestion of proteins (Lusas, 1989). Trypsin inhibitors need to be eliminated up to almost 85 % (Navicha, 2017).

Soymilk is a potential ingredient for substitution of milk powder in food products with respect to providing high nutritious quality, lowering production cost and being an alternative for vegan and vegetarian diets. Chocolate is a very popular product for people of all ages. A new type of chocolate is constantly being introduced almost every day and many are accepted by consumers. Soy chocolate is a product accepted by vegan and vegetarian consumers and is produced by various brands in different countries. But it is important to specify the physico-chemical and functional properties of soy chocolate to identift the quality of final chocolate and production cost. Rheological properties of molten chocolate are characterized to provide superior quality chocolates with good-composed texture and are affected by ingredients and its refining and conching time.

2. GENERAL INFORMATION

2.1 Chocolate

Chocolate, a popular food product is one of the most consumed snacks by consumers of all ages worldwide. The name "cocoa" comes from Mayas who were the first to convert cocoa beans to the chocolate at about AC. 600. Archeological findings indicates that they were writing the name cocoa on their potteries (Coe and Coe, 2013). Aztecs of Mexico had cultivated the cocoa trees (*Theobroma cacao*) and produced a drink named 'Chocolatl' a long time ago (Beckett, 2009).

Chocolate entered Europe through Spain in the late 1600s. The Europeans used this product as a drink, too (Moss and Badenoch, 2009). They utilized chocolate as a medicine, considering that chocolate is a panacea for all human illnesses, the domain of the apothecaries and not the confectioners (Szogyi, 1997). In 1700s, milk was used as an ingredient for this drink (Beckett, 2009). The first factory-made chocolate is reported in 1780, from a factory near Barcelona (Szogyi, 1997). In 1828, Van Houten of Holland developed the fat extraction from cocoa and the solid chocolate is attributed to Daniel Peter from Geneva (Beckett, 2009). Invention of milk chocolate was a major development in chocolate industry, which is found by Daniel Peters of Switzerland in 1876. After the dairy milk chocolate produced, its popularity increased astronomically (Beckett, 2008; Minifie, 1989), and it is still the most consumed type of the chocolate in markets (Beckett, 2009).

Milk, white and bitter chocolate types appeared after solid chocolate was found. Afterwards, many other options started to come onto the market according to consumer demands. During this improvement period, the popularity of chocolate continuously increased and the need to understand the production process and some quality factors emerged.

The scientific definition of the chocolate is a semisolid suspension of solid cocoa particles in a continual fat phase (Afoakwa, 2010). Today, worldwide popularity of chocolate stems from the stimuli that activates the pleasure center of the human

brain during chocolate consumption, which provides a general sensation of satisfaction and happiness (Afoakwa, 2014).

According to Turkish Standards Institution (TSE), the types of the chocolate are determined depending on the percentages of dry cocoa solid, solid milk particles, milk fat and cocoa butter. Chocolate types are bitter, couverture bitter, milk, couverture milk, extra milky, skim milked, creamy, vermicelli and chocolate flakes (bitter or milk) and white chocolate (Turkish Standards Institution, 2010). As reported by Turkish Food Codex, chocolate should contain at least 43 % total cocoa dry matter and at least 26 % cocoa butter (Turkish Food Codex, 2017).

The products that do not correspond to the defined percentages of cocoa solid or cocoa butter cannot be called as "chocolate". Besides, when some vegetable oils were used more than the restricted levels, the product is no more chocolate. These types of products are called as compound coatings or chocolate-derived products. These type of products name as compound coatings or chocolate-derived products (Beckett, 2008; Lonchampt and Hartel, 2004; Toker et al., 2016; Turkish Food Codex, 2017). Praline is one of these products known as compound coating. As the Turkish Food Codex states, praline consists of any combination of white, milk and bitter chocolates with minimum 25 % of the weight of the product, or a combination of these with other edible substances or fillings (Turkish Food Codex, 2017). They are referred to as bite-sized products which can contrast with the texture and taste of chocolate (Sira, 2015; Turkish Food Codex, 2017). Cocolins are chocolate-like products containing vegetable fats, produced with mixture of palm stearin (solid part of palm oil) and palm olein (liquid part of palm oil). Cocolin is generated as an alternative to the conventional chocolate due to its lower cost. Compound chocolate is mostly produced for enrobing of some products like cakes, candies, wafers and dried nuts (Toker et al., 2016).

The most known and consumed chocolate types are shown on Table 2.1 with their ingredients (Codex Alimentarius, 2003).

Table 2.1 Codex standards for bitter, milk and white chocolate types

Products	Content (% dry matter)				
	Cocoa Butter	Fat-free cocoa solids	Total cocoa solids	Milk fat	Total milk solids
Bitter Chocolate	≥18	≥14	≥35		
Milk Chocolate		≥2.5	≥25	≥2.5-3.5	≥12-14
White Chocolate	≥20			≥2.5-3.5	≥14

2.2 Cocoa and Cocoa Bean Processing

Cocoa bean, the raw material of chocolate, is processed by cleaning the beans, removing the shell and some form of roasting. At the beginning, cleaning enforces for the separation of the impurities. The importance of this step is about preventing physical damages to the machines and spoilage of chocolate with contaminants. The roasting process affects the subsequent stages in chocolate production. It allows flavour precursors to turn into the chemicals responsible for the original flavor of chocolate, removes residual moisture, reduces microbial risk and facilitates the winnowing process. Winnowing is separation of the shell from the germ. Winnowed cocoa nibs are ground for two reasons; to obtain smaller particles, which are necessary for chocolate production and to separate the cocoa liquor and butter. Grinding is also helps to break water-oil emulsions and decreasing the moisture level helps to flow the liquor more easily. There is an alkalization step applied to cocoa liquor or powder, which aims to create the desired color and taste (Beckett, 2008).

2.3 Chocolate Ingredients

2.3.1 Cocoa mass

The first material of the chocolate production line is cocoa (chocolate) mass, also called cacao liquor, that contains the basic components of final chocolate flavor. It

is traditionally conducted by finely grinding of the cotyledons of cacao beans (cocoa nibs) after roasting and winnowing (Martin Jr, 1988).
Roasted ground nib is called as "cocoa liquor" or "cocoa mass" (Beckett, 2009). Liquor roasting is the part that the nib is ground finely to transform into a liquid form. This stage requires well-controlled moisture levels. At the high moisture levels, the liquor would be a thick paste or a liquid; and at the low moisture levels flavour precursors cannot produce the desirable compounds and cause a final product having a poor flavour (Beckett, 2008).

With increasing awareness of the challenge of the transporting cocoa beans, the countries of the cocoa producers started to produce their own cocoa liquor. To have more control of the final product, the chocolate manufacturer allows only to process the beans partially (Beckett, 2008).

2.3.2 Cocoa butter

The cocoa nib is the most valued fragment of the cocoa bean. Roasting and grinding the nib aim both reducing the particle size and separating the butter from the nib. The cocoa butter (CB) is the most valuable and costly ingredient of the chocolate (Beckett, 2008). Vegetable oils except cocoa butter can be used for provide new flavors, to improve the physico-chemical characteristics of the product or to decrease the cost of the production. Improper storage conditions or poor tempering processes cause oil migration and a product defect called "fat-blooming" (Afoakwa, 2010; Beckett, 2009). Addition of different oils to the system affects this deterioration. Therefore, type and quantity of the fat and process conditions must be selected carefully.

According to TSE, vegetable oils allowed to be used in chocolate should have some features. These oils can be single or mixed state and should be equivalent to cocoa butter. They should be rich in non-lauric, symmetric mono-unsaturated triglycerides; palmitic acid-oleic acid-palmitic acid, palmitic acid-oleic acid-stearic acid and stearic acid-oleic acid-stearic acid. They can be combined with cocoa butter in every amount and should resemble to its physical properties, such as melting point, crystallization temperature, melting rate and tempering requirement.

The vegetable oils should be obtained by raffination and/or fraction methods. Enzymatic procedures must be carried out to arrange the triglyceride structure. Additional vegetable oils cannot be other oils than allowed in the standards and cannot exceed 5 % of the final product. These are illipee, palm oil, sal, shea, kokum gurgi and mango seeds (Turkish Standards Institution, 2010). Chocolate products containing vegetable oil should have an indication on the label (Turkish Food Codex, 2017).

Fats in the chocolate coatings can be categorized according to their relevance with cocoa butter, under three major types: cocoa butter equivalents (CBEs), cocoa butter replacers (CBRs), cocoa butter substitutes (CBs) (Lonchampt and Hartel, 2004). CBEs are original fats, which are totally compatible with CB and can be added in any proportion to chocolate without causing significant hardening or softening. They have the TAG (triacylglycerol) composition similar to CB and the polymorphism same as CB. CBEs are obtained from some exotic fats such as illipee, borneo, tallow, shea with fractionation. CBRs are partially compatible with CB can only be used if all the cocoa butter is replaced (Beckett, 2008; Lonchampt and Hartel, 2004). Their conformity to CB is lower than CBEs but higher than CBSs. The main CBR source is hydrogenated or fractionated palm oil. CBSs are incompatible with CB which are known as lauric oils such as coconut and kernel oil (Lonchampt and Hartel, 2004).

2.3.3 Sugar

Sucrose (saccharose), which is a disaccharide composed by glucose and fructose, is produced from sugar beet or sugar cane. In unfermented cocoa beans sucrose is predominant while fermented cocoa beans contains mainly glucose and fructose which are known as reducing sugars. These reducing sugars react with amino acids via Maillard Reaction to form components responsible for chocolate flavour. Sugars can be crystalline or non-crystalline (amorphous) structure. Sucrose has a natural crystalline structure. Crystal sugar is birefringent as it possesses a structure such that it can bend the light in a polarizing microscope while amorphous sugar can not. Quick drying of sugar does not allow the sugar molecules to form a crystal structure. Amorphous form of sugar is important for

both flavour and flow properties of the chocolate. It can absorb other flavour materials; but when ground with chocolate, the last taste would be more intense. Still, the amorphous phase of the sugar is an unstable one, and in the presence of water the sugar will turn into crystalline form again (Beckett, 2008, 2009).

Storage conditions are important for the chocolate quality. These conditions should be determined by means of sorption isotherms. High humidity content may cause microbial risk, deformation of structure and deterioration. Even the humidity decreases, this deformation does not disappear and the lumps would occur with sticky structure(Beckett, 2008). High humidity storage or rapid transition from low to high temperature cause chocolate to sweat and dissolving the sugars. Surface moisture evaporates and creates a white appearance on the surface. This defect is called as "sugar bloom" (Afoakwa, 2010).

There is also an increasing interest in sugar-free products, which affects the confectionery sector. Bulk or intense sweeteners are used in the confectionary industry to lower the calorie of the chocolate and to make it possible for the diabetics to consume chocolate. There are plenty of other sugars and sugar alcohols (polyols) that can be used for sucrose replacement in chocolate in recent years (Beckett, 2008, 2009). Sugar alcohols like maltitol, isomalt, xylitol, lactitol, sorbitol and mannitol; intense sweeteners such as acesulfame K, aspartame, sucralose and steviosides were also used to modify sucrose-free chocolates (Cikrikci et al., 2017).

2.3.4 Milk Powder

Understanding the milk ingredient and its effect on chocolate processing is highly crucial. Milk fat (MF) becomes the part of the fat phase of chocolate and composed of mostly the complex triglycerides, which also act as a flavour precursor in chocolate. MF has different stable crystal forms than CB, which prevent mixing the two fats in the solid state and slows the crystallization of CB (Beckett, 2009). Crystallization of milk fat occurs a little faster than cocoa butter in despite of its triglyceride diversity; but the complex of the two of them crystallizes more slowly than CB does alone (Metin and Hartel, 2012). Additionally, MF also

affects the hardness, tempering ability and melting point properties of the chocolate (Beckett, 2008, 2009). Generally, increasing content of MF results in softer texture (Liang and Hartel, 2004; Timms, 1980). MF plays a role as texture modifier, bloom inhibitor and cocoa butter replacer in chocolate (Metin and Hartel, 2012). On the other hand it is also known that powders with different shape and characterization used in milk chocolate potentially lead to differences in stability to formation of blooming (Liang and Hartel, 2004). TSE indicated that milk fat should not contain any other animal fat (Turkish Standards Institution, 2010).

The milk fat influences rheological, sensory, texture and particle size distribution properties of the chocolate significantly. As the free milk fat level increases, the viscosity and yield stress decrease and the final product generally has reduced hardness. Particle size is another characteristic affected by milk powder depending its powder particle characterization (Liang and Hartel, 2004).

Lactose is an ingredient of milk chocolate coming from milk powder. It consists of glucose and galactose which makes it a disaccharide (Beckett, 2008). Lactose being a reducing sugar easily undergoes Maillard reactions with proteins and it also undergoes caramelization. These reactions are important for achieving the typical flavour of milk chocolate (Beckett, 2009).

2.3.5 Emulsifiers

Emulsifiers, surface active agents, decrease the interfacial tension between continuous and dispersed phases owing to their special molecular structure (Schantz and Rohm, 2005).

Lecithin is the most extensive emulsifier in chocolate producing since the 1930s. Lecithin, a kind of phospholipid, naturally present molecule and is generally obtained from soya (Sugano, 2006). The molecule has both lipophilic and hydrophilic ends to hold fat and sugar molecules together due to its natural emulsifier characteristic. While hydrophilic side of the molecule attaches the sugar, lipophilic side of the molecule attaches to the fat phase (Beckett, 2009). Hence, it

is used to modify the flow properties of molten chocolate, susceptibility to moisture and tempering characteristics (Schantz and Rohm, 2005).

Besides, there are other surface-active agents used in chocolate. One of them is synthetic lecithin (YN) obtained from rapeseed oil and shows similar effect with soy lecithin and constant composition. Polyglycerol polyricinoleate (PGPR) is another surface-active agent used in chocolate obtained by partial esterification of condensed castor oil fatty acids with polyglycerol (Schantz and Rohm, 2005). Sunflower lecithin, citrem (citric acid ester), sucrose esters are other emulsifiers used in chocolate industry (Beckett, 2009).

2.4 Chocolate Production

2.4.1 Mixing

Fundamental step of chocolate processing is weighing and mixing the ingredients. Cocoa liquor, cocoa butter, sugar, milk powder (depends on chocolate type) and lecithin are mixed thoroughly for 12-15 minutes at 40-50 °C (Beckett, 2009; Zoumas et al., 2000). Generally, the ingredients added after the melted cocoa butter and melted cocoa liquor was mixed. Cocoa butter is sometimes added to chocolate with dry and/or liquid phase during conching (Bordin Schumacher et al., 2009). Additional lecithin can also be added at conching step in order to homogenize the product if it is necessary (Bolenz et al., 2003).

2.4.2 Refining

The aim of the refining step is decreasing the particle size in the liquor to obtain smooth texture (Martin Jr, 1988). Refining crashes crystalline sugar, cocoa matter, and milk solids (Zoumas et al., 2000). Desired particle size is the actual output of the process, especially for sweet and milk chocolates (Martin Jr, 1988). Particle size affects the final sensory and rheological properties. Besides the particle size reduction, refining also provides agglomerated particle distribution, fat coating (Beckett, 2009) and accomplished emulsification (Martin Jr, 1988). Chocolate becomes thicker by grinding. Because, the more amount of fat is required to coat

the particles, so that they can move past one another in the liquid chocolate (Beckett, 2009).

By refining process, the size of the particles reduces to less than 30 μm and crystalline sugar surfaces turned into an amorphous phase. Chocolate with large particle sizes (50 μm) cause larger pores, and crevices filled with fat are present. On the other hand, smaller particle sizes (18 μm) is more agglomerated (Delbaere et al., 2016).

There are two ways to grind the ingredients; fine (separate) or combine milling. The fine milling is conducted with only non-fat, solid ingredients preliminarily. After all the solid ingredients are milled, cocoa mass, cocoa butter and other liquid ingredients are added. The combine milling is carried out with melted chocolate including cocoa liquor and butter. Each method has some advantages and disadvantages. The fine milling method provides a more controlled particle size reduction. On the other hand, it will take a longer time to cover these particles with fat in the conching step. Combined method provides more intense chocolate flavor because the amorphous sugar pick up the closest aroma to itself. Milk powder is difficult to break because of its elastic character. Hence, it requires much longer time on grinding step (Beckett, 2008). Different types of refining equipment can use depending on refining ways. Roll refiners and ball mills are mostly known types of refiners. Hammer mills and classifier mills are used for fine milling (Beckett, 2008). Refining usually precedes the conching step (Martin Jr, 1988).

2.4.3 Conching

Conching is one of the steps that develop chocolate quality with removing undesirable acidic flavor and odor while retaining the desirable ones. Besides, it decreases the moisture level, coat the particles with fat and distributes the agglomerated particles which are gathering with presence of moisture or amorphous sugar (Beckett, 2009). This step contributes to the development of viscosity, final texture and flavor (Afoakwa, 2014). Removing the moisture and coating the particles with fat ease the flowability of the particles, so the liquid chocolate end up with lower viscosity (Beckett, 2009).

Conching process is carried out in 3 steps as dry, pasty and liquid phases. In the dry phase, components are warmed, mixed and aerated to evaporate the excessive water (milk chocolate contains an excess of moisture and undesired acids originated from the fermentation) (Beckett, 2008)) and undesired acids originated from the fermentation. In the pasty phase, melted cacao butter is added and the flavour of chocolate is generated. In the final liquid phase, particles are overlaped with fat which will outcome in a decrease of viscosity (Bordin Schumacher et al., 2009).

In some cases, especially for lab-scale or small-scale chocolate production, refining and conching processes are carried out together using a drum with a serrated internal surface. At the beginning of this process, the particles quickly shrink by compressing between the inner surface and the scrapers. However, the probability of capturing the large particles decreases with time. As a result of this, the particle size distributes in a wide-range (Beckett, 2009). This type of equipment is also known as "chocolate melanger".

2.4.4 Tempering

Different crystal packing configuration of molecules is called as polymorphism. Fats rich in symmetrical mono unsaturated fatty acids, such as cocoa butter, are highly polymorphic. Traditionally, cocoa butter polymorphs are known to occur 6 different forms (Beckett, 2009). Chocolate is tempered to attain the Form V (β_2) from all cocoa butter polymorphic forms. This form is the most desired one due to providing shiny appearance, good snap and endurance to bloom (Afoakwa, 2014). Unstable forms transform into stable forms by tempering. This process is crucial for the final product to be high in quality (Afoakwa, 2010).

Traditionally, chocolate used to be hand-tempered with marble tables to control the temperature. Today, tempering machines carry out this process. Tempering involves 3 stages; heating (50 °C) for complete melting, cooling (27 °C) for crystallization and reheating (30 °C) for melting the unstable forms (Afoakwa, 2014; Beckett, 2009). In fact, the temperatures of the tempering stages depend on

the fat phase composition. Milk chocolate needs lower temperatures to get tempered with respect to bitter chocolate. However, there is no unique condition for tempering of a certain chocolate. Different combinations of temperature and flow rate can result in a well-tempered chocolate (Beckett, 2009). Temperatures for milk chocolate are shown in Figure 2.1 according to Beckett, 2009.

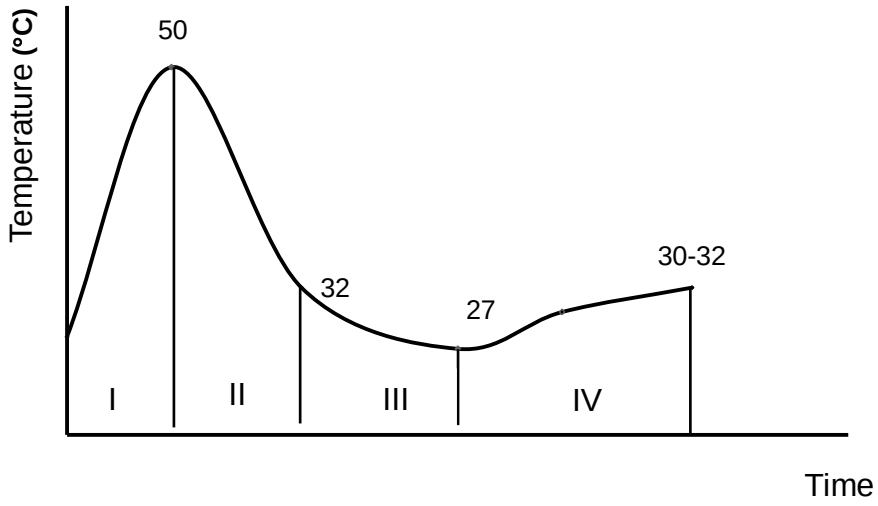


Figure 2.1 Tempering sequence for milk chocolate

As shown in Figure 2.1, in the first area, all crystals are melted. In the second one, the sensitive heat is removed but the no crystal type is formed. Both the stable and unstable crystals are formed in the third area and lastly in the forth one, the unstable crystals are melted for leaving only the stable ones.

From Form I to Form IV, butter crystallizes in a double chain form while Form V and Form VI (β-forms) crystallizes in a triple-chain. Triple-chain system has greater thermal stability due to its tight packing. The characterization of chocolate can be measured by X-ray diffraction (Afoakwa, 2010).

2.4.5 Moulding

After tempering, chocolate is moulded or enrobed before packaging. Moulding gives an obvious gloss, whilst enrobing meet with complex shapes and gives a soft structure to the finished product (Beckett, 2009).

Liquid chocolate is moulded and agitated slowly to let the bubbles out. In factories, the moulding, shaking and demoulding processes are performed automatically (Afoakwa, 2014). The bubbles should be removed as soon as possible. Moulds should not be too hot or cold to prevent detempering (Afoakwa, 2014).

2.5 Quality of Chocolate

Cocoa processing techniques and chocolate manufacturing strategies affect the quality of final product. Quality of chocolate is mostly determined with rheological and sensory properties. Flow behavior and viscosity are determined by rheological measurements and this measurement is influenced by some parameters such as particle size distribution and polymorphic structure. Sensory evaluations such as color and hardness can be made by both objective and subjective analysis which are major factors for consumers. Parameters that affect the rheology, also affect the sensory properties of the product (Afoakwa, 2010).

2.5.1 Physicochemical Properties of Chocolate

2.5.1.1 Rheological Properties of Chocolate

The 'Rheology' term was first originated in United States at 1929. The rheology science explains the flow and deformation of materials (Barnes et al., 1989; Harrison, 1940). The resistance of a liquid to flow is known as viscosity. Viscosity is the most important fluid property about the material response (Barnes et al., 1989). It is crucial to control the chocolate viscosity to obtain good quality products, especially to conduct moulding, coating, enrobing processes properly. Processing parameters such as refining and conching affect the viscosity of chocolate (Servais et al., 2003).

The true liquids, which are known as Newtonian fluids, are not affected by stirring, (Harrison, 1940). Molten chocolate is affected by mixing and is therefore called as non-Newtonian fluid. The viscosity of molten chocolate is generally measured by rotational equipments like concentric cylinder rheometers. Viscosity of chocolate is influenced by some parameters; fat content, emulsifiers, water content, conching

time, particle size distribution, temperature exposed on tempering (Chevalley, 1975).

The materials having solid and liquid character at the same time, are called as "plastic" such as molten chocolate. Plastic viscosity of chocolate is fundamental for processing conditions like enrobing, moulding and filling in rough surfaces. For rheological models, there are yield stress and plastic viscosity terms that we should to take into account to determine the behavior of molten chocolate. The yield stress value is the force required for material to start flowing. If the energy does not surpass the yield stress, the flowing does not start or if the material is flowing, it stops (Seguine, 1988). Casson, Bingham and Herschel-Bulkley models all have yield stress values (Steffe, 1996). Plastic viscosity is a measure of how easily the material flows once it starts to flow (Seguine, 1988). Apparent viscosity (η_0) can be obtained by dividing the shear rate to shear stress. For Newtonian fluids, apparent viscosity is constant while it depends on shear rate for non-Newtonian fluids (Steffe, 1996).

The rheology of molten chocolate is extremely important to define pumping, mixing and transporting characteristics. It is influenced by fat content, ingredients, grinding (particle size distribution) and production parameters (Seguine, 1988). International Office of Cocoa, Chocolate and Confectionary (IOCCC) recommends the use of concentric cylindrical systems (cup and bob) and Casson equation for the characterization of the rheological properties of molten chocolate. IOCCC recommends to take measurements at a shear rate of 2 to 50 s^{-1} (Afoakwa et al., 2009).

Chocolate is a suspension that non-fat particles dispersed in cocoa butter. Milk chocolate also contains milk solids besides other non-fat particles and milk fat with cocoa butter (Chevalley, 1975), which also known as "electrorheological fluid" (Steffe, 1996).

Bingham, Herschel-Bulkley and Casson models are the most commonly used models to define chocolate flow behavior. Equations of models are shown in Table 2.2.

Table 2.2 Models defining the molten chocolate behavior

Models	Equations
Casson model	$\tau^{0.5} = {\tau_0}^{0.5} + {\eta_{pl}}^{0.5} * \gamma^{0.5}$
Bingham Model	$\tau = \tau_0 + \eta_{pl} * \gamma$
Herschel- Bulkley model	$\tau = \tau_0 + \eta_{pl} * (\gamma)^n$

Here, τ_0 represents the yield stress, while η_{pl} is plastic viscosity, τ is shear stress and γ is shear rate (Joye, 2003).

Casson Model

Casson model was defined in 1957, and this model suggested to define the behavior of molten chocolate. In 1960, this model was adopted by the International Office of Cocoa, Chocolate and Confectionary (IOCCC) as the official method for the measurement of chocolate viscosity (Chevalley, 1975).

Casson suggested that the flow properties of dyes were determined by suspended particles in the oil. The attractive and driving forces formed between them cluster these particles. These clusters are disintegrated in large proportions by shear stress. When the sliding speed is sufficiently high, these clusters are separated into particles completely. In order to define this model mathematically, these clusters are likened to rotating bars which are proportional to the shear rate (Casson, 1959).

The Casson model defines non-Newtonian fluids with yield stress. It is considered as a non-linear Bingham model. Although this model was originally developed for cylindrical particles, it can express the rheological properties of many plastic fluids regardless of particle types As a result of these assumptions, the Casson equation was formed as follows (Joye, 2003);

$$\tau^{0.5} = {\tau_0}^{0.5} + {\eta_{pl}}^{0.5} * \gamma^{0.5} \quad (1)$$

τ : shear stress (Pa)

γ : shear rate (s^{-1})

τ_0 : Casson yield value (Pa)

η_{pl}: Casson plastic viscosity (Pa.s)

The model constants, shear stress (τ) and shear rate (γ), are obtained from Casson plot. The intercept and slope are used to acquire yield stress (τ_0) and plastic viscosity (η_{pl}) respectively (Joye, 2003). It is stated that the Casson model is suitable for many types of chocolate (Chevalley, 1975). Because it gives an appropriate characterization of chocolate handling properties (Seguine, 1988). Casson considered as a non-linear Bingham model (Joye, 2003).

Bingham Model

Bingham equation is the first model to define the flow behavior of molten chocolate (Chevalley, 1975) due to its yield stress value (Tscheuschner and Wunsche, 1979). The bingham fluids defines with plastic viscosity and yield stress as shown;

$$\tau = \tau_0 + \eta_{pl} * \gamma \quad (2)$$

τ : shear stress (Pa)

γ : shear rate (s^{-1})

τ_0 : Bingham yield value (Pa)

η_{pl}: Bingham plastic viscosity (Pa.s)

Herschel-Bulkley Model

Herschel-Bulkley model have proven to be useful to solve the engneering problems like Casson model (Steffe, 1996). This model can also be used to determine the flow behaviors of molten chocolate, with an additional parameter (n);

$$\tau = \tau_0 + \eta_{pl} * (\gamma)^n \quad (3)$$

τ : shear stress (Pa)

γ : shear rate (s^{-1})

τ_0 : Herschel yield value (Pa)

η_{pl}: Herschel plastic viscosity (Pa.s)

n : flow viscosity index

Generally, the n value ranges between 0 to 1.0. For chocolate (Steffe, 1996), n values are known to range between 0.5-0.7 mostly. n=1 means a fluid shows an ideal flow, n<1 shows the plastic flow and n>1 shows the dilatant flow (Chevalley, 1975). When 0<n<1.0 the apparent viscosity would decreases by increasing shear rates. On the other hand, when n>1.0 it would be the opposite (Steffe, 1996).

2.5.1.2 Particle Size Distribution

Size reduction starts after the fermentation of cocoa in order to facilitate the winnowing step and continues with nib grinding to obtain cocoa liquor. The smallest particles are achieved with refining step (Afoakwa, 2010). Particle size has a considerable effect on both texture and flavor of the final product (Zoumas et al., 2000). Since the particle size distribution (PSD) influences the viscosity significantly, it is considered as a key parameter for the rheology (Afoakwa, 2010). When all other conditions are constant, the apparent viscosity and the yield value of chocolate increases as the particle size decreases, since the smaller particle size means the larger surface area (Chevalley, 1975).

Practically, particle size distribution is stated as the percentage of particles below a significant particle size. A diameter is used to indicate the larger particles below which 80-90 % of all the particles may be represented. The final particle size needs to be determined whether a certain particle size range is obtained with ideal smoothness (Martin Jr, 1988). Particle size below 30 µm is desirable for chocolate. If the size is higher than 30 µm, it brings a gritty sense (Afoakwa, 2010). Particle sizes below 20-25 µm cannot be recognized by the tongue (Martin Jr, 1988). Particles below 20 µm is known to result a silky texture on mouth (Afoakwa, 2010).

In practice, particle size distribution is stated as the percentage of particles below a significant particle size. A diameter is used to indicate the larger particles below which 80-90 % of all the particles may be represented (Martin Jr, 1988).

Particle size distribution is highly substantial to determine the entire quality of sweet and milk chocolate (Zoumas et al., 2000). Consistency of molten milk chocolate is managed by particle size distribution, as it is a solid-liquid suspension. In milk chocolate production, plastic viscosity is important for pumping and mixing characteristics and yield value is important for moulding and forming (Mongia and Ziegler, 2000).

Previous studies, in which particle size distribution analysis was conducted, oil (Glicerina et al., 2014) or water (Zhao et al., 2018) were used for dispersing the chocolate. Zhao et. al. (2018) stated that as the grinding time is long then the particle size is small. According to them, 10 hours grinding time resulted in particles below 20μm, which means the silky structure was obtained. Particle size also affects the matrix of particles and liquid oil migration.

Afoakwa et al. (2008) concluded that particle size distribution is the major factor to determine the molten chocolate rheology. As the particles become finer, plastic viscosity values increase due to increasing particle numbers. The reduction of particle size also causes increasing Casson plastic value, Casson yield stress and apparent viscosity.

2.5.1.3 X-ray Diffraction Method

Fat blooming, which is characterized by a grayish haze on chocolate surface is the most common quality defect in chocolate. Improper tempering and poor storage conditions cause re-growth of the unstable crystal forms, which initiate the fat migration (Timms, 2003). Bloom formation is also feasible in systems, where triglycerides of various types are mixed. In the presence of liquid fats, the transformation of crystals from Form V to Form IV accelerates (Cebula and Ziegleder, 1993). The crystalline types of chocolate are determined with X-ray measurement. Fat is not the only component having crystalline structure. Sugar is

also in crystalline form and need to be removed before X-ray diffraction measurements, to detect only the fat crystals.

Cebula and Ziegleder (1993) found that addition of 2 % and 5 % milk fat to bitter chocolate did not affect the X-ray patterns (Cebula and Ziegleder, 1993). It is also stated that before cocoa butter is able to tolerate about 30 % milk fat without making any significant change on its polymorphism (Timms, 1980). Another study investigated the effect of the different amount of CBS on polymorphic structure of cocoa butter. Althoug the maximum amount of CBS that is allowed to add in chocolate is only 5 %, up to 20 % amount of CBS did not affect the polymorphic structure of cocoa butter (da Silva et al., 2017).

2.5.1.4 Hardness of Chocolate

The measurement of textural characteristics of chocolate is important for the evaluation of the quality and optimization of the manufacturing process. The hardness (snap/fracturability) values of different chocolates can be measured by a texture analyzer having three-point bend probe (Beckett, 2008). Different size steel balls were used in the previous instrumental measurements (Tscheuschner and Markov, 1986).

It is known that the milk components make the texture of chocolate creameier. Therefore, the change its snap while they inhibit the blooming. The particle size of the chocolate also affects the texture. If the chocolate has the maximum size of particles as 20 μm, the product's texture would be silky. On the other hand, it would be gritty when there are larger particles than 30 μm (Beckett, 2008).

2.5.1.5 Color of Chocolate

The Hunter L, a, b system, Munsell color solid system and Commission International de l'Eclariage (CIELAB) system are the color measurement methods for foods. The first of three-dimensional color measurement systems is the CIELAB system. The CIE system enables the objective measurement of the color and therefore, it is the most common method. This system defines, L*, a*, b*

values where the L* represents luminance from 0 (black) to 100 (white); a* represents the color from green to red and b* represents the color from blue to yellow. (Briones et al., 2006; Raoufi et al., 2012).

There are also whiteness index, chroma and hue angle parameters defined for color measurement. Whiteness index (WI) is used to detect the surface whiteness caused by blooming definition for chocolates. Saturation is the strangeness or weakness of the visually perceived color. Chroma (C*) value is determined as the index of color saturation or intensity which is calculated in terms of the distance of L* axis in the a* and b* plane. Hue angle (h°) is defined as the angle between the hypotenuse and the 0° on the a* axis (McGuire, 1992). Hue and chroma values are known to provide functions more closely associated with human perception (Weatherall and Coombs, 1992).

Raofi et al. (2012) declared that chocolates with fine particles appears lighter and more saturated and gives higher C*, L*, and WI values. It is also stated that when the moisture content exceeds 5 %, the color of chocolate would be darker. PGPR causes fluctuation of the color.

2.5.2 Sensory Properties of Chocolate

Chocolate is mostly consumed for its superb sensory properties. These properties such as taste, aroma and texture are characterized during production (Hoskin, 1994). The appearance of chocolate is evaluated according to whether it has rich color, blooming or smooth surface. Bloomed chocolate is considered as a poor quality product. High-quality chocolate should have shiny and smooth appearance without any "off-taste" and "off-aroma". Breaking with a good snap without any grittiness is also a quality parameter for a chocolate (Hoskin, 1994). Instruments can evaluate texture and color, objectively. Besides these properties can also be evaluated by sensory analysis with trained or un-trained panelists, subjectively. Sensory analysis should be as objective as possible. However, it only provides a single facet of data about the sample (Mongia and Ziegler, 2000).

Cıkrıkcı et.al. (2017) investigated the sensory properties of sugar-free dark chocolates produced with sweeteners and bulking agents instead of sucrose. They stated that the chocolates prepared with partial stevia had acceptable scores which were close to the control chocolate. Cebula and Ziegleder (1993) stated that addition of milk fat to bitter chocolate resulted in a softer texture (Cebula and Ziegleder, 1993). Komes et.al. (2013) indicated that enriched chocolates such as milk chocolate with dried prunes and dark chocolate with raisins received poor values while plain dark and milk chocolates received high scores according as sensory evaluation tests.

2.6 Functional Properties of Chocolate

Plant derived flavanoids act as antioxidants and they have radical scavenging and free radical reducing properties (Afoakwa, 2010). Recent studies stated that flavanoid-rich food products like chocolate, show beneficial health effects such as blood pressure lowering and cardio-protective effects (Cooper et al., 2008). Cocoa phenolics are considered as chemo-preventive agents due to their high antioxidative characteristics (Carnésecchi et al., 2002). Total phenolic content of cocoa bean is estimated as 12-18 % by dry matter. Approximately 60 % of these phenolics are procyanidin, catechin and epicatechin (Zzaman et al., 2014).

Flavanols (flavan-3-ols or catechins) are compounds that are the subclass of flavanoids and flavanoids are the subclass of polyphenols (Figure 2.2). Flavanols can be found as monomeric forms which are (-)-epicatechin and (+)-catechin. They also can also be found as dimeric forms or polymeric combinations known as procyanidins (Cooper et al., 2008). Catechin and epicatechin are easily absorbed in body due to its molecular size and they are more active as biologically (Zzaman et al., 2014).

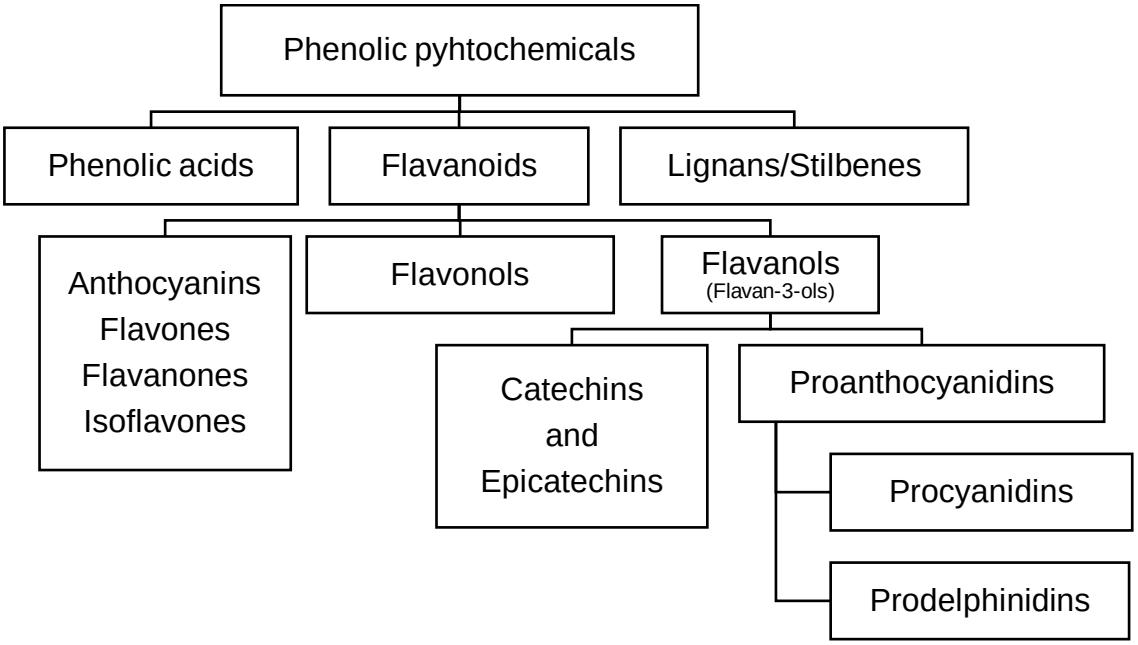
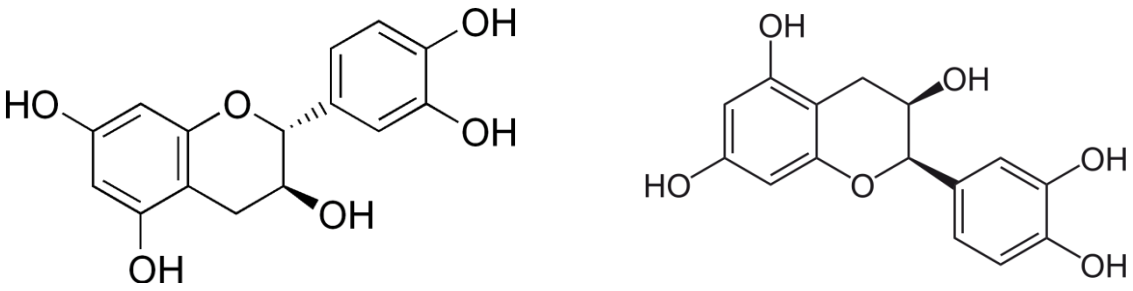


Figure 2.2 Classification of phenolic phytochemicals (Bueno et al., 2012)

Anthocyanins are responsible for purple color of cocoa beans while catechin and epicatechin are colorless. Molecules containing subunits smaller than three are soluble and they cause astringency in cocoa. Oxidation, enzymatic browning or exudation during fermentation reduces soluble polyphenols. This reduction causes the astringency to turn into the original chocolate flavor and the purple color to brown color. Fermentation also causes production of free amino acids via enzymatic hydrolysis. These free amino acids and reducing sugars are also flavor precursors of chocolate (Beckett, 2009). Reducing sugars in cocoa beans react with amino acids during Maillard reaction to form many of the components found in chocolate flavor (Martin Jr, 1988).



(a) (b)

Figure 2.3 Structure of the (a) (+)-catechin and (b) (-)-epicatechin

The antioxidant character of chocolate comes from cocoa ingredient. As a result of that, milk chocolate has lower antioxidant character than bitter chocolate (Cooper et al., 2008).

2.5.1 Total Phenolic Content of Chocolate

One method for determination of the content the phenolics in foods was first found by Folin and his colleagues at Harvard Medical School as a colorimetric method. The method is based on the specification of the color intensity by a spectrophotometer. Folin and Ciocalteu suggesting a new reagent modified this method as Folin-Ciocalteu. A standard curve is used to relate the absorbance with concentration in this method. Generally, gallic acid (Martini et al., 2018) and catechin (Brcanovic et al., 2013) are used to generate the curve. Results are given as Gallic acid equvalent or catechin equivalents (Vermerris and Nicholson, 2007). Folin-Ciocalteu method is still the most common method to determine total phenolic content of foods.

Phenolic content was determined before gravimetrically (Selamat et al., 2002) and spectrophotometrically (Brcanovic et al., 2013) in cocoa and its products. Unfermented cocoa was found to have 120-180 g/kg polyphenolic content with gravimetric method (w/w) (Selamat et al., 2002). Spectrophotometric methods express the result as Gallic acid equivalent/g sample or Catechin equivalent/g sample.

A previous study showed that the cocoa bean processing caused the decrease of the total phenolic content. According to the analysis, the total phenolic content of cocoa bean is 9.64 mg/g, but the alkalized cocoa powder has 3.74 mg/g total polyphenolic content (Jolić et al., 2011). According the study by Komes et.al (2013), the addition of dried fruits to chocolate caused an increase in the total polyphenolic content (TPC) of final product. The enrichment of chocolate resulted in the increase of TPC. The bitter chocolate with dried prunes was found to have 6.08 mg/GAE while the plain bitter chocolate has 4.60 mg GAE/g total phenolics. TPC of the milk chocolate increased from 2.12 mg GAE/g to 2.34 mg GAE/g by the addition of dried cranberries. The study also mentioned that the milk

chocolates had lower values of total polyphenolic content due to lower content of cocoa liquor.

2.5.2 Antioxidant Capacity of Chocolate

Oxygen is a vital molecule for energy production in living cells. Reactive oxygen and free radicals are formed during normal cellular metabolism in living organisms. Smoking, air pollution and ultraviolet light also cause formation of reactive oxygen and free radicals. Radicals are highly unstable and sensitive molecules, thereby their electrons interact with other molecules in the cell and produce oxidative stress. Oxidative stress occurs due unbalanced antioxidant and pro-oxidant levels. Oxidative stress products trigger various diseases such as Alzheimer and Parkinson (Aitken et al., 1995).

Antioxidants have beneficial effects due to transferring a hydrogen atom or an electron to reactive species. Owing to this act of antioxidants, the antioxidant capacity methods divided into two basic methods, which are Hydrogen-atom transfer (HAT) and Electron transfer (ET) methods. HAT-based methods mostly utilize kinetic reactions based on competing antioxidant and substrate for peroxyl radicals. ET methods are based on color change by virtue of oxidant reduction ability of antioxidant. TRAP (total peroxyl radical trapping antioxidant parameter, ORAC (oxygen radical absorbance capacity) are included to HAT methods while ABTS (2,2"-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) and DPPH (1,1-diphenyl-2-picrylhydrazyl) are included to ET methods (Apak et al., 2016).

2.5.2.1 CUPRAC Assay

CUPRAC (CUPric Reducing Antioxidant Capacity) is one the assays used to determine total antioxidant capacity. Copper(II)–neocuproine (Cu(II)–Nc) reagent is used for this method, which is cheap, easily accessible and suitable almost for all antioxidants. This method based on Neocuproine-Cupper(II) complex to reduced to Neocupraine-copper(I) in precence of antioxidants. The reduced component gives maximum absorbance at 450 nm which consist of Neocuproine (2,9-dimetil-1,10-fenantrolin) and Copper(I) (Apak et al., 2004).

2.5.2.2 ABTS Assay

TEAC (Trolox Equivalent Antioxidant Capacity)-ABTS method is another assay to determine total antioxidant capacity of foods. The assay utilizes the hydrogen transmitter antioxidants to decrease the absorbance of ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) which is a chromogen radical cation. Total antioxidant capacity is expressed in terms of Trolox Equlivalent with help of the standard curve (Miller et al., 1993).

2.5.2.3 DPPH Assay

DPPH (1,1-diphenyl-2-picrylhydrazyl) is a stable free radical. This method is based on measuring the scavenging effects of antioxidants on the DPPH radical. The radical solution is prepared with solving it in methanol or ethanol. This purple colored radical interacts with hydrogen donors and it is reduced to hydrazine. With the addition of antioxidant to the DPPH radical solution, the absorption decreases and the color changes from purple to yellow in the presence of antioxidants. This method is known as an easy and valid method to evaluate the radical scavenging ability of antioxidants (Sánchez-Moreno et al., 1998).

The Antioxidant Capacity Studies From Literature

A previous study stated that plain bitter chocolate has 4.55 mmol Trolox/L and milk chocolate has 2.32 mmol Trolox/L when the extract obtained with acetone. On the other hand, enriched chocolates containing dried fruits did not contribute to the total antioxidant capacity of chocolate. It was demonstrated that cocoa and cocoa derived products has marvelous antioxidant properties. The study also concluded that acetone is a better solvent than methanol and water (Komes et al., 2013). Another parameter affecting the antioxidant capacity of chocolate is the process conditions. According to Gultekin-Ozguven, the antioxidant capacity of final chocolate decreased as temperature and pH increased during production. The study also confirmed the conching process did not affect the antioxidant capacity significantly (Gultekin-Ozguven M., 2016). Another study investigated whether the antioxidant capacity change during the storage or not. Results showed that there

was no significant difference between the antioxidant capacity values of the fresh product and the stored product at 20 °C for 180 and 360 days (Laličić-Petronijević et al., 2016).

The QUENCHER Approach

The measurement of total antioxidant capacity is useful to understand health potential of the foods. Although there are several described assays for total antioxidant capacity analysis, there are many opposing opinions about them. It is unclear which chemical mechanism is suitable, which functions are physiologically suitable and whether they are sensitive, precise, effortless, reproducable and cheap. Antioxidants can be soluble, semi-soluble or insoluble. The food matrix contains these types of antioxidants together and there is no unique solvent to solve all in once (Gökmen et al., 2009). To overcome this problem, Gokmen et al. (2009) suggested a new approach for measure the total antioxidant capacity without extraction stage. The new method called 'QUENCHER' because of it is QUick, Easy, New, CHEap and Reproducible method. The specifically exemplified for ABTS and DPPH methods and stated that it also applicable for FRAP (ferric reducing antioxidant power), DMPD (*N*,*N*-dimethly-*p*-phenylenediamine dihydrachloride) and ORAC (oxygen radical absorbance capacity) methods. It is also demonstrated that QUENCHER method is practicable for CUPRAC method (Tufan et al., 2013). In previous studies, QUENCHER method has been reported to be useful in establishing the correlation between the databases that determine antioxidant activity of diet and some disease markers. Although many methods are used to determine antioxidant activity in foods, it is known that there are some doubts about the suitability of chemical mechanisms, physiological suitability of measured function, sensitivity, reproducibility and cost (Gökmen et al., 2009).

Studies dealing with antioxidant measurements assumes that different extraction methods represent the true antioxidant capacity of food. These foods, however, contain complex antioxidant compounds which are completely soluble or insoluble in the solvent. There is no solvent type that can completely dissolve these antioxidant compounds embedded in the whole food structure. The chemical hydrolyses used to overcome this problem changed the structure of food, resulting

in extracts which could not represent the actual amount of antioxidants after digestion or storage. In order to avoid the problems mentioned, the spectrophotometric measurement method, which is known as QUENCHER method, suggests to mix free radicals directly with solid sample (Gökmen et al., 2009).

2.6 Enrichment of Chocolate

European Consensus Document defines the functional foods as a food that has beneficial feature on one or more target functions in human body, improve the health and decrease the risk of disease beyond its adequate nutritional effects (Diplock et al., 1999). According to American Dietetic Association, functional foods are modified foods or food ingredients that may improve the body health beyond its natural nutrients it (Bloch and Thomson, 1995). Recently, polyphenols are in demand due to their high antioxidant capacity and potential health benefits (Wollgast and Anklam, 2000).

Some ingredients are added to chocolate to increase its functional and/or nutritional properties to meet some special needs of consumer diets (lactose-free, vegan, gluten-free etc.). There are various studies about enrichment of chocolate in the literature. A previous study mentioned that chocolate is a good food matrix for typical dairy probiotic products. Dark chocolate enriched with microencapsulated probiotic bacteria provides high protection to bacteria with good sensory and compositional characteristic (Mirković et al., 2018). In another study, dark chocolate enriched with turmeric and green tea. As result of this enrichment, total phenolics of chocolate increased (Martini et al., 2018). Milk chocolate is enriched with inulin, one of the bulk sweeteners. Results show that final product has acceptable sensory properties with low calorie (Konar, 2013).

Soy products are commonly used for enrichment due to their rich nutritional content and good functional characteristics. The raw full-fat soy flour is known to be a great form for baking, textured soy flour is used as meat-extender or replacer, soy protein concentrate is handled for infant formulas, soy protein isolate is

employed for meat or dairy alternatives and soymilk is utilized as cow's milk substitute with growing attention (Golbitz and Jordan, 2006).

2.7 Soybean (*Glycine max*)

For almost 5000 years, Chinese people utilize soybean (*Glycine max*) as an important source of nutrition. Ancient writings, which date back to B.C. 2800s, mentioned soybean as one of the divine grains for Chinese civilization. The use of soybeans from the Asian continent in food was at the beginning of the last millennium. In 1800s Asians began to emigrate to Europe and North America, and they introduced the soybean and its products starting from their neighborhoods (Golbitz and Jordan, 2006). In Turkey, the cultivation of soybeans has started with the encouragement of the Ministry of Agriculture and Rural Affairs, and sugar factories in 1980s (Oner, 2006).

2.7.1 Nutritional Aspects of Soybean

Soybean contains valuable components, which are almost totally disclosed chemically. These components have nutritional and physiological functions and almost all of them have beneficial health effects as characterized by the preventive potential for life-style-related (adult) diseases (Gupta, 2012). Soybeans contain about 35-40 % protein, 15-20 % fat, 30 % carbohydrates (17 % dietary fiber (Sugano, 2006)), 10-13 % moisture, and around 5 % minerals and ash (Peter Golbitz, 2006) depending on growing location, season, and environmental stress (Sugano, 2006).

Soybean has a premier position among all agricultural crops mainly by being a source of high quality proteins (Gupta, 2012). Soy proteins, which are acceptable for almost all diets, provides an alternative to animal proteins as they are lactose and cholesterol-free (Riaz, 2006).

2.7.2 Nutritional and anti-nutritional components of Soybean

Present studies indicate that soy foods and isoflavones have some good effects on human health. Soy proteins provide hypo-cholesterolemic effect by activating liver low-density lipoprotein receptors (Lovati et al., 1987). Tofu consumption, at least once a week, related with 15 % lowering the breast cancer risk for women (Wu et al., 1996). It is proved that isoflavones show a protective effect on prostate cancer (Nagata et al., 2007). Studies also support the positive role of soy consumption in the prevention of bone loss (Xiao, 2008).

2.7.2.1 Isoflavones

Isoflavones are called phyto-estrogenes because of their estrogen-like structure and effect. Soybeans contain three type of isoflavones as four chemical forms: aglycon forms (daidzein, genistein and glycitein), glucoside forms (daidzini genistin and glycitin), acetylglocoside formss and molanylglucoside forms. It is known that they possess antifungal and antioxidant activity (Wang and Murphy, 1994). Potential adverse effects of soybeans have been increased with data indicating that soy proteins and isolates stimulating tumor growth in rat having estrogen-positive breast cancer cells. But these data are not supported for humans. Hence, data do not reflect situation in pre- or postmenopausal women body. Datas also recommended neither soy nor isoflavones affect on thyroid action in healthy adults which has been investigated for 70 years. Even the patients of thyroid do not need to avoid from soyfoods (Messina 2006).

Soybean variety, processing conditions and additives affect the isoflavone content of the final product. Heating and soaking process is important variables causing the losses of the isoflavone profile. A previous study examined the effect of heat treatments (temperature-time combination) on the isoflavone content of soymilk. The heat treatment at 95 °C for 45 minutes accepted as the optimum process conditions for soymilk which gave an increase in the genistein content. It was also stated that severe heat treatment leads to Maillard-type reactions and autodegredation resulting in a decrease of genistein. (Huang et al., 2006).

2.7.2.2 Lipoxygenase Enzymes

Lipoxygenase enzymes catalyze the oxidation of polyunsaturated fatty acids. There are 3 different isoenzymes, which are known as Lipoxygenase-1 (LOX-1), Lipoxygenase-2 (LOX-2) and Lipoxygenase-3 (LOX-3) having different pH optima, thermal stability and substrate specificity (Engeseth et al., 1987). LOX-1 has the smallest size, displays the maximum activity at pH 9.0, turn the linoleic acid into 13-hydroperoxide derivative. LOX-2 known as the isoenzyme having the large size, displaying the maximum activity at pH 6.8, forming 13- and 9-hydroperoxide compounds. LOX-3 is the most active isoenzyme concerning carotenoid cooxidation and oxodioneic acid production. It has the maximum pH level around a range about 7.0, produces 9-hydroperoxide products moderately (Ramadoss et al., 1978). Zhu S. et al. concluded that 100 °C for 15 minutes heat treatment is enough for inactivation of lipoxygenase, trypsin inhibitors and urease (Zhu et al., 1996).

These enzymes also responsible the "beany-flavor" of soymilks (Engeseth et al., 1987). They should be inactivated almost completely (99 %) to eliminate the off-flavour compounds (Navicha et al., 2017). Heat treatment and changing pH levels cause the proteins tend to be insoluble, thereby reduced soymilk yield and chalky feeling in the mouth occurs. To prevent these problems, partly inactivation and deodorization is applied on soymilk (Giri and Mangaraj, 2012).

Navicha W. et al. investigated the effect of roasting on soybeans with several temperature and time combinations on LOX activity of soymilk. The results showed that the roasting at 110 °C for 80 minutes significantly reduced the LOX activity. As temperature raised, nutritional value, yield, solid content and color values decreased (Navicha et al., 2017). Zhu S. et al. reported that dry extrusion at 96 °C had resulted significant decrease in LOX-3 activity which is the most heat sensitive isoenzyme and at 107 °C had resulted to inactivate the LOX-1 remarkably (Zhu et al., 1996). Alhendi et al. investigated the inactivation the LOX activity of whole soybeans with pulsed light application with different time and distances. 150 s treatment from 9 cm distance resulted with 0.0 % residual LOX activity (Alhendi et al., 2018).

2.7.2.3 Trypsin Inhibitors

Besides having rich protein content, soybeans also include the trypsin inhibitors. Trypsin inhibitors inhibit the action of trypsin secreted from pancreas which is necessary to digest the proteins. Soy proteins may pass through the body without digestion if trypsin inhibitors are not destroyed. These inhibitors can be inactivated by heat treatment. Temperature-time combination is important for the inactivation of the trypsin inhibitors and at the same time for not to loose essential amino acids and production cooked flavor (Lusas et al., 1989).

Heckler et al. concluded that the heat treatment of soymilk with 90 % inhibition of trypsin inhibitors is good for nutritional quality (lysine) (Hackler et al., 1965). In 1989, Wilson J. reported that soymilk heat treatment should be based on 85 % inactivation of trypsin inhibitors (TI) to determine temperature and time (Wilson, 1989).

Baker and Mustakas reported that 100 °C for 15 minutes was enough to achieve total inactivation of TI (Baker and Mustakas, 1973). In another study, microwave (2450 MHz, 1000 Watts) and conventional heat treatments were applied on soybeans with different time and temperature combinations to compare trypsin inhibitor activity (TIA) reduction. Conventional heat treatment at 100 °C for 30 minutes resulted 1 % and microwave treatment at 100 °C for 8 minutes resulted 3 % residual TIA content (Vagadia et al., 2018)

2.7.3 Soybean Products

Traditional soy foods made from the whole soybean are soymilk, tofu, miso and tempeh. Some soy-based foods are made with soy protein ingredients, such as soy protein isolate, soy protein concentrate, and soy flour (Peter Golbitz, 2006). Concentrated and isolated soy proteins are equal to the protein quality of milk, meat, and eggs, and they are easily digested by humans (Riaz, 2006). Besides their nutritional contribution, soybean products are also used for their functionalities in foods. Accordingly, a number of dairy substitution products have been developed with soy protein products in consequence of their functionality and

high nutrition values (most of the active phytochemicals including isoflavones) (Enders, 2001). These products can be used in most food formulations and recipes as substitution for cow’s milk. They help lowering the costs, improving nutrition, reducing allergy response, and improving the functionality of foods (Enders, 2001).

2.7.3.1 Soymilk and Its Powder

Soymilk is substantially the water extract of soybeans which is the most popular soyfood (Huang et al., 2006). It is an alternative to dairy milk especially for individuals who exhibit allergic reactions to cow's milk or need adequate proteins in their diets (Liu, 1997). Soymilk contains more protein, iron, unsaturated fatty acids, niacin and less calories and amounts of fat, carbohydrates, and calcium than cow’s milk and human milk (Chen, 1989). Soymilk can be obtained by different methods depending grinding conditions, heat treatment and other ingredients. Cornell Method, which is also known as "hot-grind" method, was first described at Cornell University by Wilkens and his colleagues in 1967. The method includes a hot grinding step and a heat treatment after the slurry filtered with cloth after the inactivation of lipoxygenase were done completely at 80-100 °C for 10 min. The soymilk is sterilized after bottled and sealed at 121 °C for 12 min (Liu, 1997). Traditionally, soymilk was being prepared with cold grinding method (Chen, 1989). Heating process affects principally the microbiological activity, nutritional quality of proteins, color and flavors of the soymilk (Huang et al., 2006).

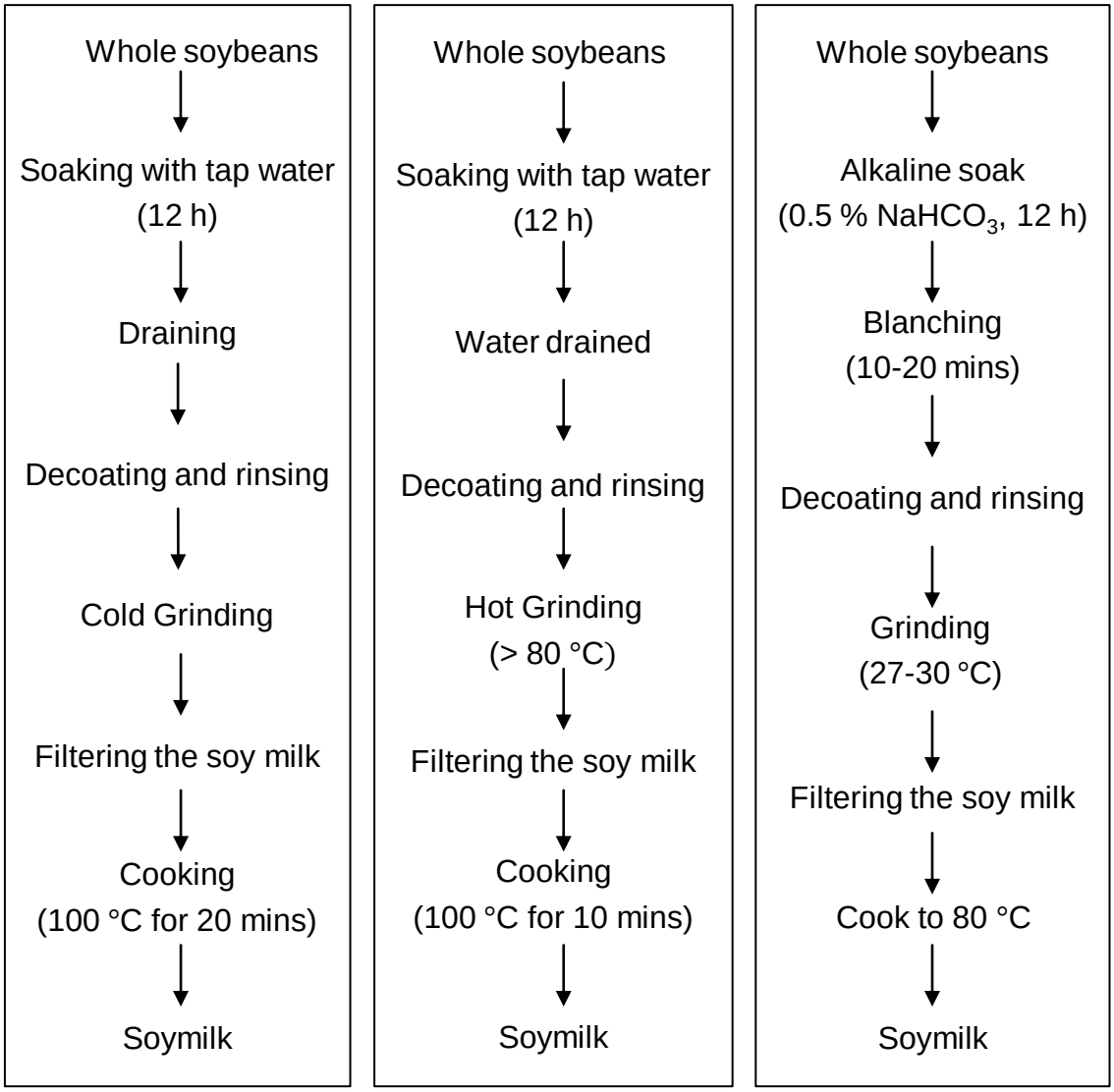


Figure 2.4 Preparation of Soymilk with Traditional (a), Cornell (b), Illonis (c) methods (Abagoshu et al., 2017; Chen, 1989)

Soymilk powder is mentioned as a very good source of nutrient (low-cost protein and polyphenols) for malnourished population in the developing countries. It can fortificate the food with high vitamin A, vitamin B-complex, iron and zinc (Mazumder and Hongsprabhas, 2016).

Jinapong N. et al. stated that the instant soymilk powder production with ultrafiltration, spray drying and fluidized bed agglomeration steps is possible (Jinapong et al., 2008). Giri S. and Mangaraj S. reported that the soymilk and soymilk powder production according to various methods.

2.8 Soy Chocolate

Soymilk is a potential ingredient for substitution of milk powder in milk chocolate to lower the cost. It can be beneficial the usage of soymilk instead of cow's milk due to its milky appearance and nutritional value. Obatoye A.O. and Ogunwolu S.O. (2014) studied production of milk chocolate containing soy milk powder with different ratios. As soy milk powder ratio increased, nitrogen, moisture, fat and protein contents increased (Obatoye and Ogunwolu, 2014). Another study revealed that higher conching time provided smaller particle size for soy chocolate with decreased hardness (Zarić et al., 2015).

The Aim of the Study

The objective of this study is to determine the soy-chocolate and milk chocolate quality produced with different ingredient types (skimmed milk powder, whole milk powder, soymilk powder and soy protein isolate) and with different production conditions (conching for 2-4-6 hours). For the study, soymilk powder was produced and chemical, functional and physical properties was determined. Effects of the ingredient types and production conditions on rheological properties, particle size distribution, polymorphism and sensory poperties of chocolates were investigated. Three rheological models was used to determine the rheology of chocolate.

3. MATERIALS AND METHOD

3.1 Materials

The soybean (Nazlocan cv.) samples were obtained from The Eastern Mediterranean Agricultural Research Institute in Adana, Turkey. Soymilk and soymilk powder were produced under laboratory conditions. Cocoa liquor, cocoa butter and chocolate seeds were obtained from Barry Callebout (Eskisehir, Turkey) and powdered sugar (Kenton, Istanbul, Turkey) was bought from regional markets. All the chocolates used for this thesis were produced as laboratory scale. Whole milk powder (Ulker, Ankara, Turkey) and skimmed milk powder (Slava, Karaman, Turkey) were used to produce milk chocolate. Soy protein isolate (Alfasol, Turkey) and soymilk powder were used as a substitute of milk powder. A lyophilizator (Christ 2B, Germany) used to dry soymilk dried after freezed in a refrigerator (Arcelik, Turkey). Other drying processes conducted with a oven (MMM Venticel, Germany).

Chemicals were bought from Merck (Germany) and Sigma Aldrich (USA). Hexane, NaOH, HCl, potassium sulfate, petroleum ether, sodium tetraborate, borax, phosphate dibasic, sodium phosphate monobasic, linoleic acid (L1376), Tween20, tris (hydroxymethyl) aminomethane, tyripsine bovine pancreas (T8003), BAPA, dimethylsulfoxide, sodium carbonate, cellulose, Folin-Ciocalteu reagent, neocuproine, DPPH, ABTS and trolox were purchased from Sigma; boric acid, copper (II) sulfate pentahydrate, glcial acetic acid, ethanol, potassium persultate, copper (II) chloride, sodium acetate and gallic acid were bought from Merck.

3.2 Methods

3.2.1 Soymilk powder production

Soymilk production was conducted by Cornell method. Soybeans were cleaned and soaked in water for 24 hours. Soaked beans were drained, rinsed and peeled to make hot grinding. Beans were ground by a blender (ISOLAB Laborgerate GmbH, Turkey) for 2 minutes at 80-90 °C. Ground slurry was filtered by a muslin cloth and soymilk was obtained. Soymilk was heated to 100 °C in a water bath for 20 minutes (Clifton, UK). The time was counted after the central temperature of soymilk had reached to 98 °C. The aim of the heat treatment was to eliminate the lipoxygenase and trypsin inhibitor enzymes. After the heat treatment, soymilk was transferred to plastic beakers and covered with parafilm (ISOLAB, Turkey). Small holes were drilled on the parafilm by a needle to enable the drying process. Soymilks were frozen in a freezer (Arcelik, Turkey) at -24 °C overnight and dried by a lyophilizator (Christ 2B, Germany) under 0.02 atm pressure at -55 °C for 48 hours. Dried samples were ground by coffee grinder machine (Sinbo, Turkey) and sieved to 212 µm size.

3.2.2 Analysis of Powder Products

3.2.2.1 Physical Analysis of Powder Products

In order to determine the physical properties of skimmed milk powder (SMP), whole milk powder (WMP), soymilk powder (SoMP) and soy protein isolate (SPI) samples, bulk density, tapped bulk density, solubility, wettability values were specified. Then, Carr Index and Hausner Ratio values were calculated.

The bulk density values of the powder ingredients were measured by the addition of samples with specific weight to a graduated beaker according to the method Jinapong et al. (2008). Results were shown as g/ml. The tapped bulk density values were determined by weighing the samples into graduated beaker and the beaker was tapped 70 times on a plastic surface according to the method of Dirim et al. (2012). The final volume was read and results were expressed as g/ml (Dirim

and Caliskan, 2012; Jinapong et al., 2008). The density values were measured as 3 replications.

The wettability value was determined by the addition of 5 grams of powder sample into the 100 ml distilled water. Then, it was slightly stirred by a magnetic stirrer (Daihan MSH-20A, Korea). The time that all powder particles penetrate into water was evaluated visually and recorded (Nguyen et al., 2018). 1 g sample was carefully put into the beaker containing 100 ml of pure water. A magnetic stirrer was used to mix samples and determine the solubility values of samples. The suspensions were transferred into centrifuge tubes and centrifuged (Sigma 3-18K, USA) at 5000 rpm for 5 minutes. 25 ml of supernatants were taken into a petri dish and dried at 105 °C to the constant weight. Solubility (%) was calculated from weight loss. Wettability and solubility values were measured as 3 replications.

Hausner Ratio (HR) is a useful description, which does not require a separate test. HR can be derived from bulk density tests, which is the ratio of bulk density over the tapped bulk density (Equation 4) (Ortega-Rivas et al., 2006). This value was used to evaluate the flowability of products (Nguyen et al., 2018).

$$\mathrm{HR} = \frac{\rho_{\mathrm{tapped}}}{\rho_{\mathrm{bulk}}} \tag{4}$$

The Carr Index (CI) is another description obtained by density values. The Carr index was calculated according to Equation 5, represents the compressibility of the product (Nguyen et al., 2018).

$$\mathrm{CI}\ (\%) = \frac{\rho_{\mathrm{tapped}} - \rho_{\mathrm{bulk}}}{\rho_{\mathrm{bulk}}} \tag{5}$$

The color values of the ingredients are important because they affects the color of the final product. Color values of powder products were determined by a colorimeter (Minolta Spectrophotometer CM-3600j, Japan) at room temperature. Results were expressed according to CIELAB system scale (L*, a*, b*, C* and h° values) where L* is brightness, a* is green to red color scale, b* is blue to yellow color scale, h° is the color tone (Equation 6) and C* is saturation (Equation 7).

Color measurements were done as 6 replications. C* and h° values are calculated as shown below;

$$hue\ angle\ (h°) = \arctan\ (b^*/a^*) \quad (6)$$

$$Chroma\ (C^*) = [(a^*)^2 + (b^*)^2\ ]^{0.5} \quad (7)$$

3.2.2.2 Chemical Analysis of Soymilk Powder

Proximate analysis

Proximate analysis was performed to specify the contribution of the nutritional value of soymilk powder on soy-chocolate. Moisture values were determined gravimetrically for all the powder products used in chocolate production. According to AACC (1999) Method 82-21.01, the samples were dried at 105 °C to the constant weight. The result was calculated by dividing the final weight to initial weight and multiplied by 100. Protein content of soymilk powder was determined by AACC (1999) 46-12 Kjeldahl method containing the digestion, distillation and titration steps. Fat content was determined by AACC (1999) 30-10.01 method which is based on the principle of extracting the oil in the sample dissolved by petroleum ether with the Soxhlet device (Electro-mag, Turkey). AACC (1999) 08-16.01 method was conducted for determination of the ash content. Approximately 1 g sample was weighed into ash crucibles and burnt for 3 hours in the ash oven (Protherm, Turkey) at 600 ∓ 15 °C. Carbohydrate content was calculated after protein, fat, ash and moisture content were specified.

The pH of the samples was measured from their suspensions (1:100 (w/v) by using a pH-meter (PL-700AL, Turkey) from their suspensions). Measurements were made as 3 replications. The results were shown as the average of the measured values.

Nitrogen solubility index (NSI) was determined with method AACC (1999) 46-23.01. The sample was weighed, diluted by adding water slowly, then centrifuged

at 1500 rpm for 10 minutes and Kjeldahl standard protein analysis method (46-16.01) was monitored. Calculation is done as follows:

$$Water\ soluble\ N\ (\%) = \frac{(\mathrm{B} - \mathrm{S})\ \mathrm{x}\ \mathrm{N}\ \mathrm{x}\ 0.14}{\text{sample weight}}\ \mathrm{x}\ 100 \quad (8)$$

B = volume of alkaline back-titration of blank (ml)
S = volume of alkaline back-titration of sample (ml)
N = normality of alkaline

$$\mathrm{NSI}\ (\%) = \frac{\%\ \text{water soluble N}}{\%\ \text{total N}}\ \mathrm{x}\ 100 \quad (9)$$

Protein dispersibility index (PDI) was determined by using the AACC (1999) 46-24.01 method. 20 g soymilk powder was weighed and stirred manually after 50 ml pure water until the pasty consistency was obtained. Distilled water was added to pasty sample and the volume was completed to 300 ml. The suspension was stirred by a mechanic stirrer (IKA-Werke RW16, Germany) at 8500 rpm rate for 10 minutes at room temperature. The temperature of the suspension was recorded as 48 °C at the end of the time. The slurry was put into a centrifuge tube. Then, it was centrifuged at 2700 rpm for 10 minutes. Supernatant was filtered before analysis. The standard Kjeldahl method (46-16.01) was applied on the supernatant. According to the method, 15ml of the supernatant corresponds to 1 g of the sample. The calculation of PDI was done according to Equation 10 and 11.

$$\%\ \text{Water dispersible protein} = \frac{(\mathrm{B} - \mathrm{S})\ \mathrm{x}\ \mathrm{N}\ \mathrm{x}\ 0.014\ \mathrm{x}\ 6.25}{\text{sample weight}}\ \mathrm{x}\ 100 \quad (10)$$

B = volume of alkaline back-titration of blank (ml)
S = volume of alkaline back-titration of sample (ml)
N = normality of alkaline

$$\mathrm{PDI}\ (\%) = \frac{\%\ \text{water dispersible protein}}{\%\ \text{total protein}}\ \mathrm{x}\ 100 \quad (11)$$

Lipoxygenase Activity

The lipoxygenase (LOX) enzyme activity assay was carried out for both soymilk powder and soy protein isolate. The latter was used directly, because it does not contain any fat, but soymilk powder was defatted with hexane prior to the analysis and sieved to 212 µm pore size (Engeseth et al., 1987). Heat treatment was applied on samples for different times to observe the activity of lipoxygenase enzyme. Soymilk samples were subjected to a temperature of 98-100 °C for 0, 5, 10, 15 and 20 minutes and then lyophilized to analyze. To calculate the residual percentage of lipoxygenase enzyme activity, the activity of raw soybean was also determined. The lipoxygenase activity was determined using the spectrophotometric method for LOX-1 and LOX-3 (Axelrod et al., 1981).

Firstly, 0.2 M pH 9.0 borate buffer for the LOX-1 determination, 0.2 M pH 6.5 phosphate buffer for the LOX-3 were prepared as a method, which was first described by Axelrod et al. (1981), and then developed by Engeseth et al. (1987). Substrate, which was 0.017 % linoleic acid solution, was prepared according to Alhendi et al. (2018). In this method, stock solution was prepared by the addition of 50 µl linoleic acid (cis-9, cis-12-octadecadiene acid) (L1376, Sigma, USA), 50 µl Tween20 (Sigma, USA) and 50 ml of the previously prepared buffer solution. The final stock solution was prepared by mixing 5 ml of deionized water and 20 ml of buffer solution from the prepared stock solution. This final stock solution was used as the substrate for the analysis (Alhendi et al., 2018).

Extraction of the enzyme was performed by several modifications of the method specified by Axelrod B. et al. (1981). 1 g sample was mixed with 50 ml of pH 6.5 0.2 M sodium phosphate buffer. Then, stirred by a magnetic stirrer at room temperature for 30 minutes. The suspension was centrifuged (13000 rpm, 4 °C, 10 min). The supernatant was used as liquid enzyme extract.

For determination of enzyme activity, 2 ml of stock solution and 0.95 ml deionized water were mixed and transferred into cuvettes and the reaction started after the addition of 50 µl enzyme extract. Spectrophotometer (Thermo Scientific, USA) was used to measure the absorbance change at every 30 seconds for 5 minutes. 234

and 280 nm wavelengths were used for LOX1 and LOX-3 activities respectively. Deionized water was used for the blank measurement. 0.001 absorbance change was accepted as 1 unit of LOX activity and LOX unit/minute was calculated. Results were expressed as LOX Residue % (Axelrod et al., 1981).

Trypsin Inhibitor Activity

Samples prepared for lipoxygenase enzyme activity and soy protein isolate were also used to specify trypsin inhibitor activity spectrometrically according to method 22-40.01 (AACC (1999). Tris buffer, substrate solution, trypsin solution and enzyme extract solution were prepared before analysis.

For preperation of tris buffer (0.05 M pH 8.2), 2.53 g calcium chloride and 6.05 g tris were dissolved in 900 ml of distilated water. pH was adjusted to 8.2 and volume was completed to 1 L. 40 mg of BAPA was dissolved completely in 1 ml of dimethyl sulfoxide and completed with tris buffer to 100 ml to prepare substrate solution. Trypsin solution was prepared with 4 mg trypsin dissolved in 200 ml 0.001 M HCl. Acetic acid solution (30 %) was prepared with 70 ml of deionized water was added to 30 ml of glacial acetic acid. 1 g of defatted and sieved (212 µm) sample was mixed with 50 ml 0.01 N NaOH solution for 3 hours on a magnetic stirrer to perform the enzyme extraction.

0.6, 1.0, 1.4 and 1.8 ml of extracted enzyme were put into the test tubes, followed by the addition of deionized water up to 2 ml. A test tube was prepared only with 2 ml of deionized water for blank. 2 ml of trypsin solution was added to each tube and vortexed. All the tubes were placed in water bath and it was ensured that the internal temperature of tubes reached to 37 °C. Then, 5 ml of the substrate at 37 °C was added to tubes, vortexed and waited at 37 °C for 10 minutes. Finally, 1 ml of acetic acid solution was added and vortexed to stop the reaction. The absorbances were measured by a spectrophotometer at 410 nm wavelength. 0.01 absorbance increase was expressed as the Trypsin Inhibitor Unit (TIU). The TIU/ml results were plotted against the ml values to make the calculations. The results were calculated as TIU/g dry sample.

3.2.2.3 Functional Properties of Powder Products

Emulsion capacity and stability, water holding capacity and oil binding capacity of powder products were determined.

For emulsion capacity (EC) measurements, suspension containing 1 % powder product (w/v) were prepared and 2.5 ml of this suspension was transferred into the 50 ml centrifuge tubes and homogenized for 2 minutes using 50 % power in the ultrasonic homogenizer. Then, 2.5 ml corn oil was added and homogenized again with 50 % power for 2.5 minutes. Emulsions were centrifuged at 5000rpm for 3 minutes and final emulsion volumes were measured (Johnson et al., 1981). The emulsion capacity (%) was measured in 3 replicates as the ratio of the height of the emulsified layer to the height of the tube content. To determine emulsion stability (ES), 2.5 ml of 1 % (w/v) suspension and 2.5 ml of corn oil were emulsified using an ultrasonic homogenizer (Bandelin Sonoplus HD 2070, Germany) for 30 minutes in a 80 °C water bath. Then, it was brought to room temperature and centrifuged at 5000 rpm for 3 minutes. The emulsion stability (%) was measured in 3 replicates as the ratio of the content of the emulsified layer to the height of the tube content.

The analysis of the water holding capacity (WHC) was performed according to Nguyen et al. (2017) modified for soymilk powder and soy protein isolate samples. 1 g sample was added to centrifuge tube; 20 ml of distilled water was added and stirred in the shaker at room temperature for 30 minutes. The suspension was centrifuged at 10000 rpm for 10 min. The remaining liquid was then filtered through filter paper and the remaining weight was measured. Measurements were performed in 3 replications. The water holding capacity expressed as the amount of water bound in the dry matter per g sample, was calculated as shown in Equation 12.

$$WHC(g\ water/g\ dry\ matter) = \frac{\text{last weight} - \text{tare} - \text{sample weight}}{\text{sample weight}} \text{x } 100 \qquad (12)$$

Oil binding capacity (OBC) analysis for soymilk powder and soy protein isolate samples were analyzed according to Nguyen et al. (2017) et al. 10 ml of corn oil was added to 1 g sample and vortexed. For half an hour, the samples were kept at room temperature and vortexed at every five minutes. The resulting suspension was centrifuged at 10000 rmp for 10 min. After centrifugation, the oil fraction was evacuated, the remaining part was filtered with filter paper and weighed. Measurements were done as 3 replications. OBC was calculated in dry matter as shown in Equation 13.

$$OBC\ (g\ oil/g\ dry\ matter) = \frac{\text{last weight} - \text{tare} - \text{sample weight}}{\text{sample weight}} \text{x } 100 \quad (13)$$

3.2.3 Chocolate production

Four different chocolates containing four types of powder ingredients including skimmed milk powder, whole milk powder, soymilk powder and soy protein isolate were prepared according to the formulations indicated in Table 3.1.

Chocolate samples were produced as 300 g at a time. The cocoa butter (72 g) was firstly melted by benmari style in metal pod and the cocoa mass (82.5 g) was added after all the butter melted. The mixture of the sugar (90 g) and powder (54 g) was added after sieving to avoid the agglomeration of particles. Lastly, the lecithin (1.5 g) was added. Lastly, the mixture was transferred to the chocolate melanger (Premier, USA).

Samples were subjected to 3 different conching times for each formulation. Skimmed milk powdered chocolates were processed for two hours (SMC2), four hours (SMC4) and six hours (SMC6); whole milk powdered samples were processed for two hours (WMC2), four hours (WMC4) and six hours (WMC6); soy milk powdered samples were processed for two hours (SoMC2), four hours (SoMC4) and six hours (SoMC6); chocolates prepared with soy protein isolate were processed for two hours (SPC2), four hours (SPC4) and six hours (SPC6). In this process, refining and conching steps were carried out simultaneously without heating.

Table 3.1 Chocolate formulations

Products	Name	Conching time	Cocoa Liquor	Cocoa Butter	Powder Ingredient	Sugar	Lecithin
Whole Milk Chocolate	WMC2	2	27.5 %	24 %	18 %	30 %	0.5 %
	WMC4	4	27.5 %	24 %	18 %	30 %	0.5 %
	WMC6	6	27.5 %	24 %	18 %	30 %	0.5 %
Skimmed Milk Chocolate	SMC2	2	27.5 %	24 %	18 %	30 %	0.5 %
	SMC4	4	27.5 %	24 %	18 %	30 %	0.5 %
	SMC6	6	27.5 %	24 %	18 %	30 %	0.5 %
Soymilk Chocolate	SoMC2	2	27.5 %	24 %	18 %	30 %	0.5 %
	SoMC4	4	27.5 %	24 %	18 %	30 %	0.5 %
	SoMC6	6	27.5 %	24 %	18 %	30 %	0.5 %
Soy Protein Chocolate	SPC2	2	27.5 %	24 %	18 %	30 %	0.5 %
	SPC4	4	27.5 %	24 %	18 %	30 %	0.5 %
	SPC6	6	27.5 %	24 %	18 %	30 %	0.5 %

Tempering stage was performed by a tempering machine (Chocovision R2, USA) and temperature change was controlled with a infrared thermometer. Tempering was carried out using seeds to growth right crystal type. Initially, temperature was raised to 45 ∓ 0.1 °C to ensure that no crystal was left and decreased gradually to 27.4 ∓ 0.1 °C to produce the Form V crystal type. Then, samples were heated again to 30 °C to melt the unstable crystal types. Tempered chocolates were moulded and tapped slightly to remove bubbles left inside. Samples were cooled to the room temperature.

3.2.4 Physicochemical Properties of Chocolates

3.2.4.1 Moisture Content and Water Activity

It is known that the high moisture levels cause to form aggregated sugar particles and gritty texture in chocolate. Rising moisture level on the sugar particle surfaces increases friction and apparent viscosity (Afoakwa, 2010). Therefore, it is crucial to know the moisture content and the water activity of the chocolates. Moisture

content analysis of chocolate samples was conducted according to AACC (1999) 44-01.01 gravimetric method. Samples were weighted in dried moisture dishes. Uncovered dishes were placed in the oven and dried at 105°C to the constant weight. Final weight of dishes was measured after they were cooled to room temperature in a desiccator. The result was expressed as moisture content % of sample. Water activity of chocolates was measured by the water activity device (Novasina, Labtouch aw, Germany). Both of the measurements were done as 3 replicates.

3.2.4.2 Color of Chocolates

Color measurement was performed by a spectrophotometer (Konica Minolta CM-3600d, Japan) device after the calibration was done by white and black apparatus. L*, a*, b* values of CIE lab system scale of samples were determined at room temperature. C* and h° values were calculated from collected datas. All measurements were done as 6 replicates.

3.2.4.3 Texture Profile Analysis of Chocolates

Texture Profile Analyzer (TA Plus, UK) was for determination of the hardness (N) values of the chocolates. was used for this purpose. The fracture values of the specimens were considered as hardness and Three Point Bend probe was used as probe to break the samples. The speed of the probe was 2 mm/s from the top to break the sample with a maximum power of about 40 N. The hardness value was expressed as the force (N) used by the probe to break the chocolate.

3.2.4.4 Rheological Properties of Chocolates

The flow behavior of molten chocolates was measured by a rheometer using a cup and bob geometry (Malvern Kinexus, Japan). Shear stress was measured as a function of increasing shear rate from 0.1 to 100 s^{-1} (ramp up) at 40 °C for 2 minutes ramp time. The device was calibrated with "zero gap" before each measurement. Results were fitted into Casson, Herschel-Bulkley and Bingham models which are widely used rheological models for chocolate. Apparent viscosity and yield stress values were calculated from the collected data as shear rate versus shear stress. All the measurements were done as 3 replicates.

3.2.4.5 Particle Size Distribution

Particle size distribution measurements of chocolate samples were done by the laser diffraction technique, which is known as low-angle laser light scattering. This standard technique is widely preferred for analysis, characterization and quality control around the world by diverse industries. Malvern Mastersizer instrument is used for analysis ranging 0.1-2000 µm particle size. According to this technique, the principle is that diffraction angle is proportional to particle size which is highly reproducible (Afoakwa, 2010).

This analysis was performed by using particle size analyzer (Malvern, Mastersizer 3000, UK). Since it was conducted in distilled water, the chocolate samples were defatted by hexane prior to analysis. Defatted chocolate powder samples were sieved to 212 µm pore size to obtain a standard particle size. Sieved sample was weighed 0.1 grams for analysis and then pure water (1.330 refractive index) was added and ultrasonic dispersion was applied with 70 % power for 3 minutes. Results were obtained at 25 °C and 1.395 refractive index with 2 replicates. D_{90} (90% <size), D_{50} (50% <size), $D_{3,2}$ (Sauter mean diameter), $D_{4,3}$ (DeBroukere mean diameter) results were obtained from the analysis. Since the D_{90} parameter represents large particle content in the chocolate industry, it is used as a representative for all four parameters.

3.2.4.6 X-ray Diffractometer

The X-ray diffraction method based on collecting the diffracted rays directed to the sample and it is used to identify the polymorphic fat crystals composing the chocolate. X-rays are generated from X-ray tubes and filtered to obtain monochromatic radiation, collimated and directed to the sample. When the conditions satisfy the Bragg's Law, the interaction of the incident rays with the sample, produces a diffracted ray. The Bragg's law (Equation 14), relates the wavelength of radiation to diffraction angle and lattice spacing in a crystalline sample. These diffracted beams are detected, handled and counted. All possible diffraction ways of the lattice are achieved for the powder product owing to the random orientation of the powdered material by scanning the sample in the 2θ angle range. Conversion allows the identification of the diffracted peaks (Klug and Alexander, 1974; Warren, 1990).

$$2d \sin\theta = n\lambda \quad (14)$$

In the X-ray analysis, it is essential to extract the sugar since the peaks formed by the sugar crystal obscures the peaks of the cocoa butter crystal peaks. Chocolate samples were ground by a coffee grinder (Sinbo, Turkey) and kept in cold water stirring slowly at 4 °C for 4 hours to separate the sugar. The water was removed by vacuum filter. The washing procedure was repeated twice to be sure that all the sugar was removed. Then, the samples were allowed to dry at room temperature for 48 hours. Dried samples were ground and milled again with coffee grinder and stored in a refrigerator at 4 °C until the analysis. De-sugared chocolate powders were analyzed by a X-ray diffractometry (Rigaku Ultima-IV, Japan) using Bragg-Brentano geometry for powder samples. Experimental results were obtained by using a Cu-K ray, (λ=1.151418 Å) in 2θ scale from the range of 2-50° at 25 °C with 1°/min acquisition time.

3.2.5 Functional Properties

Total phenolic content (TPC) determination was performed by Folin-Ciacalteu method and total antioxidant capacity determination (TAC) of chocolates was carried out by three different assays, which were DPPH, CUPRAC and ABTS methods. The extract preparation for all these analyses was performed according to QUENCHER method. Chocolate samples were defatted by hexane and then diluted with cellulose (1:15, w:w).

3.2.5.1 Total Phenolic Content (TPC)

This analysis was performed by applying a number of modifications to the method described by Dogan (2015). Sodium carbonate solution (75 g/L) was prepared before analysis. 3.75 g Na_2CO_3 was dissolved in 50 ml of pure water. Folin Ciocalteu Phenol Reagent (2N) (Sigma, USA) was diluted to 1:10 (v/v) by distilled water to make it 0.2 N. The Folin-Ciocalteu solution is sensitive to light and so it was kept in the dark until usage.

1.25 ml Folin reagent (0.2 N) was added to 10 mg of diluted solid sample. The mixture vortexed and kept in the dark for 5 min. 1 ml of sodium carbonate solution was added to mixture and vortexed. The last mixture was put in the shaker for 1 hour in dark. To assess the results, a calibration curve of gallic acid (3,4,5-trihydroxy-benzoic acid) was generated. Results are given in mg GAE/g dry matter (GAE: gallic acid equivalent) (Dogan, 2015).

3.2.5.2 Total Antioxidant Capacity (TAC)

DPPH Assay

This analysis was performed according to Serpen A. et al. (2012). 40 mg/L $DPPH^{+\bullet}$ stock solution was prepared with dissolving the DPPH reagent in the mixture of water:ethanol (1:1) (v/v). This solution can be used for 24 hours if stored in the

dark. $DPPH^{+\bullet}$ solution was used after the dilution with a mixture of water:ethanol (1:1) (v/v) until a 0.750 absorbance was obtained at a wavelength of 525 nm.

10 mg diluted solid sample of was mixed with 10 ml of $DPPH^{+\bullet}$ stock solution and put in a shaker (Edmund Bühler GmbH, Germany) for 2 hours in the dark. At the end of the period, mixture was centrifuged at 10000 rpm for 2 minutes. The supernatant filtered with a 0.45 µm microfilter and absorbance was read at 525 nm wavelength. A Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) calibration curve was generated to calculate the results (Serpen et al., 2012). The results of TAC_{DPPH} were expressed as mM Trolox/g dry sample.

ABTS Assay

$ABTS^{+\bullet}$ solution was prepared according to Acar et al. (2009). The ABTS reagent was dissolved in pure ethanol and then, the same amount of water was added. Lastly, potassium persulfate ($K_2S_2O_8$) was added and mixed. The final solution contained 7 mM ABTS and 2.45 mM potassium persulphate. This solution can be used after it kept in the dark for 12-16 hours and be available for reaction for the next 24 hours. $ABTS^{+\bullet}$ solution was diluted with water:ethanol (1:1) mixture until 0.700 absorbance was obtained at a wavelength of 734 nm.

This analysis was performed according to Serpen. et al. (2012). 10 mg of $ABTS^{+\bullet}$ stock solution was added to the solid sample diluted with 10 mg cellulose and mixed in the dark for 26 min. At the end of the period, mixture was centrifuged at 10000 rpm for 2 minutes. Supernatant was filtered through 45 µm microfilter and the absorbance values were determined at a wavelength of 734 nm (Tufan et al., 2013). A Trolox calibration curve was generated to calculate the results. The results of TAC_{ABTS} were expressed as mM Trolox/g dry sample.

CUPRAC Assay

This analysis was performed according to Tufan et al. (2013). 0.4262 g $CuCl_2.2H_2O$ was dissolved in 250 ml distillated water to prepare Copper(II)chloride

solution (0.2 M). It was kept in the dark due to its light-sensitive character. 19.27 g NH_4Ac was dissolved in 250 ml distillated water to obtain 1 M pH 7.0 ammonium acetate solution. Neocuproin solution ($7.5x10^{-3}$ M) was also prepared solving the 0.078 g of neocuproin (2.9-dimethyl-1,10-phenanthroline) in 50 ml 96 % ethyl alcohol.

1 ml of copper (II) chloride, 1 ml of neocuproin solution, 1 ml ammonium acetate solution and 1.1 ml water:ethanol (1:1) (v/v) mixture was added to 10 mg diluted solid sample and this mixture was vortexed for 1 min. Mixture was put in the shaker for 30 min in dark. After the reaction, mixture centrifuged in with 10000 rpm for 2 minutes. Supernatant was filtered through 45 µm microfilter. Absorbance values were determined at 450 nm wavelength. A Trolox calibration curve was generated to calculated the results. The results of TAC_{CUPRAC} were expressed as mM Trolox/g dry sample.

3.2.6 Sensory Properties of Chocolates

Sensory analysis was conducted with 10 untrained panelists. The scale used to perform sensory analysis (Appendix A-1) of chocolate samples was shown and explained to the panelists before the evaluation. Appearance, texture, flavour, odor and after taste attributes were chosen to evaluate the sensory properties of products. The assessment was conducted using 1 to 5 point scale. Samples (7 cm x 4.8 cm x 0.4 cm) were served at room temperature and under white fluorescent lighting. The form that panelists filled in is given on Appendix A.

3.2.7 Statistical Analysis

After taking the measurements, mean and standard deviation were calculated and Minitab 18 (Pennsylvania, USA) software was used for one-way analysis of variance (ANOVA) and Tukey multiple comparison test was used to determine the differences between the results. Differences were considered significant for $p \leq 0.05$. All statistical results are given in Appendix C.

4. RESULTS AND DISCUSSION

4.1 Chemical Properties

4.1.1 Proximate Analysis of Soymilk Powder

Moisture content values were determined on wet basis for all powder ingredients used in chocolate production. Moisture levels were found as 5.5 % in SMP, 2.8 % in WMP, 3.4 % in SoMP and 5.9 % in SPI samples. The highest amount was found in SPI and the lowest value was found in WMP samples. The moisture content on the non-fat samples was higher than the fat including samples due to their free hydrophilic sites.

The amount of ash in soymilk powder was 6.06 %, the amount of fat was 18.14 %, the amount of protein was 44.43 %; and the carbohydrate amount was calculated as 27.96 %. All values are given on wet basis and shown in Table 4.1. Obatoye and Ogunwolu (2014) produced soymilk by Illonis method and dried the milk at 60 °C for 14 hours in oven to use it in soy-cow milk chocolate. They stated that nutritional value of soymilk powder was 2.69 % moisture, 3.60 % ash, 44.10 % protein, 21.05 % fat and 21.60 % carbohydrate (Obatoye and Ogunwolu, 2014). Another study has previously reported that soymilk powder, which had dried by spray-drying method, had 19.9 % fat content (Ishiwu et al., 2014). The amount of fat in soy milk plays a crucial role for physical characterization such as texture and sensorial properties.

The process conditions mostly affect protein, fat, fiber and viscosity, while the amount of moisture and carbohydrate are minimally affected (Giri and Mangaraj, 2012). Jinapong et al. (2008) specified the proximate content of spray dried soymilk powder as 48.79 % protein, 28.77 % fat, 4.97 % ash and 17.47 % carbohydrate content on dry basis (Jinapong et al., 2008).

Table 4.1 Nutritional value of soymilk (wet basis %)

Proximate analysis	Content (%)
Moisture	3.40 ∓ 0.025
Fat	18.14 ∓ 0.064
Ash	6.06 ∓ 0.140
Protein	44.43 ∓ 0.378
Carbohydrate	27.96 ∓ 0.130

Results are given with mean values and standard deviations over three replicates.

The pH of the soymilk was determined as 6.87 before drying. The pH values determined for soymilk powder and soy protein isolate were for 1:100 (w:v) suspensions. The soymilk suspension had a pH of 6.90, while the soy protein isolate suspension had a pH of 7.85. In previous studies, it was stated that soymilk obtained by hot grinding method should have a pH between 6.7-7.2 at the end of the process (Giri and Mangaraj, 2012). This shows that the soymilk obtained in this study was in the appropriate pH range.

Protein dispersibility index (PDI) was found as 72.01 % in SoMP. Zhu et al. (1996) stated that the PDI value was inversely proportional to the lipoxygenase inactivation. They reported that increasing the lipoxygenase inactivation from 90 % to 100 % caused to reduce PDI values approximately to the half (Zhu et al., 1996). PDI is highly influenced by pH (Volkert and Klein, 1979) and process conditions (Dubois and Hoover, 1981).

The nitrogen solubility index (NSI) was found as 18.20 % for SoMP. Van Burren et al. (1964) obtained soymilk powders by different heat applications and drying processes. They stated that freeze-dried soymilk powder that was heat-treated for 10 minutes at 120 °C was found to have 30 % NSI values. They stated that increasing temperature caused the NSI values to decrease. Spray dried powders had 6 % NSI according to them (Van Buren et al., 1964). The NSI value is used to decide in which product the soy ingredient should be added.

The anti-nutritional factors are destroyed by heat, thus the low PDI and NSI values are also evaluated as an indicator of inactivation of these factors (Dubois and Hoover, 1981).

4.1.2 Lipoxygenase Enzyme Activity

High temperature applications in soymilk production leads to reducing functionality and forming the volatile organic compounds responsible for undesired cooked taste-odor and protein denaturation (Giri and Mangaraj, 2012). On the other hand, sufficient heat treatment is needed to eliminate the anti-nutritional contents such as lipoxygenase enzyme and trypsin inhibitors. Heat treatment application was optimized considering these parameters.

The lipoxygenase residue of heat-treated soymilk samples for different times (5, 10, 15, 20 minutes), raw soybean and soy protein isolate were indicated in Table 4.2.

Table 4.2 Lipoxygenase residue in soymilk powder products

Sample	Lipoxygenase-1	Lipoxygenase-3
Raw soybean	100	100
0'	37.6 ∓ 1.14	33.4 ∓ 3.30
5'	20.8 ∓ 1.49	17.9 ∓ 0.71
10'	16.0 ∓ 2.04	13.6 ∓ 1.07
15'	7.2 ∓ 0.13	4.6 ∓ 1.31
20'	0.9 ∓ 0.02	-
SPI	3.9 ∓ 0.58	2.9 ∓ 0.61

Results are given with mean values and standard deviations over three replicates.

The soymilk subjected to heat treatment for 20 minutes was found to have 0.9 % LOX-1 activity residue while LOX-3 activity was not detected. The residual LOX values were found significantly lower after the heat treatment. In this case, the amount of time and temperature determined to ensure the inactivation (20 minutes at 98-100 °C) of the lipoxygenase enzyme was concluded to be sufficient.

According to Zhu et al. (1996) 30 minutes at 98.9 °C heat treatment resulted 4 % LOX-1 and 0 % LOX-3 residual content. The lower value of our results is thought to caused by the overnight soaking and hot-grinding stages before production.

4.1.3 Trypsin Inhibitors

The Trypsin Inhibitor Activity (TIU/mg) of the soymilk samples exposed to heat treatment for different times (5, 10, 15, 20 minutes) were found as shown in Table 4.3.

Table 4.3 Trypsin inhibitor activity reduction

Sample	TIU/mg
0'	76.54 ∓ 2.10
5'	49.90 ∓ 1.59
10'	21.42 ∓ 1.21
15'	11.14 ∓ 0.89
20'	1.11 ∓ 0.10
SPI	5.45 ∓ 0.26

Results are given with mean values and standard deviations over three replicates.

Johnson et al. (1981) stated that 99 °C heat treatment at 6.7 pH for almost 20 minutes resulted approximately 10 % of residual trypsin inhibitors (Johnson et al., 1981). Our results are more satisfying (98.5 % inactivation) than this value which was caused by soaking and hot grinding steps. The time-dependent change of the trypsin inhibitor activity is shown in the figure below.

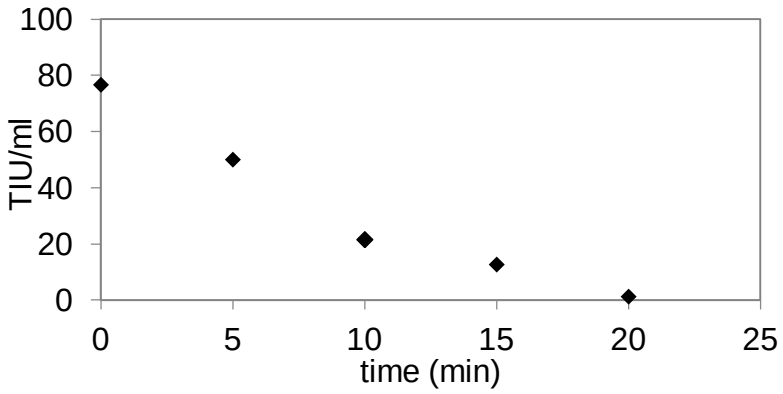


Figure 4.1 Trypsin inhibitor change with time

4.2 Physical Properties of Powder Ingredients

The bulk densities were found 0.415 g/ml for SMP, 0.326 g/ml for WMP, 0.253 g/ml for SoMP and 0.234 g/ml for SPI samples. Santana et al. (2017) reported that the bulk density values were related to the particle size. Even small differences in the bulk density values affect the flowability of the product greatly (Santana et al., 2017). The tapped bulk density was found 0.632 g/ml for SMP, 0.516 g/ml for WMP, 0.419 g/ml for SoMP and 0.330 g/ml for SPI samples. A previous study reported that loose bulk density of spray-dried soymilk powders were changed between 359-470 kg/m^3 while tapped bulk densities were changed 532-640 kg/m^3 (Nguyen et al., 2018). Spray-dried powders have non-homogeneous particle dispersion that are the cavity of big particles are filled in small particles, resulting the high bulk density (Schubert, 1987). The density values were obtained to understand the flow characteristics and cohesiveness of the powders. Bulk and tapped bulk densities of powder ingredients were significantly different from each other ($P<0.05$).

The brightness (L*), green to red color degree (a*), blue to yellow color degree (b*), the chromatic appearance (C*), and hue angle (h°) values were shown in Table 4.4 for powder ingredients. The results showed that SoMP sample had high brightness value (84.6) although it was lower than SMP (94.1), WMP (94.1) and SPI (85.2) samples. A previous study reported that the L* values of spray dried soy beverage powders were varied between the range of 86.2 to 88.8 (Giri et al., 2017) which are higher than our results. According to the study by Johnson et al. (1981), L* values of heat treated soymilks were decreased with the increasing temperature of heat treatment (Johnson et al., 1981). L*, a* and b* values in soymilk are considered as an indicator of browning reaction. Heating process resulted in a color change from green (negative a*) to red (positive a*) by increasing the a* value. Heating also increased the b* value which means higher yellowness (Kwok et al., 1999). The highest b* value was obtained in SoMP samples. SMP and WMP samples had lower b* values than SoMP and SPI samples. a* and b* values of each sample were found to be significantly different ($P<0.05$). According to Tukey grouping, SMP and WMP samples; SoMP and SPI

samples were similar with regard to L* values while they were different with regard to a* and b* values.

Table 4.4 Color values of powder products

Products	L*	a*	b*	C*	h°
SMP	94.1 ∓ 0.36[a]	-2.2 ∓ 0.24[a]	12.3 ∓ 0.73[a]	12.5 ∓ 0.76[a]	100.0 ∓ 0.51[a]
WMP	94.1 ∓ 0.33[a]	-1.4 ∓ 0.15[b]	10.2 ∓ 0.43[b]	10.3 ∓ 0.45[b]	98.0 ∓ 0.59[b]
SoMP	84.6 ∓ 0.19[b]	1.2 ∓ 0.10[c]	21.6 ∓ 0.33[c]	21.6 ∓ 0.33[c]	86.7 ∓ 0.26[c]
SPI	85.2 ∓ 0.58[b]	0.7 ∓ 0.03[d]	15.8 ∓ 0.14[d]	15.8 ∓ 0.15[d]	87.5 ∓ 0.13[c]

Results are given with mean values and standard deviations over three replicates.
Means with different letters are significantly different (P<0.05) as Tukey's HSD.

The C* value, which describes the degree of saturation, purity or intensity of color, increases by increasing the heating time. High C* values can be evaluated as high degree of browning in soymilk (Kwok et al., 1999). h° value of raw soymilk was reported before as 108° describing with yellow region (90°-108°). It is also known that upon heating, the h° value of soymilk decreases towards to reddish-yellow region (below 90°). According to our results, h° value was in reddish-yellow region due to heat treatment. C* values were found to be significantly different for each ingredient (P<0.05) while h° values of SoMP and SPI samples were not significantly different from each other.

In a porous system, wettability, which means liquid penetration as a result of capillary action, is generally dependent on the particle size, surface area, density, porosity and surface activity (Santana et al., 2017). This value defines the capacity of particle to absorb water on its surface. Wettability was measured visually and results were expressed as time of the powder particle absorb the water. Long wetting time means less wettability of the powder product. Particle size is the primary factor affecting wettability (Koç et al., 2014). Smaller particles have a larger specific surface area (the ratio of the surface area to the mass), so that each particle cannot be wetted individually. The nature of the particle surface also affects the wettability. For instance, wettability reduces in the presence of free fat on the surface (Santana et al., 2017). The wettability values measured are shown

in Table 4.5 along with other physical properties. SPI samples had the lowest wettability due to its protein character. Previous studies showed that the powder ingredients obtained by freeze-drying method have more porous structure (Caparino et al., 2012). Thus, soymilk powder had a higher wettability than other powder ingredients as expected. Wettability is also affects the solubility character of powders. The poor wettability feature causes a poor solubility. SPI showed poor wettability character, so that it showed poor solubility. Solubility character of powder is affected by particle size and heat treatments. Lower the particle size, lower the solubility and and flowability of powders. (Koç et al., 2014). Generally, porous systems are expected to have high solubility (Rogers et al., 2008). But soymilk powder did not show good solubility character despite its high porous structure.

Previously, Ishiwu et al. (2014) compared the cow's milk powder and spray-dried soymilk powders in terms of solubility and wettability. They concluded that cow's milk powder had better solubility and wettability than soymilk powders (Ishiwu et al., 2014). Although soymilk powder production process was different from our study, the results were similar. This is because of the surface area, surface charge, density, porosity and surface activity also affects the solubility (Koç et al., 2014). Caparino et al. (2012) reported that the solubility of freeze-dried mango powders was significantly lower than the solubility of spray-dried mango powders. They claimed that the cell structures of the freeze-dried mango powders were not disrupted, so that less solids dissolve to be supernatant (Caparino et al., 2012). A previous study reported that soya protein isolate had 17.4 % solubility (Kinsella, 1979) which is very close to our results (17.01 %). Statistically, wettability and solubility values of samples were significantly different from each other ($P<0.05$).

Table 4.5 Physical properties of powder products

Sample	Carr Index (CI) (%)	Hausner Ratio (HR)	Bulk density (g/ml)	Tapped bulk density (g/ml)	Wettability (s)	Solubility (%)
SMP	36.7 ∓ 0.46^{a}	1.55 ∓ 0.010^{bc}	0.415 ∓ 0.009^{a}	0.63 ∓ 0.009^{a}	1026 ∓ 5.5^{a}	77.92 ∓ 0.080^{a}
WMP	38.3 ∓ 0.38^{b}	1.62 ∓ 0.017^{b}	0.326 ∓ 0.005^{b}	0.52 ∓ 0.015^{b}	1235 ∓ 20.2^{b}	70.00 ∓ 0.070^{b}
SoMP	42.3 ∓ 0.46^{c}	1.75 ∓ 0.044^{a}	0.253 ∓ 0.004^{c}	0.42 ∓ 0.025^{c}	766 ∓ 17.2^{c}	40.00 ∓ 0.020^{c}
SPI	31.9 ∓ 0.53^{d}	1.47 ∓ 0.056^{c}	0.234 ∓ 0.005^{d}	0.33 ∓ 0.009^{d}	1607 ∓ 11.4^{d}	17.08 ∓ 0.070^{d}

Results are given with mean values and standard deviations over three replicates.

Means with different letters are significantly different ($P<0.05$) as Tukey test.

The Hausner Ratio (HR) has a great potential to be a fingerprint criterion in assessing the behavior of powder products. HR is calculated using bulk and tapped bulk densities and describes the mobility of the powder product (Ortega-Rivas et al., 2006). HR also indicates the powder cohesiveness. The decreasing HR is regarded as reduction of cohesiveness (Abdullah and Geldart, 1999). The powders are divided into four different groups in order to evaluate the flowability with respect to HR values. Powders with a HR less than 1.25, flow more easily and powders with HR greater than 1.4, have problems in flowing. Previously, it was stated that HR is very sensitive to particle shape, so this ratio can be used also as the powder shape index (Ortega-Rivas et al., 2006).

The Hausner Ratio ranges defining flowability of powders are shown below (Ortega-Rivas et al., 2006);

1.0 < HR < 1.1	Easy flowing
1.1 < HR < 1.25	Moderately flowable
1.25 < HR < 1.40	Difficult to flow
HR > 1.4	Very difficult to flow

Hausner ratio values of powder ingredients are shown in Table 4.5. The values showed that SMP was better in flowability than WMP and SoMP had the most difficult flow behavior while SPI had the easiest one. This is clarified by the fact that soy-oil increases the cohesiveness and adhesiveness of powder products more than milk fat does. Particle shape also affects the flow. Spherical particles flow more easily than porous particles (Koç et al., 2014). HR is known to decrease with increasing particle size, which also means that diminish the cohesiveness (Abdullah and Geldart, 1999). Fat-containing samples (WMP and SoMP) show more viscous (cohesive) character and they had more worse flow behavior than other ingredients. Spray-dried soymilk powders were reported to have HR between 1.416-1.603 by Nguyen et al. (2018). The fat-containing samples used in this study had higher HRs than these values while SMP and SPI had lower. Jinapong et al. (2008) found that HR of spray-dried soymilk powder was 1.67

(Jinapong et al., 2008). According to Tukey grouping test, SoMP was statistically similar to WMP and SPI samples. In order to define the cohesiveness levels, the Hausner Ratio ranges were determined as follows (Jinapong et al., 2008);

HR < 1.25	free flowing
1.2 < HR <1.4	transitional
HR > 1.4	high cohesive

According to these ranges, all of the powder products had high cohesiveness level. WMP and SoMP samples were found to exhibit higher cohesiveness due to their fat content.

To determine the flowability levels, the Carr Index (CI), which is also known as compressibility index, are grouped as follows (Jinapong et al., 2008);

CI <15	Very good flowability
15 < CI <20	Good flowable
20 < CI <35	Fair flowability
35 < CI <45	Bad flowability
CI > 45	Very bad flowability

Carr Index (%) values of powder ingredients are shown in Table 4.5. According to these values, there is no powder ingredient that can flow very good or good. SPI sample showed fair flow and SMP, WMP and SoMP samples showed bad flow attribute. Compressibility index values of spray-dried soymilk powder were reported to change in a range of 29.40-37.60. In another study, CI value of spray-dried soymilk powder was found as 40 % (Jinapong et al., 2008). Generally, spray-dried products are spherical in shape (Koç et al., 2014) which may cause the product to flow more easily. In contrast, freeze-dried samples have more porous structure with poor flow character. It is easier to compress the spherical and smooth particles when compared to porous particles. Both HR and CI values

showed that SoMP had the poorest flow behavior. All the samples were significantly different from each other ($P<0.05$) with respect to Carr Index value.

Powder ingredients are thought to affect the flow properties of chocolate due to their flow characteristics. Fat-containing powder samples are expected to improve the flow properties of molten chocolate with cocoa butter. This will be discussed in the section on chocolate rheology results.

4.3 Some Functional Properties of Powder Ingredients

Functionality is defined as any characteristic that affects the application and utilization of the product apart from its nutritional value, which is important to decide a food ingredient to use in different food mediums (Martinez, 1979). As functionality, water holding capacity, oil binding capacity, emulsion capacity and emulsion stability of SoMP and SPI were evaluated. These values are shown in Table 4.6.

Table 4.6 Functional properties of SoMP and SPI ingredients

Parameter	SoMP	SPI
WHC*	0.96 ∓ 4.210	3.52 ∓2.360
OBC**	1.42 ∓ 0.687	2.26 ∓ 0.954
EC (%)	51.7 ∓ 2.08	41.0 ∓ 1.00
ES (%)	81.5 ∓ 1.72	65.5 ∓ 2.04

Results are given with mean values and standard deviations over three replicates.

* g water/g dry matter

** g oil/g dry matter

Water holding capacity (WHC) was found 0.96 g water/g dry matter for SoMP samples, while 3.52 g water/g dry matter was found for SPI samples. Generally, increasing fat content results lower WHC due to reducing available hydrophilic binding sites to hold by protein (Heywood et al., 2002). Nguyen D.Q. et al. (2017) reported that the WHC of spray-dried soymilk powder ranged from 0.7 to 1.10 g/g (Nguyen et al., 2018). Enders (2001) previously reported that soy protein isolate

has a high water-binding capacity and can reach up to 400 % (Enders, 2001). The results found by the study of Yalçın S. (2011) are close our results. They reported that the water holding capacity was 338 % (3.38 g/g) of the infrared treated soy flour samples (Yalcın, 2011). It is known that the lyophilized samples have more open porous structure and improved reconstitution properties (Ortega-Rivas et al., 2006). Water holding capacity is also related to the agglomeration of the particles. Agglomerated particles cause increased particle size and water retention between the cell walls (Nguyen et al., 2018). In our study, SoMP had good water holding capacity values but the SPI had greater capacity than SoMP.

The oil binding/holding capacity (OBC) was found as 1.42 g oil/g dry matter for SoMP, while 2.26 g oil/g dry matter for SPI. Yalcın S. (2012) reported that the oil binding capacity of the soybean oil for Adasoy variety was found to be 113.1 % (1.13 g/g) and 130.5 % (1.31 g/g) for the Nazlıcan variety. Similar results were obtained in our study with the Nazlıcan variety results, which is the same variety that used in this study. Generally, oil binding is commonly related to the physical entrapment of oil by the protein. The rising protein content results with higher OBC of product (Heywood et al., 2002).

The amount of water released by centrifugation from the emulsion was used to calculate the emulsion capacity (EC). The results of the analyzes showed that SoMP samples had an EC of 51.7 % and SPI samples had 41 %. A previous study conducted this measurement with sunflower flour, concentrate and isolate. Sunflower flour has superior emulsifying capacity which had less protein content than others. The reason is the preparation process of isolates and concentrates causes denaturation of proteins and their protein content consists of less soluble ones than flours (Wilding et al., 1984). It was stated before that EC of powders are related to their fat content. Increasing fat content of powder increases the EC (Han and Khan, 1990). It was also seen in our results that SoMP had higher fat content than SPI, hence it had higher EC.

Emulsion stability (ES) analyses were conducted by calculating the percentage of oil released from the centrifuged emulsion after thermal application (Table 4.6). According to the results, it was concluded that SoMP samples had an emulsion

stability of 81.5 % and SPI samples had 65.5 %. It has been previously reported that there was a good correlation between emulsion capacity and stability in soy products (Wilding et al., 1984). In this study, emulsion stability and capacity were found correlated. Soy proteins are used to enhance and stabilize the emulsions and improve the texture of food systems. pH affects the capacity and stability of emulsions significantly (Kinsella, 1979; Volkert and Klein, 1979). Zayas and Lin (1989) studied the emulsion capacity and stability of corn germ proteins and they concluded that increasing fat content made a positive effect on emulsion stability which was related to viscosity of emulsion (Zayas and Lin, 1989). The present study also concluded the same way. SoMP was more stable than SPI due to its fat content.

4.4 Moisture Content and Water Activity of Chocolates

The moisture contents and the water activities of the chocolates are shown in Table 4.7.

Table 4.7 Moisture content and water activity values of chocolate samples

Sample	Moisture content (%)	Water activity
SMC2	3.33 ∓ 0.007	0.233 ∓ 0.0035
SMC4	3.16 ∓ 0.040	0.219 ∓ 0.0036
SMC6	3.71 ∓ 0.096	0.219 ∓ 0.0042
WMC2	2.94 ∓ 0.016	0.223 ∓ 0.0025
WMC4	2.73 ∓ 0.006	0.225 ∓ 0.0023
WMC6	3.14 ∓ 0.015	0.242 ∓ 0.0071
SoMC2	2.72 ∓ 0.020	0.268 ∓ 0.0243
SoMC4	2.42 ∓ 0.030	0.239 ∓ 0.0610
SoMC6	2.44 ∓ 0.030	0.224 ∓ 0.0130
SPC2	1.72 ∓ 0.021	0.239 ∓ 0.0112
SPC4	2.26 ∓ 0.055	0.222 ∓ 0.0053
SPC6	2.19 ∓ 0.005	0.238 ∓ 0.0061

Results are given with mean values and standard deviations over three replicates.

Moisture content of chocolate samples changed between 1.72-3.71 % and water activity values ranged between 0.219-0.268 which is a very narrow range. Chocolate water activity generally ranges between 0.4-0.5. This value is influenced by producing parameters such as conching and refining (Konar, 2013). It was proven that 0.4–0.5 water activity enables Salmonella to survive in chocolate products although it cannot grow in these conditions (Beckett, 2009). The values obtained in this study were lower than this range. However, no correlation could be established between water activity and moisture content values and conching times in this study.

4.5 Color and Texture Profile Analysis

The color values varied in very small ranges. It was observed that these values increased or decreased during the conching process. The color values are shown in Table 4.8.

Table 4.8 Color values of chocolates

Products	L*	a*	b*	C*	h°
SMC2	30.8 ∓ 0.24^{bc}	7.2 ∓ 0.10^{cd}	7.4 ∓ 0.13^{cd}	10.3 ∓ 0.15^{cde}	45.7 ∓ 0.30^{bc}
SMC4	32.3 ∓ 1.72^{bcd}	8.1 ∓ 0.62^{a}	9.0 ∓ 1.29^{de}	10.8 ∓ 0.54^{def}	45.8 ∓ 1.60^{bcd}
SMC6	31.4 ∓ 0.14^{cd}	7.6 ∓ 0.34^{e}	7.7 ∓ 0.61^{f}	9.0 ∓ 0.37^{g}	43.5 ∓ 0.51^{d}
WMC2	29.5 ∓ 0.63^{a}	7.2 ∓ 0.51^{a}	7.2 ∓ 0.62^{ab}	12.1 ∓ 1.37^{a}	48.1 ∓ 2.00^{bcd}
WMC4	29.8 ∓ 0.75^{a}	7.5 ∓ 0.17^{a}	7.8 ∓ 0.61^{a}	10.4 ∓ 0.30^{a}	45.9 ∓ 1.12^{bcd}
WMC6	30.3 ∓ 1.17^{cd}	7.3 ∓ 0.07^{d}	7.5 ∓ 0.36^{def}	9.7 ∓ 0.49^{ef}	44.4 ∓ 1.30^{cd}
SoMC2	30.8 ∓ 0.38^{ab}	7.2 ∓ 0.24^{bc}	7.9 ∓ 0.47^{b}	10.8 ∓ 0.48^{b}	45.5 ∓ 0.62^{ab}
SoMC4	28.0 ∓ 1.66^{bc}	6.7 ∓ 0.46^{b}	6.6 ∓ 0.83^{def}	10.7 ∓ 0.50^{bc}	47.1 ∓ 0.98^{cd}
SoMC6	29.5 ∓ 0.39^{bcd}	6.6 ∓ 0.23^{e}	6.2 ∓ 0.39^{de}	10.6 ∓ 0.94^{cde}	45.1 ∓ 1.86^{cd}
SPC2	30.1 ∓ 1.015^{cd}	6.9 ∓ 0.29^{e}	6.8 ∓ 0.45^{cd}	10.1 ∓ 0.79^{bcd}	44.9 ∓ 0.84^{a}
SPC4	29.3 ∓ 2.073^{d}	7.5 ∓ 0.52^{cd}	7.5 ∓ 0.86^{def}	9.5 ∓ 0.91^{ef}	44.5 ∓ 1.62^{d}
SPC6	29.3 ∓ 0.478^{d}	6.8 ∓ 0.24^{de}	6.8 ∓ 0.57^{ef}	9.6 ∓ 0.55^{fg}	44.8 ∓ 1.69^{cd}

Results are given with mean values and standard deviations over three replicates.

Means with different letters are significantly different (P<0.05) as Tukey test.

The color changes are not related to the parameters in chocolate processing. Normally, the properties of the components used in chocolate affect the color value. But, no significant differences were observed between the color values of the chocolates produced with different conching times. Although, the lowest brightness value was observed in the SoMC4 samples, no correlation could be established between the ingredients of the chocolates and no significant difference ($P>0.05$) was observed.

Texture analyses were evaluated as snap of the chocolate bars. Maximum load was defined as hardness of the chocolates. Results showed that SPC chocolates were the hardest and SoMC chocolates were the softest samples. Generally, fat-containing samples were softer and easy to fracture. SMC chocolates were found to be softer than SPI chocolates due to its milk content, which causes softening of the structure (Afoakwa, 2010). The hardness of chocolates decreased as increasing conching time for all chocolates. The reduction in particle size (refining) and the smoother particles (conching) in the chocolates result in a more silky texture of final product (Afoakwa et al., 2009; Beckett, 2009). Therefore, a softer texture and more easily snap products were obtained after 6 hours of conching for all samples.

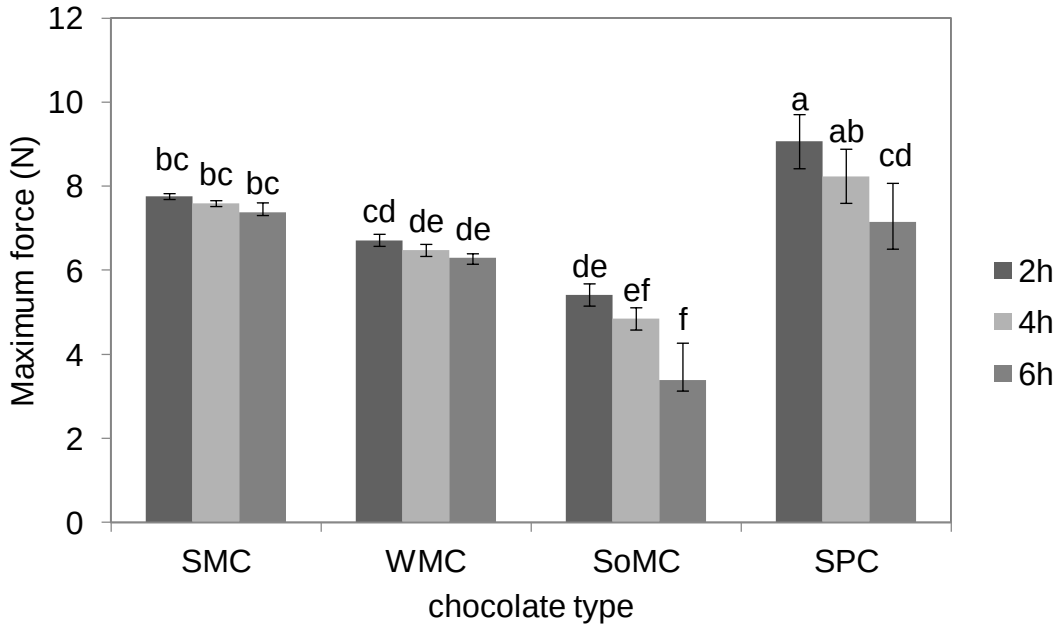


Figure 4.2 Hardness values of chocolates

4.6 Rheological Properties

The data obtained from rheological measurements were evaluated according to Casson, Herschel-Bulkley and Bingham models and rheological parameters were calculated. Molten chocolate is known to exhibit non-Newtonian flow behavior. In this study, molten chocolate samples showed the same attribute. The rheological measurements of the chocolates were found to be compatible with Casson, Herschel-Bulkley and Bingham models due to the fact that the correlation coefficients were close to 1 (R^2>0.99). In the literature, it was reported that particle size affects the plastic viscosity and yield stress values which are important rheological parameters (Afoakwa et al., 2008). Table 4.9 shows the model constants according to Casson, Herschel-Bulkley and Bingham models of molten chocolates with different formulations and conching times.

Casson model is the first to express the rheological properties of molten chocolates (Chevalley, 1975) and still the most common model. In this section; Casson, Herschel-Bulkley and Bingham viscosities were determined at 50 s^{-1} shear rate and 40 °C for 2-4-6 hours conched samples (Table 4.10) and evaluated with particle size distribution values. Results showed that the Casson, Herschel-Bulkley and Bingham models were providing a perfect fit to describe the flow behavior of molten chocolate regardless of the powder ingredient type.

A certain force is needed to exceed a certain threshold for molten chocolate to start flowing. This threshold is known as yield stress (τ_0), which is influenced by the producion conditions and ingredient types. According to Casson model, the yield stress values of 2 hours conched samples were significantly different (P<0.05) from each other. But 6 hours conched SMC, WMC and SoMC samples were similar considering Tukey grouping. Casson, Herschel-Bulkley and Bingham models showed that yield values were significantly different between 2 and 6 hours conched products for all the chocolate types in all models. Yield stress of samples are shown in Table 4.9.

Table 4.9 Casson, Herschel-Bulkley and Bingham model constants and correlation coefficients for chocolates obtained at 40°C

Sample	Casson		Herschel-Bulkley			Bingham	
	τ_0(Pa)	R^2	τ_0(Pa)	n	R^2	τ_0(Pa)	R^2
SMC2	23.55 ∓ 0.591^{a}	0.994	29.33 ∓ 0.518^{a}	0.771 ∓ 0.0063	0.998	39.13 ∓ 0.768^{a}	0.992
SMC4	14.97 ∓ 0.488^{efg}	0.996	21.04 ∓ 0.592^{cde}	0.864 ∓ 0.0004	0.999	24.83 ∓ 0.598^{def}	0.997
SMC6	16.58 ∓ 0.516^{cd}	0.995	22.51 ∓ 0.420^{cd}	0.854 ∓ 0.0044	0.999	26.32 ∓ 0.609^{d}	0.997
WMC2	7.30 ∓ 0.148^{i}	0.996	11.47 ∓ 0.143^{g}	0.895 ∓ 0.0101	0.999	13.88 ∓ 0.613^{h}	0.998
WMC4	8.05 ∓ 0.458^{i}	0.995	12.20 ∓ 0.573^{g}	0.893 ∓ 0.0006	0.999	14.23 ∓ 0.824^{h}	0.998
WMC6	15.30 ∓ 0.585^{def}	0.994	20.59 ∓ 0.714^{def}	0.862 ∓ 0.0046	0.999	23.57 ∓ 0.528^{efg}	0.997
SoMC2	14.13 ∓ 0.625^{fg}	0.996	19.40 ∓ 0.754^{ef}	0.850 ∓ 0.0011	0.999	23.12 ∓ 0.523^{fg}	0.997
SoMC4	13.50 ∓ 0.422^{gh}	0.995	18.72 ∓ 0.488^{f}	0.863 ∓ 0.0010	0.999	21.82 ∓ 0.827^{g}	0.997
SoMC6	16.34 ∓ 0.509^{cde}	0.995	21.62 ∓ 0.572^{cd}	0.847 ∓ 0.0011	0.999	24.96 ∓ 0.735^{de}	0.997
SPC2	12.34 ∓ 0.394^{h}	0.996	22.68 ∓ 0.273^{c}	0.954 ∓ 0.0194	0.998	24.72 ∓ 0.372^{def}	0.998
SPC4	17.71 ∓ 0.608^{c}	0.996	25.33 ∓ 0.973^{b}	0.873 ∓ 0.0068	0.999	29.60 ∓ 0.526^{c}	0.997
SPC6	19.80 ∓ 0.646^{b}	0.995	27.00 ∓ 0.647^{b}	0.854 ∓ 0.0024	0.999	31.67 ∓ 0.618^{b}	0.997

** Results are given with mean values and standard deviations over three replicates.

** Means with different letters are significantly different (P<0.05) as Tukey test.

Apparent viscosities were calculated according to the models. For all models, apparent viscosities of molten chocolates determined for constant shear rate of 50 s^{-1} at 40°C, which are shown in Table 4.10.

Table 4.10 Apparent viscosities of chocolates at 50 s^{-1} shear rate

Sample	Casson viscosity (Pa.s)	Herschel- Bulkley viscosity (Pa.s)	Bingham viscosity (Pa.s)
SMC2	3.849 ∓ 0.010[b]	3.879 ∓ 0.008[b]	3.885 ∓ 0.080[b]
SMC4	2.709 ∓ 0.018[d]	2.749 ∓ 0.018[d]	2.722 ∓ 0.019[d]
SMC6	2.583 ∓ 0.005[d]	2.633 ∓ 0.022[d]	2.606 ∓ 0.020[d]
WMC2	2.159 ∓ 0.072[f]	2.187 ∓ 0.081[f]	2.160 ∓ 0.078[f]
WMC4	1.854 ∓ 0.021[g]	1.883 ∓ 0.021[g]	1.863 ∓ 0.023[g]
WMC6	2.174 ∓ 0.030[f]	2.203 ∓ 2.203[f]	2.188 ∓ 0.030[f]
SoMC2	2.405 ∓ 0.038[e]	2.438 ∓ 0.038[e]	2.420 ∓ 0.038[e]
SoMC4	2.242 ∓ 0.014[f]	2.275 ∓ 0.015[f]	2.255 ∓ 0.015[f]
SoMC6	2.199 ∓ 0.016[f]	2.230 ∓ 0.016[f]	2.210 ∓ 0.017[f]
SPC2	4.381 ∓ 0.109[a]	4.430 ∓ 0.109[a]	4.406 ∓ 0.109[a]
SPC4	3.313 ∓ 0.030[c]	3.360 ∓ 0.030[c]	3.328 ∓ 0.40[c]
SPC6	3.187 ∓ 0.030[c]	3.232 ∓ 0.031[c]	3.205 ∓ 0.28[c]

Results are given with mean values and standard deviations over three replicates.
Means with different letters are significantly different (P<0.05) as Tukey test.

The viscosity decreases as the shear rate increases in chocolates (Afoakwa et al., 2009). This was observed the same in all chocolate types and conching times. According to the Tukey grouping test, the viscosities of 2, 4 and 6 hour conched chocolates showed significant differences (P<0.05), except for WMC6 and SoMC6 samples, between the ingredient type and all models gave consistent results (lettering) with each other. Except for WMC samples, the 2 and 6 hours conched chocolates showed significant difference (P<0.05) in the viscosities while no significant difference was observed (P>0.05) in the viscosities between the 2 and 4 hour conched chocolates. WMC samples showed no significant differences between 2 and 6 hour processing time. Casson, Herschel-Bulkley and Bingham viscosities of molten chocolates were calculated and shown in Figure 4.3 with error bars.

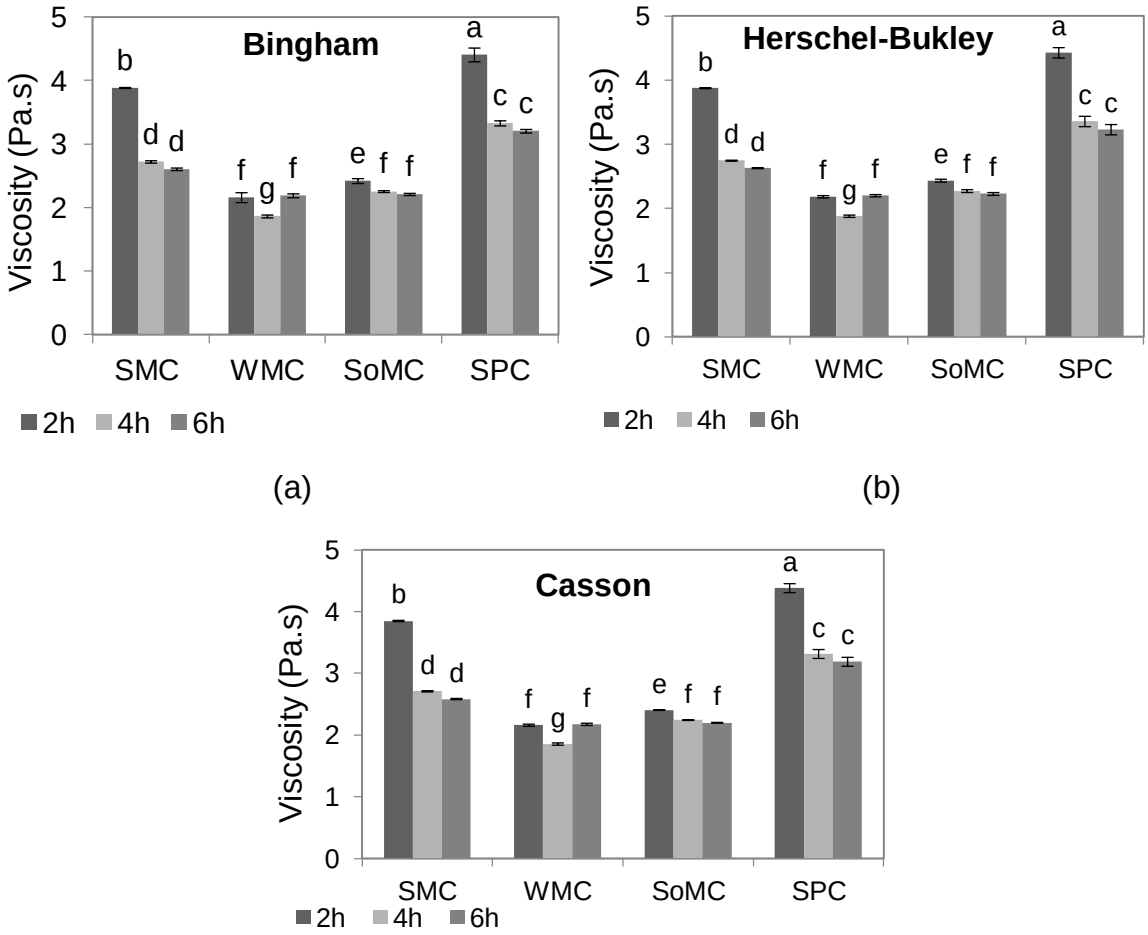


Figure 4.3 Viscosity values of chocolates according to Bingham (a), Herschel-Bulkley (b) and Casson models (c)

The apparent viscosity of chocolate is usually affected by its fat content and surfactants (e.g. lecithin) (Gonçalves and Lannes, 2010). The highest viscosity among all the samples was observed in SPC2 chocolate samples for all models. Another high value was obtained by SMC2 samples. These two samples do not contain any other fat than cocoa butter. Viscosity of samples WMC and SoMC was lower than the other two samples. In this case, it can be concluded that the presence of soy and milk fats have a lowering effect on the viscosity of molten chocolates. Cıkrıkcı et al. (2016) concluded that as a result of the high amount of fat in the white chocolate samples, the particle-particle interactions were reduced, the mobility increased, and the viscosity decreased (Cikrikci et al., 2017). Similarly, in the samples prepared with soymilk powder and whole milk powder, the mobility increased and the viscosity decreased due to the increase of fat phase. Furthermore, emulsifiers are known to influence the molten chocolate flow

characteristics (Beckett, 2009) and the soymilk powder had an extra lecithin content than other ingredients naturally. A previous study mentioned that spray dried soymilk powder contains 0.2 % lecithin content (Ang et al., 1985). This also may had contributed to reduction of the viscosity on SoMC samples.

Refining and conching processes are essential steps for the development of viscosity, texture and formation of the final flavor. Diminishing acidity, eliminating undesired volatile compounds, decreasing moisture, development the unique flavor of chocolate, obtaining the smoother surfaced particles and improving the flow properties are carried out by these processes (Afoakwa, 2010; Afoakwa et al., 2009). Comparing the conching times, it was observed that the apparent viscosity decreased with increasing conching time except for WMC samples. Because the milk fat has a tendency to coat the particles and replace the cocoa butter that surrounds the particles. The increase of apparent viscosity of WMC samples may be attributed to releasing cocoa butter from particles. The process also contribute to release of moisture, crystallizing the amorphous lactose, forming a bridge between the milk powder particles and increasing the viscosity of the chocolates (Afoakwa et al., 2009). In this case, increasing viscosity of WMC samples may also be explained by increasing amorphous form of lactose. The apparent viscosity (μ_0) against shear rate (γ) was used to define the rheological behavior of 2, 4 and 6 hours (Figure 4.3-4.5) conched chocolates by Casson model.

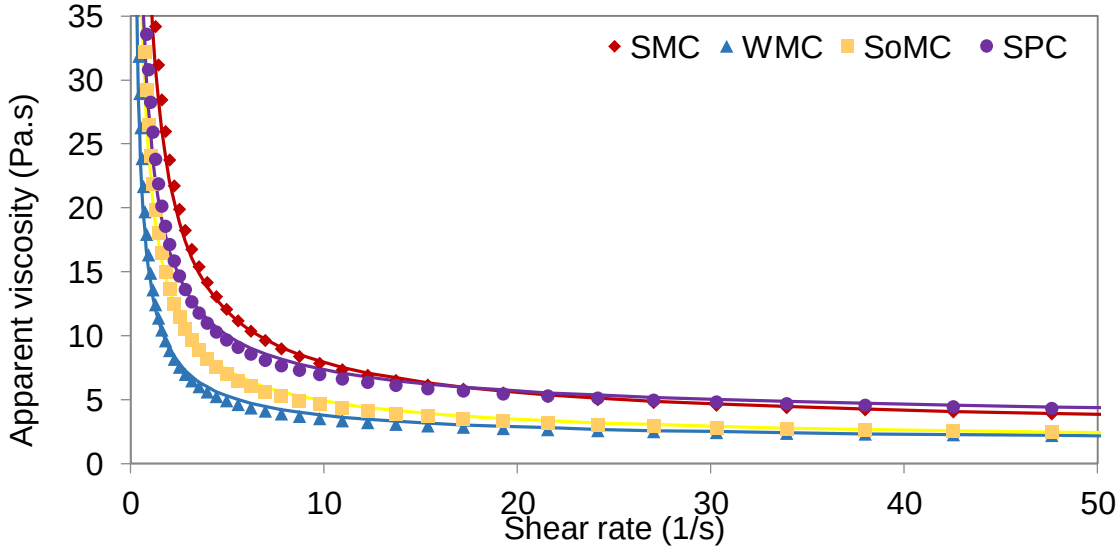


Figure 4.4 Apparent viscosity versus shear rate of 2 hours conched chocolates by Casson model

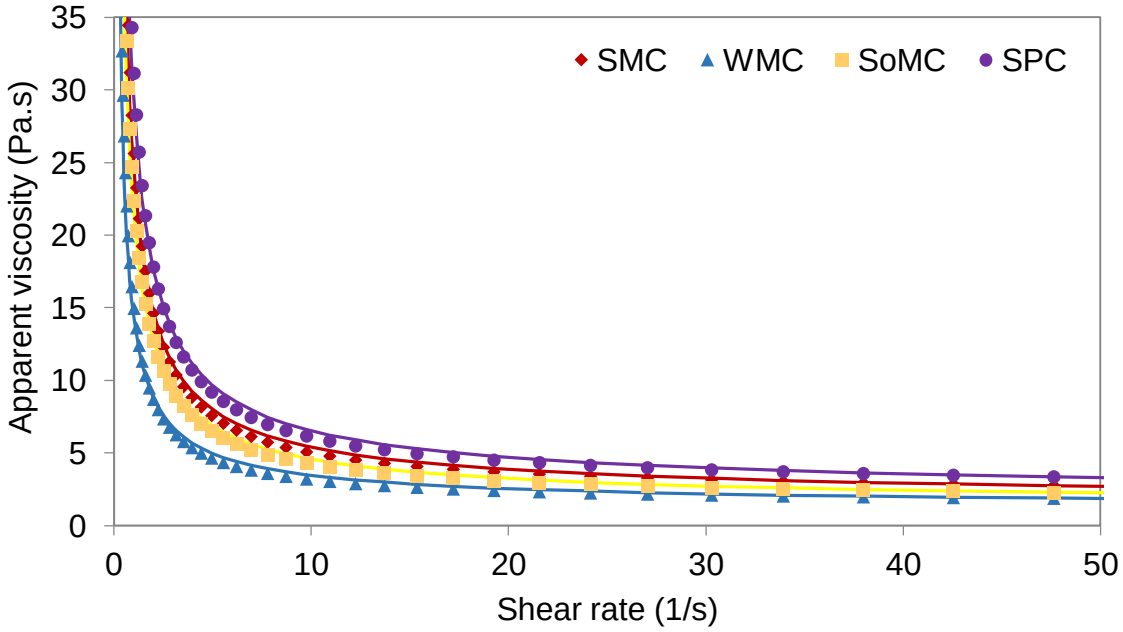


Figure 4.5 Apparent viscosity versus shear rate of 4 hours conched chocolates by Casson model

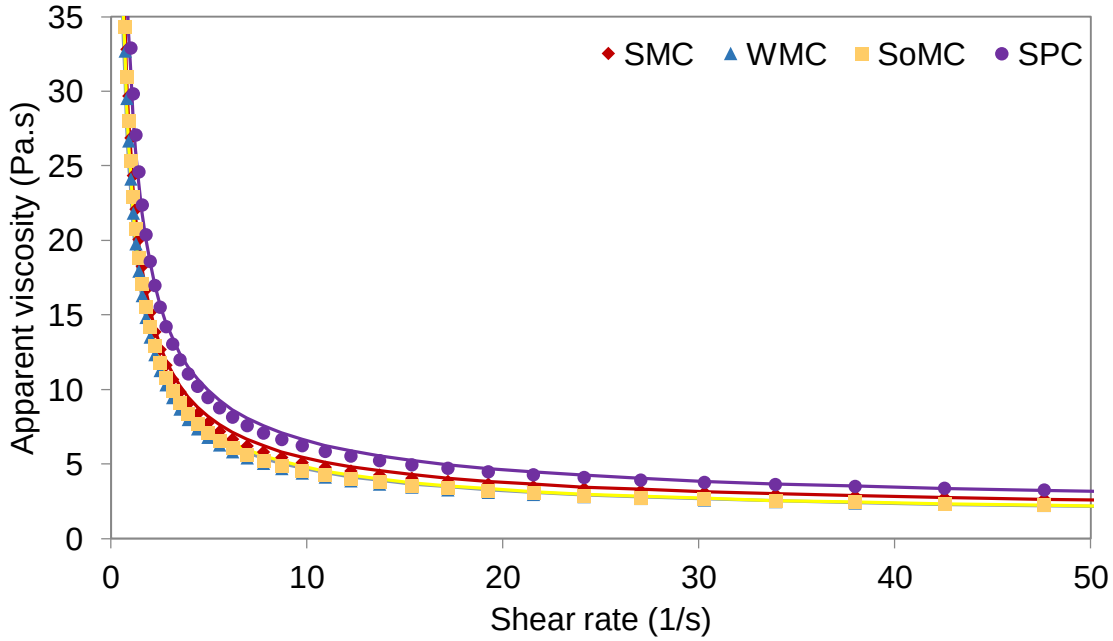


Figure 4.6 Apparent viscosity versus shear rate of 6 hours conched chocolates by Casson model

In the graphs, the symbols represent the apparent viscosities, while the lines represent the Casson model. The apparent viscosity decreases as the shear rate increases, which demonstrates the pseudoplastic nature of chocolate (Glicerina et al., 2014). As can be seen from the graphs above, the apparent viscosities of the samples gave more similar results with each other as the process time increases.

Yield stress is the necessary energy that initiates the chocolate flow and influences the coating of solid surfaces. According to Afoakwa et al. (2008), increasing particle size caused significant reductions in yield stress. Particle size reduction causes to increase the particle-particle interactions. Yield stress values were related to increasing specific surface area. Statistically, ANOVA results showed that yield stress value of Casson, Herschel-Bulkley and Bingham models were significantly influenced ($P<0.05$) by ingredient type and conching time. All models were consistent with each other.

Fat-containing powder ingredients (SoMP and WMP samples) had higher HR and CI values which means lower flowability and higher cohesiveness for a powder. However, they have improved the flow properties of the product when they added to chocolate. SoMC and WMC samples had lower viscosity than others which included more cohesive ingredients.

4.7 Particle Size Distribution

Particle Size Distribution (PSD) results were obtained as $D_{[3,2]}$, $D_{[4,3]}$, D_{10}, D_{50} and D_{90} and specific surface area, which were presented Table 4.11. D_{10}, D_{50}, D_{90} represent the 10 %, 50 % and 90 % of all particles finer than that size respectively. $D_{[3,2]}$ represents the Sauter mean diameter and $D_{[4,3]}$ represents the DeBroukere mean particle diameter.

Table 4.11 Particle size distribution data for chocolates

Samples	D[3;2] µ	D [4;3] µ	Dv(10) µ	Dv(50) µ	Dv(90) µ	Dv(97) µ	ssa*
SMC2	5.32 ∓ 0.085	16.60 ∓ 0.000	2.35 ∓ 0.035	9.82 ∓ 0.283	37.50 ∓ 0.707^{cd}	68.10 ∓ 0.000	1141 ∓ 19.8
SMC4	4.82 ∓ 0.064	16.35 ∓ 2.475	2.17 ∓ 0.057	8.88 ∓ 0.106	33.40 ∓ 1.697^{de}	72.35 ∓ 8.415	1246 ∓ 17.0
SMC6	4.62 ∓ 0.035	16.10 ∓ 0.000	2.07 ∓ 0.028	8.42 ∓ 0.007	35.70 ∓ 0.990^{cd}	84.60 ∓ 0.707	1300 ∓ 9.9
WMC2	4.59 ∓ 0.127	13.70 ∓ 1.273	2.04 ∓ 0.042	8.44 ∓ 0.403	29.10 ∓ 3.394^{efg}	60.45 ∓ 9.546	1308 ∓ 36.8
WMC4	4.72 ∓ 0.106	15.15 ∓ 0.354	2.11 ∓ 0.064	8.72 ∓ 0.191	32.20 ∓ 1.697^{def}	72.45 ∓ 3.465	1273 ∓ 28.3
WMC6	4.29 ∓ 0.141	12.05 ∓ 0.636	1.91 ∓ 0.057	7.55 ∓ 0.339	25.55 ∓ 1.485^{g}	48.70 ∓ 3.111	1401 ∓ 46.0
SoMC2	5.37 ∓ 0.028	16.35 ∓ 0.354	2.43 ∓ 0.007	9.65 ∓ 0.092	36.15 ∓ 0.495^{cd}	68.70 ∓ 0.424	1119 ∓ 6.4
SoMC4	4.88 ∓ 0.042	15.25 ∓ 4.031	2.20 ∓ 0.014	8.53 ∓ 0.049	27.05 ∓ 1.061^{fg}	49.75 ∓ 7.566	1230 ∓ 11.3
SoMC6	4.46 ∓ 0.007	11.65 ∓ 0.071	2.01 ∓ 0.007	7.68 ∓ 0.021	24.30 ∓ 0.141^{g}	43.10 ∓ 0.141	1346 ∓ 1.4
SPC2	8.05 ∓ 0.170	28.70 ∓ 2.546	3.40 ∓ 0.099	20.40 ∓ 0.424	60.35 ∓ 2.758^{a}	96.80 ∓ 17.253	746 ∓ 16.3
SPC4	7.22 ∓ 0.163	21.20 ∓ 0.566	2.97 ∓ 0.085	16.80 ∓ 0.566	43.45 ∓ 0.495^{b}	61.00 ∓ 0.566	832 ∓ 18.4
SPC6	6.72 ∓ 0.057	19.60 ∓ 0.566	2.77 ∓ 0.021	15.15 ∓ 0.212	40.10 ∓ 0.141^{bc}	58.30 ∓ 0.566	893 ∓ 7.8

* ssa: specific surface area (m^2/kg)

Results are given with mean values and standard deviations over two replicates.

Means with different letters are significantly different (P<0.05) as Tukey test.

Some reported studies stated that D_{90} indicates the large particle content and can be used as a representative for all four parameters (D_{10}, D_{50}, $D_{[3,2]}$, $D_{[4,3]}$) in the chocolate industry. D_{90} has been reported to correlate the sensory character with micrometer measurements for the largest particles. PSD properties have high impact on flow and sensory properties (Servais et al., 2003). D_{90} values of all chocolate samples were shown in Figure 4.7 with error bars.

Results showed that D_{90} values were significantly different ($P<0.05$) for ingredient types and process time. Specific surface area and D_{90} values were also influenced by ingredient type and conching process significantly ($P<0.05$). It was observed that specific surface area and D_{90} had an inverse relationship.

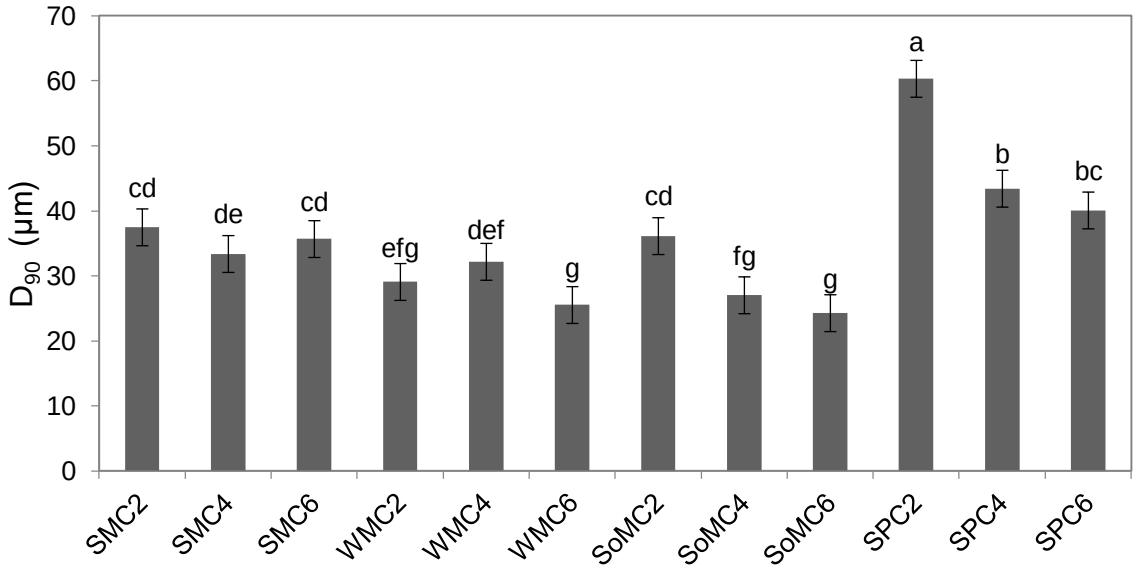


Figure 4.7 D_{90} values of chocolates

SoMC and SPC samples showed a significant decrease from 2 to 6 hours conching time ($P<0.05$) in D_{90} values while SMC and WMC did not. In all samples, specific surface area values of 6 hours conched samples were higher than 2 hours conched samples as opposed to D_{90} values. D_{90} is directly proportional to D_{10}, D_{50}, $D_{[3,2]}$ and $D_{[4,3]}$ but inversely proportional to specific surface area (Afoakwa et al., 2008). Increasing conching time led to significant reduction ($P<0.05$) in D_{90} values and a significant rise ($P<0.05$) in specific surface area values for SoMC and SPC when the 2 and 6 hours conched samples compared. Although a significant reduction was achieved with conching, SPC samples had higher D_{90} and also the

maximum particle size among the samples. The desired particle size (30 μm) was achieved for WMC and SoMC samples while SMC and SPC samples were higher than this value.

Particle size was also reduced by conching process caused by powder ingredients. Afoakwa et al. (2008) stated that particle size distribution (PSD), is affected by Casson plastic viscosity with considerable interactions. PSD results showed that the process extension led to reduce the particle size. As the particle size decreases, the free-fat decreases and the viscosity increases due to increased surface area of the particles to be covered by fat (Afoakwa, 2010; Beckett, 2009). Although the particle size decreased by the process time, viscosity decreased due to the fact that conching process enables the smooth particle formation. Thus, the particle surfaces become smoother while the particle size reduced which leads to reduce the viscosity. Previously, it was stated that increasing particle size caused drastically decreased plastic viscosity while increasing fat content reduces specific surface area (Afoakwa et al., 2008).

4.8 Polymorphic Characteristic of Chocolate Products

Analysis of polymorphic profiles showed that all the chocolate types crystallized in the β form as described before (Beckett, 2009). This can be explained by soy and milk fat do not have much effect on chocolate crystal type due to their low content compared to cocoa butter. Cocoa butter was the dominant fat phase in the system. According to these data, tempering process has been performed successfully. Chocolates were evaluated visually in terms of appearance and no blooming defect was observed.

β form is described as one strong peak at 4.6 Å (Beckett, 2009) which is known as the most stable crysyal type of chocolate. The polymorphic profiles according to X-ray diffraction patterns are shown in Figure 4.8.

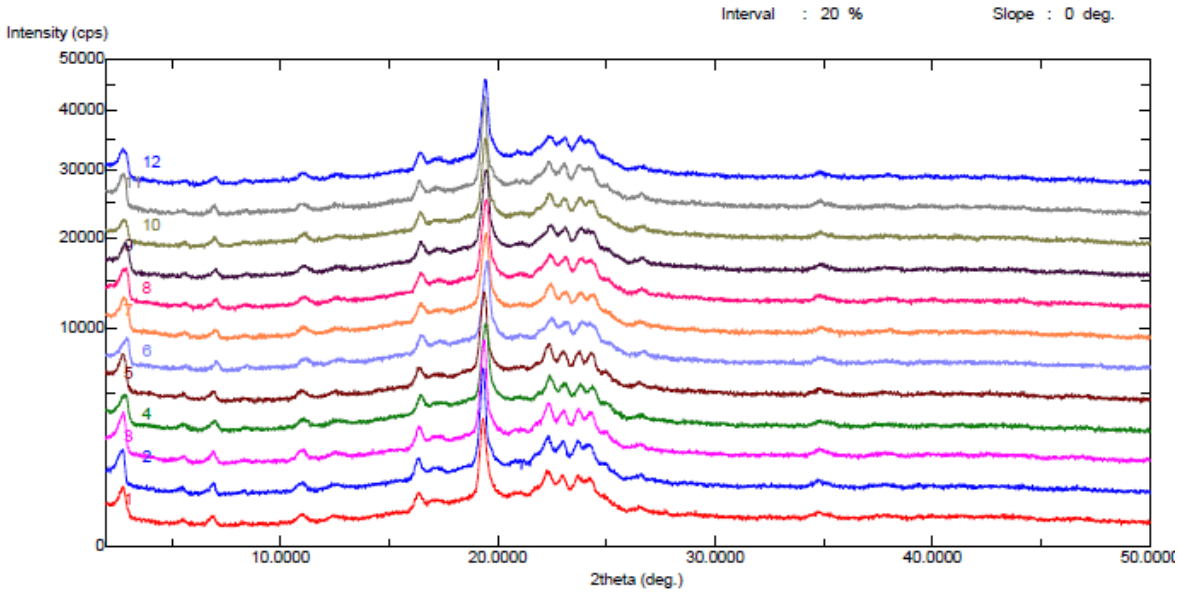


Figure 4.8 X-ray patterns of chocolates

All of the samples gave the same pattern at around 4.6 Å. This means that tempering step was conducted properly and chocolate samples are in the most stable form. But, the analyzes of chocolate samples were conducted after production. However, the effect of storage on the crystal structure and stability of soy-chocolate is uncertain. Thus, in the future studies, the investigation of storage effect would be beneficial.

4.9 Functional Properties of Chocolates

These analyses were carried out in non-fat chocolate samples. Because it is known that the presence of fat in functional analysis makes the results uncertain. The fat of chocolate was separated in two steps using 1:10 (w/v) and 1:5 (w/v) ratios with hexane.

4.9.1 Total Phenolic Content

The total phenolic content (TPC) results was found between 80.10 mg GAE/g dry weight (SPC6) and 129.33 mg GAE/g dry sample (SoMC2). Table 4.12 shows the total phenolic content of chocolates prepared by different ingredient type and conching times.

Table 4.12 Total phenolic contents of chocolates (mg GAE/g dry sample)

Sample	Conching times		
	2	4	6
SMC	112.90 ∓ 1.015^{c}	106.07 ∓ 1.447^{de}	109.30 ∓ 1.200^{de}
WMC	110.67 ∓ 1.242^{c}	102.90 ∓ 1.682^{ef}	105.17 ∓ 0.73^{def}
SoMC	129.33 ∓ 1.193^{a}	117.70 ∓ 1.760^{b}	104.70 ∓ 2.070^{ef}
SPC	101.00 ∓ 1.910^{f}	82.73 ∓ 2.420^{g}	80.10 ∓ 0.917^{g}

Total phenolic content of SoMC and SPC were decreased with conching time, but the decrease of TPC of SMC and WMC samples were not linear. 2 hours conched samples showed that SoMC samples had higher TPC, but at the end of 6 hours conching period, SMC and WMC samples had higher values than SoMC. It is thought that this may be the result of the difference of sensitivity degree of the phenolic substances in milk and soymilk. According to the results, SoMC samples affected from the conching time significantly ($P<0.05$) while SMC, WMC and SPC samples did not. SoMC2 samples gave the highest and SPC6 gave the lowest TPC value among all the samples.

TPC of chocolate samples were analyzed statistically (two way ANOVA), for ingredient type and process time, the results are given in Appendix C. Statistically, it was found that total phenolic content values of chocolates with different ingredient and process times were significantly different from each other ($P <0.05$). The calibration curve of gallic acid is given in Appendix D.

4.9.2 Total Antioxidant Capacity

The total antioxidant capacity (TAC) was determined by DPPH, ABTS and CUPRAC methods and evaluated in terms of mM Trolox/g dry samples as given in Table 4.13.

Table 4.13 Total Antioxidant Capacity of chocolates (mM Trolox/g dry sample)

Sample	ABTS	DPPH	CUPRAC
SMC2	5.25 ∓ 0.136^{cd}	5.17 ∓ 0.125^{de}	5.18 ∓ 0.025^{gh}
SMC4	5.81 ∓ 0.336^{bcd}	5.69 ∓ 0.108^{bcd}	5.71 ∓ 0.035^{d}
SMC6	5.27 ∓ 0.108^{cd}	5.09 ∓ 0.090^{de}	5.11 ∓ 0.025^{hi}
WMC2	5.29 ∓ 0.531^{cd}	5.10 ∓ 0.752^{de}	5.13 ∓ 0.017^{ghi}
WMC4	$5.80 \mp 0.060b^{cd}$	5.57 ∓ 0.101^{bcde}	5.59 ∓ 0.015^{e}
WMC6	5.75 ∓ 0.125^{bcd}	5.38 ∓ 0.170^{cde}	5.43 ∓ 0.031^{f}
SoMC2	5.98 ∓ 0.501^{abc}	5.92 ∓ 0.159^{abc}	5.94 ∓ 0.083^{c}
SoMC4	6.79 ∓ 0.283^{a}	6.58 ∓ 0.093^{a}	6.61 ∓ 0.046^{b}
SoMC6	6.50 ∓ 0.126^{ab}	6.28 ∓ 0.110^{ab}	6.29 ∓ 0.032^{a}
SPC2	5.08 ∓ 0.367^{d}	4.96 ∓ 0.025^{e}	5.03 ∓ 0.032^{i}
SPC4	5.77 ∓ 0.206^{bcd}	5.64 ∓ 0.080^{bcde}	5.70 ∓ 0.036^{d}
SPC6	5.40 ∓ 0.236^{cd}	5.22 ∓ 0.030^{cde}	5.23 ∓ 0.031^{g}

TAC by DPPH method ranged from 4.96 mM Trolox/g dry sample (SPC2) to 6.58 mM Trolox/g dry sample (SoMC4). TAC by ABTS method ranged from 5.08 mM Trolox/g dry sample (SPC2) to 6.79 mM Trolox/g dry sample (SoMC4). The results obtained with CUPRAC ranged from 5.03 mM Trolox/g dry sample (SPC2) to 6.61 mM Trolox/g dry sample (SoMC4).

The results show that the highest values were obtained by ABTS method and the lowest results were obtained by DPPH method. Although the results obtained by CUPRAC method were found to be lower than the ABTS method, it was found to be higher than the results obtained from the DPPH method. All methods were resulted in SoMC samples gave significantly high ($P<0.05$) antioxidant capacity among all the samples.

Komes et al. (2013) determined the antioxidant capacity of milk chocolates according to ABTS method using acetone, methanol and water as solvent for extraction step. They obtained the highest value by acetone extraction and this value was found to be 2.32 mmol/L Trolox (Komes et al., 2013). The same study showed that this value was approximately 3 times higher than the value obtained

by water extraction and approximately 2 times greater than the extraction by methanol. These results indicate that the extraction stage can change the antioxidant capacity values to a great extent and that there are losses at this stage. The results in our study ranged from 4.96 mmol Trolox/L (SPC2) by DPPH to 6.79 mmol Trolox/L (SoMC4) by ABTS. These results are much higher than the results obtained by Komes et al. (2013). Oracz and Nebesny (2016) investigated the effect of roasting conditions (temperature, time and relative humidity) of cocoa beans on the amount of antioxidant content. They conducted the study with the cocoa extracts obtained with 70 °C water. The results varied for ABTS method between 0.48-1.41 mmol TE/g dry sample and for DPPH method 0.32-1.37 mmol TE/g dry sample (Oracz and Nebesny, 2016). These results obtained for cocoa beans are considerably lower than our results. This shows how the QUENCHER method prevent the losses from extraction step and also the isoflovane content of SoMC samples contributed its antioxidant content.

The total antioxidant capacity results showed that the results are not directly related to the conching time. In a previous study, the effect of the conching properties on the antioxidant properties of the chocolates were investigated and it was stated that the conching time and temperature did not have a significant effect on the antioxidant capacity, but with the 3 hours of conching, antiradical properties were developed, significantly (Di Mattia et al., 2014). As a result of analysis, the total antioxidant capacity values of all sample types increased at first. However, the values decreased at further process. In our study, the conching process was carried out for the melted chocolate without external heat, the temperature was maintained by friction only after the initial ingredients were melted (approx. 65 °C). It is thought that this temperature may negatively affect the amount of antioxidants. Nevertheless, no linear relationship was found between this process and TAC values.

TAC of chocolate samples were analyzed statistically (two-way ANOVA), for ingredient type and process time, the results are given in Appendix C. Results were evaluated two-way ANOVA for ABTS, DPPH, CUPRAC and TAC analysis ingredient type and process time. Statistically, it was found that TAC values of chocolates with different ingredient and process times were significantly different

from each other according to ABTS, DPPH and CUPRAC methods ($P<0.05$). The calibration curves of Trolox were prepared for each method (Appendix D).

4.10 Sensory Analysis of Chocolates

Sensory analysis is one of the most important analyzes to determine the consumability of products. Ingredient type is major parameter affecting the sensory perception of chocolate. In this study, sensory evaluation was conducted only to evaluate the different ingredient type in order to compare the consumability of soy-included products with milk chocolates.

Sensory analyzes of chocolates were evaluated in terms of aftertaste, appearance, flavor, odor and odor parameters and scores of samples are shown in Figure 4.9.

A previous study investigated the difference of taste between conched and uncounched chocolates. They stated that conched chocolate was described as less bitter taste and soft structure than unconched samples. They also reported that there was a detectable flavor change between the samples (Hoskin, 1994). It was mentioned before D_{90} was correlated with the sensory character and PSD properties have high impact on flow and sensory properties. Therefore only 6 hours conched chocolatees were used in sensory analysis, which had lower particle size.

Statistical analysis was performed to observe the differences between the samples. Statistically, there was no significant difference ($P>0.05$) from all samples in terms of ingredient. Moreover none of the results were below 3. Therefore, it can be said that all the samples have acceptable after taste, appearance, flavour, texture and odor characteristic. SoMC samples gave the highest flavour and acceptance level among the samples. This shows that soymilk can be use as milk substitution in terms of taste of chocolates. However SoMC samples had the lowest apparance value. SPC samples had lowest aftertaste and texture level. This may be caused by its high particle size. Because particle size is known to affect texture considerabily. According to results, the substitution of

soymilk powder with milk powder is acceptable in terms of taste. However, the results can be interpreted as the texture and appearance properties of soy-chocolates can be improved.

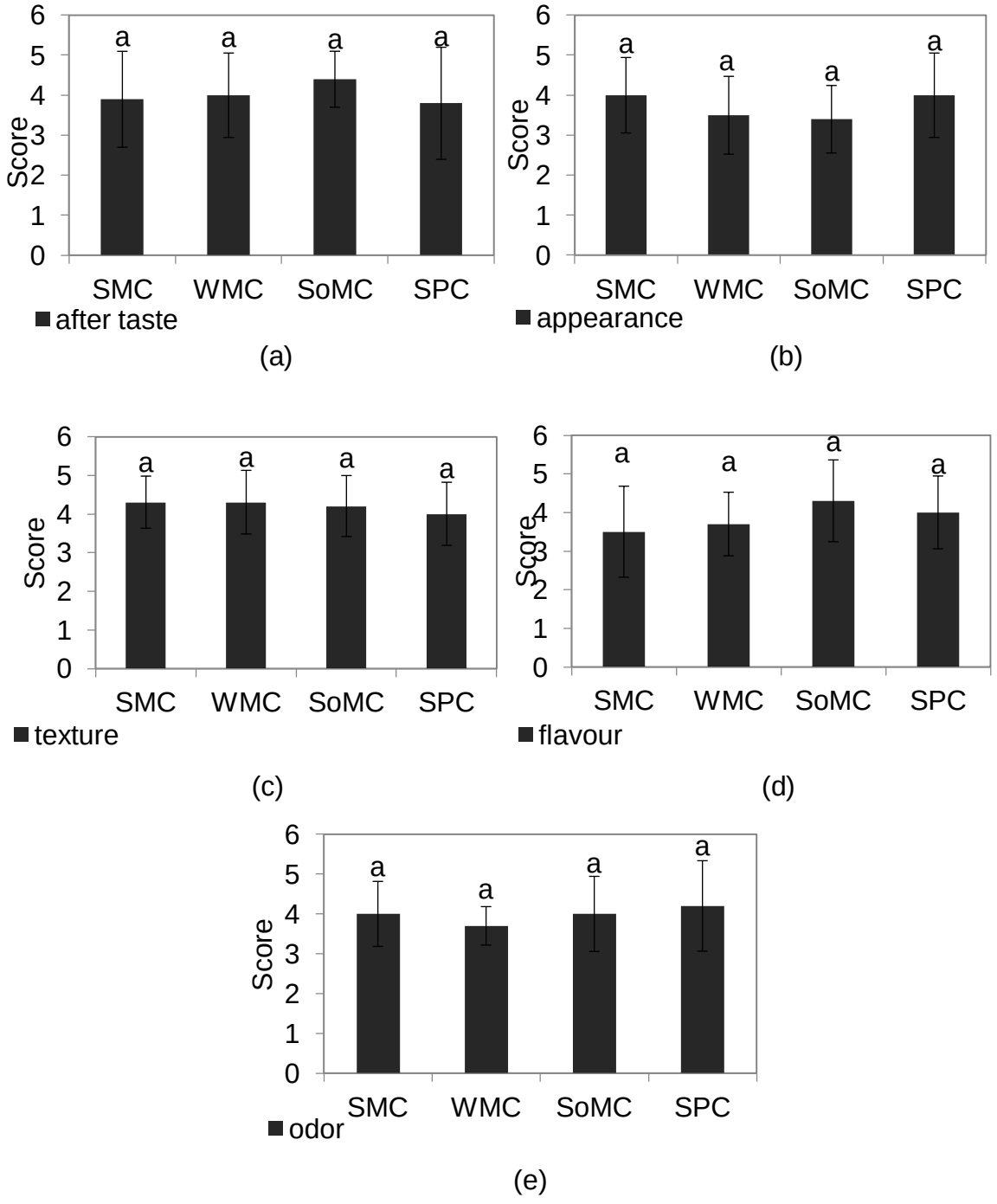


Figure 4.9 Sensory analysis scores a) after taste, b) appearance, c) texture, d) flavour, e) odor

5. CONCLUSION

Chocolate is the most famous snack in the world that has consumer in any age. Therefore, one of the most developed and innovated products of the confectionery industry is chocolate. With increasing interest in functional products and different diets, the emergence of different food products has also increased. Soy products has a great potential to use in food products to enhance the functionality. Soymilk, is considered as "light milk", because it does not cause lactose intolerance and bloating due to its lactose and cholesterol free content. Today, soybean ingredients are widely used as milk substitution in many foods because of its rich protein and isoflovane content and functional characteristics. In this study, it was aimed to combine the popular chocolate product with the functional soy products as an alternative to milk chocolate and to search the effects of this substitution on the quality and functionality of chocolate. Quality parameters were evaluated with chocolate rheology, particle size distribution, polymorphism and texture while the functionality of chocolate was evaluated as total phenolic content and antioxidant capacity.

Soymilk powder was produced as lab-scale for this study and proximate composition were determined. Soymilk powder had 3.40 % moisture, 18.14 % fat, 6.06 % ash, 44.43 % protein and 27.96 % carbohydrate content. Soy protein isolate was also used as a substituent in chocolate and skimmed milk powder and whole milk powder was used to produce control chocolates. Physical analysis were conducted for all powder ingredients and functional analysis were carried out for SoMP and SPI samples. As physical characteristics, Hausner Ratio and Carr Index values were calculated for powder samples, which are defines the flow property and cohesiveness character of powder products. Water holding capacity (WHC), oil binding capacity (OBC), emulsion capacity (EC) and emulsion stability (ES) were determined to define the functional characteristics of SoMP and SPI samples. Although the SPI samples had higher WHC and OBC, SoMP samples had higher EC and ES. Chocolates were produced lab-scale for this study with different ingredients (SMP, WMP, SoMP, SPI) and conching times (2, 4, 6 hours).

The rheology of chocolates were fitted to three models; Casson, Herschel-Bulkley and Bingham models. All the models gave high correlation coefficients (R^2>0.99). Viscosity is a major parameter of rheology and vital to determine to define the flow characteristics of molten chocolate. The apparent viscosities of chocolate samples were determined at 50 s^{-1} shear rate for all models. SPC samples gave the highest viscosity values for all process times. SoMC and WMC gave lower viscosity values than others due to their fat content.

The yield stress values were obtained from models and compared by their ingredient type and conching time. Yields stress values were affected both the ingredient type and conching process (P<0.05) significantly. Particle size is another criterion affects the rheological behavior of molten chocolates. Particle size were evaluated by D_{90} value which presents the diameter of 90 % of the particles below this size and represents the other diameters. D_{90} is known to show inverse attitude with specific surface area. D_{90} and specific surface area values were also evaluated statistically, and they were found to be affected both process time and ingredient type significantly (P<0.05). Increasing process time resulted lower sized particles and higher specific surface area. Smaller particles evoke the rising viscosity due to larger area needs more fat content. However, the chocolate samples showed the opposite manner. Decreasing particle size caused lower apparent viscosities. This result was attributed to conching process creates smoother particles that exhibits lower apparent viscosity. X-ray diffraction patterns showed that all the chocolate samples had the Form V crystal type. It signs the proper tempering process. Sensory properties were indicated that all the chocolate samples had high scores from panelists. All samples were acceptable and no significant difference was observed between the samples (P<0.05).

Functional characteristics of chocolates were evaluated with total antioxidant capacity (TAC) and total phenolic content (TPC). ABTS, DPPH and CUPRAC methods were used to determine TAC of chocolates. According to TAC results, SoMC samples had highest antioxidant capacity level among the samples. This is thought to be caused from isoflovanes of soybean. SPC samples had the lowest level of TAC, which is thought to proteins of soybeans masking the antioxidant behavior of chocolate. Among the methods, ABTS gave the highest level of TAC.

CUPRAC values were lower than ABTS and DPPH values were the lowest. All the methods were affected from ingredient type and conching process significantly ($P<0.05$) and the models were consistent with each other. However, it is difficult to say that conching has a direct effect on the antioxidant capacity of chocolates. Because there was no proper increase or decrease of TAC in the samples. TPC values showed that SoMC2 had the highest level and SPC6 had the lowest level. Such as TAC analysis, TPC analysis were significantly ($P<0.05$) affected by conching time and ingredient type, but there was no proper effect.

Consequently, the soymilk powder used in this study affected the chocolate highly similar with whole milk powder on account of rheology and texture, but with greater protein content and antioxidant capacity levels. This study contributed to understanding rheological character and antioxidant capacity of soy-included chocolates. These findings can be utilized to improve the soy-included chocolates or other functional chocolates.

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APPENDIX A

Table A.1 Sensory analysis identifying scale

APPEARANCE
5. Shiny and smooth surface with homogenous chocolate color, favorably to ingredient type
4. Very slight reduction in homogeneous color and sparingly reducing in brightness
3. Deterioration in homogeneity of smooth surface in its own color
2. Matte surface with little whiten (flowering) areas and uneven structure
1. Unacceptable whiteness, excessive roughness and matte surface and uneven structure in the color of chocolate
TEXTURE
5. Ideal hardness and brittleness with expected snap quality; soft melting in the mouth, considerable sense of ingredient taste
4. Depending on the type of ingredient, slightly chalky structure in mouth; a bit decrease in hardness
3. Decrease/increase in hardness and friability; sticky/granular taste in the mouth
2. Significant granular or sticky taste, evidently soft/hard structure , melting so easily
1. Extremely hard/soft, brittle, excessively loose, sticky and oily/dry in the mouth
FLAVOUR
5. Typical chocolate flavor with sense of ingredient
4. A slight decrease of chocolate flavour with specific ingredient in flavor
3. Predominant ingredient flavor, with slightly sense of chocolates own flavour
2. Significant foreign flavor formation, greatly sense of bitter component flavor
1. Unacceptably disturbing foreign flavor, excessively oxide/rancid taste
ODOR
5. Distinctive, intense chocolate odor and the feeling of ingredient smell
4. Very little reduction in chocolate's own odor and the feeling of ingredient smell
3. The smell of chocolate is not felt, the ingredient smell is undesirable
2. Obvious foreign odor formation, away from its own odor
1. Unacceptably disturbing foreign smell with oxide odor

Table A.2 Sensory analysis evaluation form

Name and surname:

Date:

Please evaluate the given samples according to the scoring form.

	385	721	206	119
Appearance				
Texture				
Flavour				
Odor				
After taste				

APPENDIX B

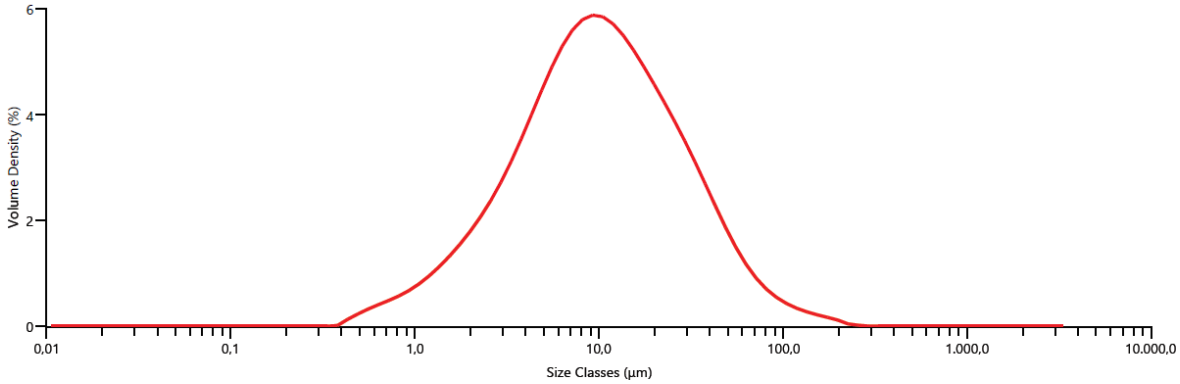

Figure B.1 Particle size distribution of SMC2

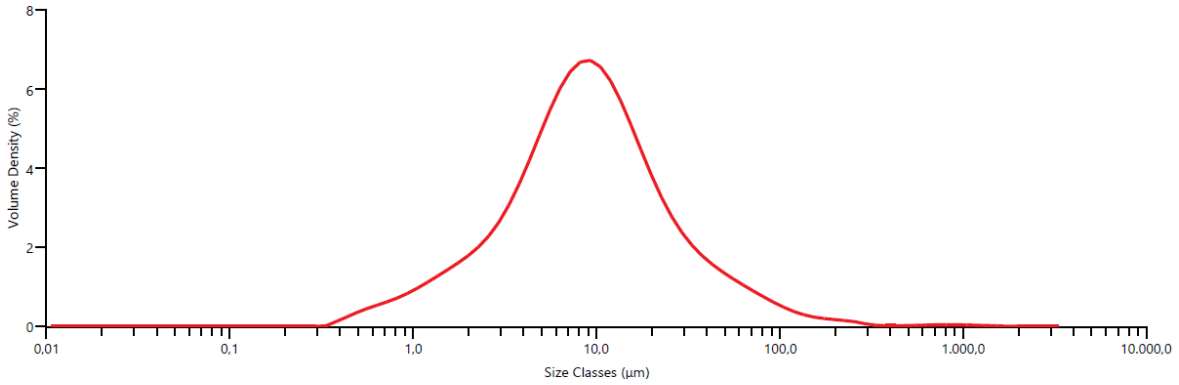

Figure B.2 Particle size distribution of SMC4

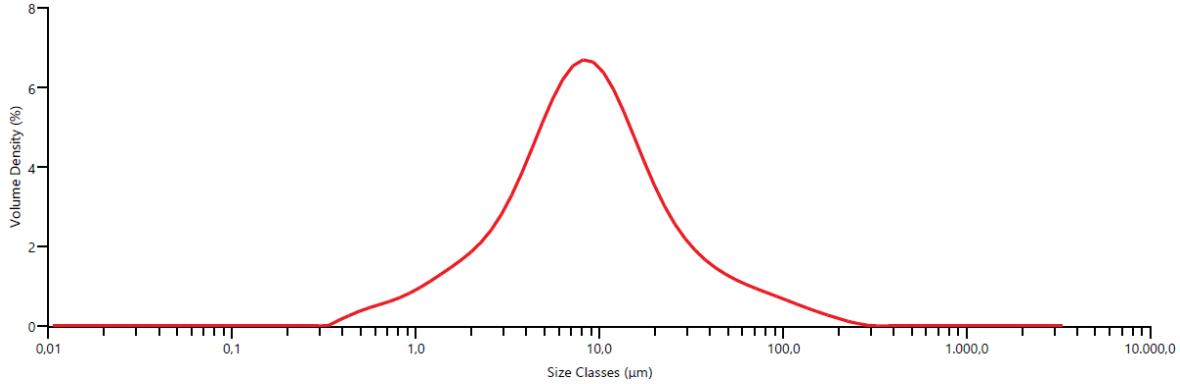

Figure B.3 Particle size distribution of SMC6

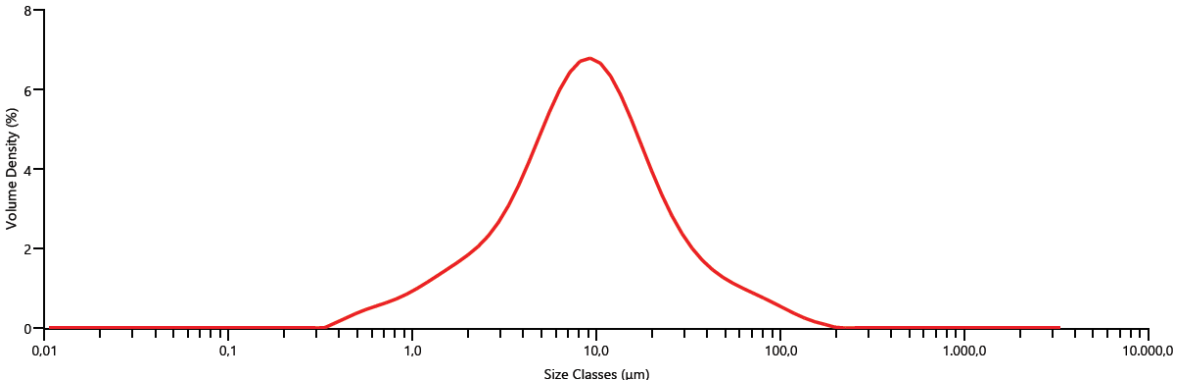

Figure B.4 Particle size distribution of WMC2

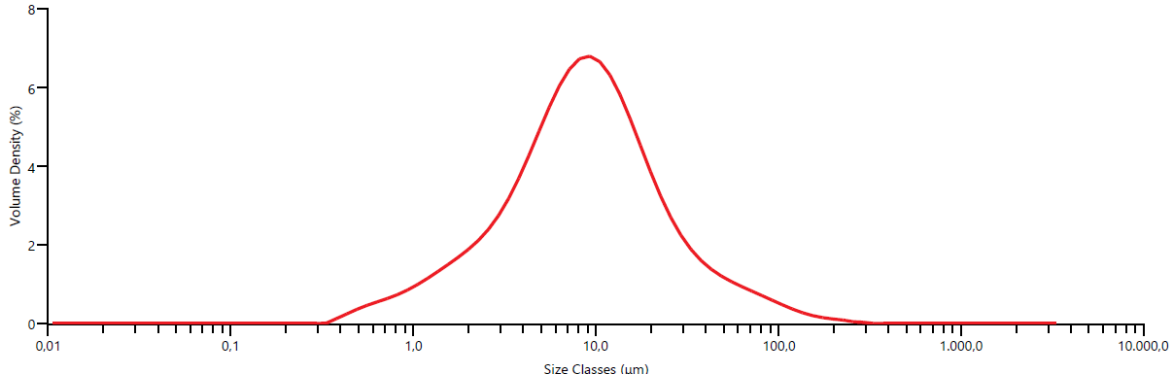

Figure B.5 Particle size distribution of WMC4

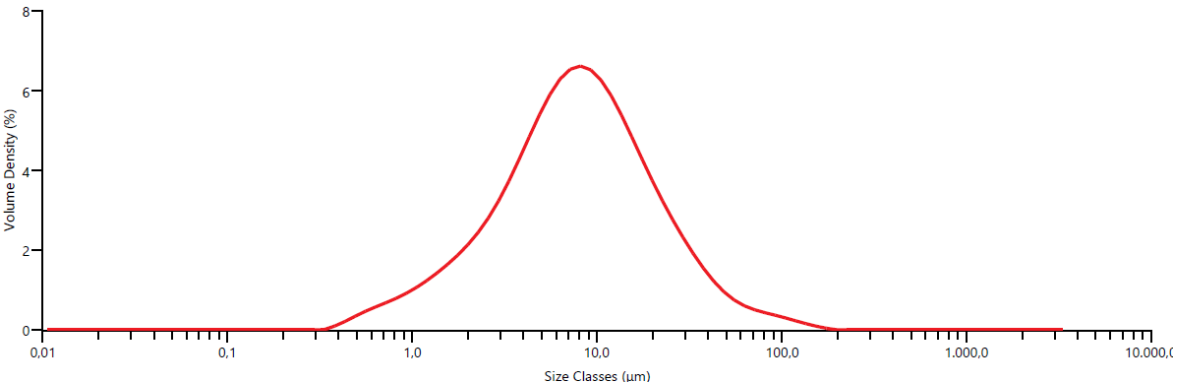

Figure B.6 Particle size distribution of WMC6

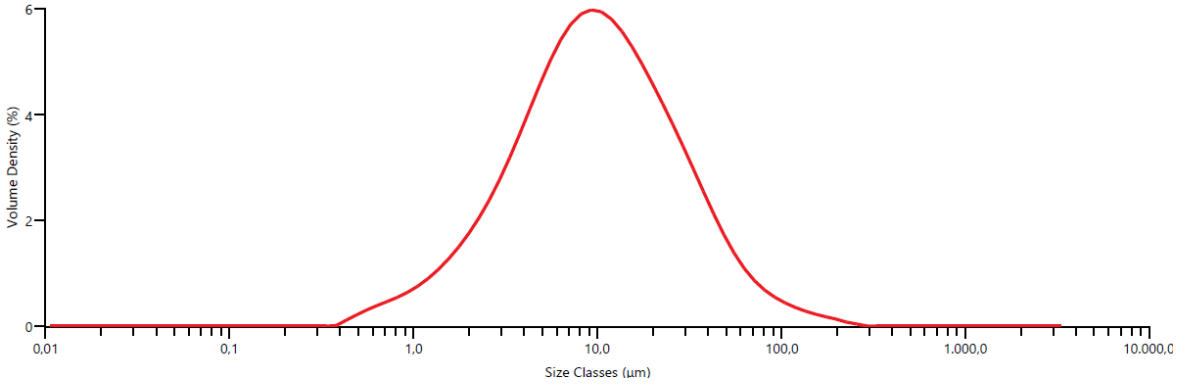

Figure B.7 Particle size distribution of SoMC2

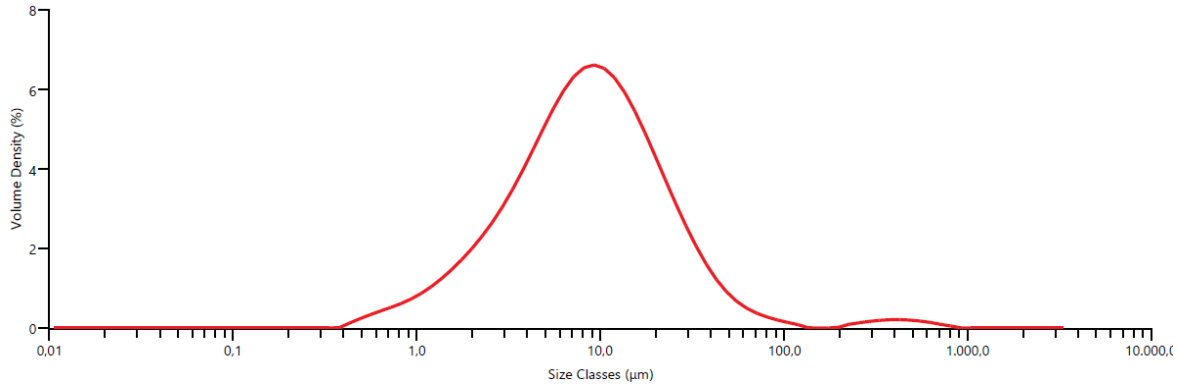

Figure B.8 Particle size distribution of SoMC4

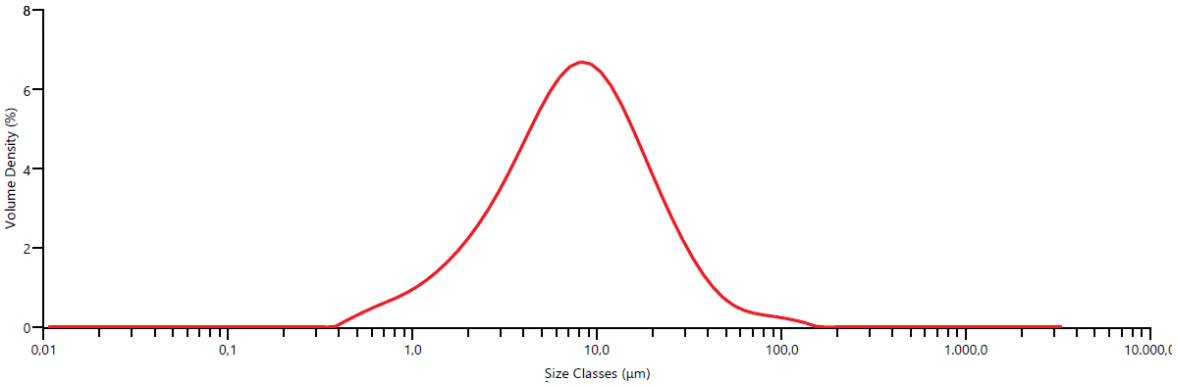

Figure B.9 Particle size distribution of SoMC6

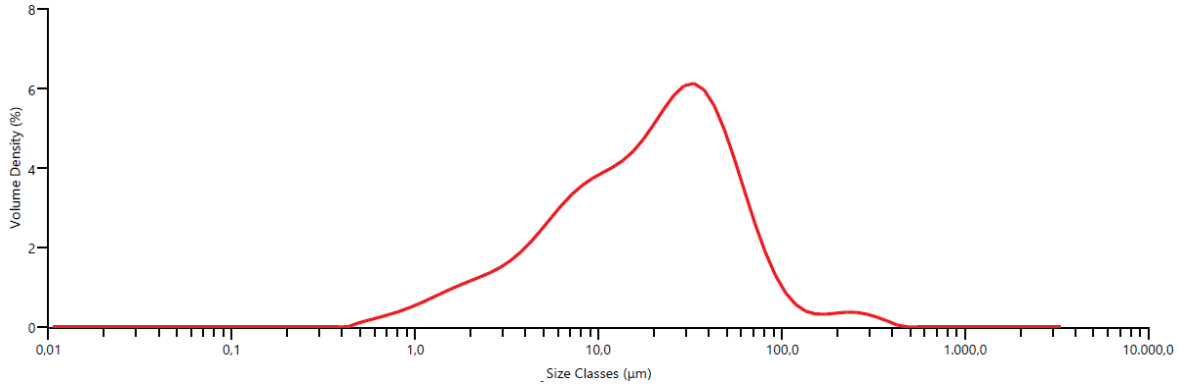

Figure B.10 Particle size distribution of SPC2

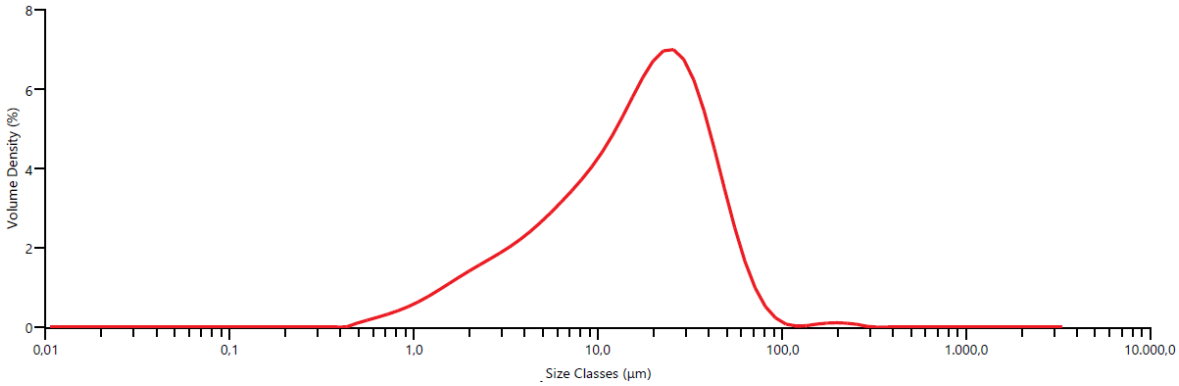

Figure B.11 Particle size distribution of SPC4

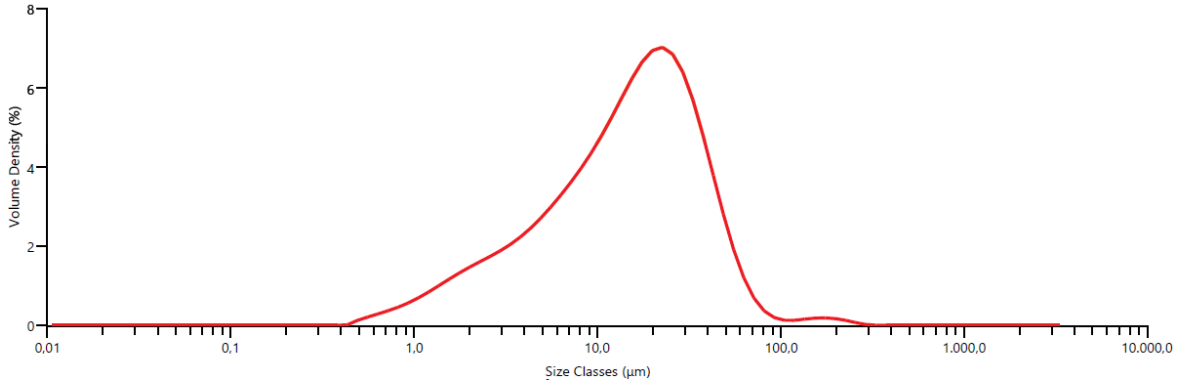

Figure B.12 Particle size distribution of SPC6

APPENDİX C

Table C.1 General Linear Model: Casson yield value versus ingredient; process time

Factor Information

Factor	Type	Levels	Values
C1	Fixed	4	SMC; SoMC; SPC; WMC
C3	Fixed	3	2; 4; 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
C1	3	334,769	111,590	426,28	0,000
C3	2	78,244	39,122	149,45	0,000
	6	268,998	44,833	171,27	0,000
C1*C3					
Error	24	6,283	0,262		
Total	35	688,293			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,511638	99,09%	98,67%	97,95%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	14,9727	0,0853	175,59	0,000	
C1					
SMC	3,427	0,148	23,20	0,000	1,50
SoMC	-0,315	0,148	-2,13	0,043	1,50
SPC	1,647	0,148	11,15	0,000	1,50
C3					
2	-0,618	0,121	-5,13	0,000	1,33
4	-1,415	0,121	-11,74	0,000	1,33
C1*C3					
SMC 2	5,868	0,209	28,09	0,000	2,00
SMC 4	-2,016	0,209	-9,65	0,000	2,00
SoMC 2	0,091	0,209	0,44	0,667	2,00
SoMC 4	0,255	0,209	1,22	0,235	2,00
SPC 2	-3,658	0,209	-17,51	0,000	2,00
SPC 4	2,510	0,209	12,02	0,000	2,00

Table C.2 General Linear Model: Herschel yield value versus ingredient; process time

Factor Information

Factor	Type	Levels	Values
C1	Fixed	4	SMC; SoMC; SPC; WMC
C3	Fixed	3	2; 4; 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
C1	3	261,70	87,234	190,78	0,000
C3	2	41,33	20,663	45,19	0,000
	6	616,95	102,825	224,88	0,000
C1*C3					
Error	24	10,97	0,457		
Total	35	930,95			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,676203	98,82%	98,28%	97,35%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	21,067	0,113	186,93	0,000	
C1					
SMC	0,037	0,195	0,19	0,849	1,50
SoMC	-0,303	0,195	-1,55	0,133	1,50
SPC	3,937	0,195	20,17	0,000	1,50
C3					
2	-0,336	0,159	-2,11	0,046	1,33
4	1,448	0,159	9,08	0,000	1,33
C1*C3					
SMC 2	8,559	0,276	31,00	0,000	2,00
SMC 4	-0,039	0,276	-0,14	0,889	2,00
SoMC 2	-1,708	0,276	-6,19	0,000	2,00
SoMC 4	-0,588	0,276	-2,13	0,044	2,00
SPC 2	-1,988	0,276	-7,20	0,000	2,00
SPC 4	-1,122	0,276	-4,06	0,000	2,00

Table C.3 General Linear Model: Bingham yield value versus ingredient; process time

Factor Information

Factor	Type	Levels	Values
ingredient type	Fixed	4	SMC; SoMC; SPC; WMC
process time	Fixed	3	2; 4; 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
ingredient type	3	923,27	307,758	835,41	0,000
process time	2	99,21	49,603	134,65	0,000
ingredient type*process time	6	544,56	90,760	246,37	0,000
Error	24	8,84	0,368		
Total	35	1575,88			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,606954	99,44%	99,18%	98,74%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	24,821	0,101	245,36	0,000	
ingredient type					
SMC	5,272	0,175	30,09	0,000	1,50
SoMC	-1,523	0,175	-8,69	0,000	1,50
SPC	3,846	0,175	21,95	0,000	1,50
process time					
2	0,392	0,143	2,74	0,011	1,33
4	-2,201	0,143	-15,38	0,000	1,33
ingredient type*process time					
SMC 2	8,649	0,248	34,91	0,000	2,00
SMC 4	-3,065	0,248	-12,37	0,000	2,00
SoMC 2	-0,573	0,248	-2,31	0,030	2,00
SoMC 4	0,719	0,248	2,90	0,008	2,00
SPC 2	-4,339	0,248	-17,51	0,000	2,00
SPC 4	3,141	0,248	12,67	0,000	2,00

Table C.4 General Linear Model: Casson yield versus D90; SSA

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
D90	22	423,91	19,27	0,55	0,807
Error	1	34,80	34,80		
Total	23	458,70			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
5,89888	92,41%	0,00%	*

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	14,99	1,22	12,32	0,052	
D90					
24,2	1,92	5,77	0,33	0,796	1,91
24,4	1,23	5,77	0,21	0,866	1,91
24,5	0,09	5,77	0,01	0,990	1,91
26,3	-1,61	5,77	-0,28	0,827	1,91
26,6	0,97	5,77	0,17	0,894	1,91
26,7	-7,59	5,77	-1,32	0,414	1,91
27,8	-1,02	5,77	-0,18	0,888	1,91
31,0	-6,42	5,77	-1,11	0,466	1,91
31,5	-7,63	5,77	-1,32	0,412	1,91
32,2	-0,25	5,77	-0,04	0,972	1,91
33,4	-7,09	5,77	-1,23	0,435	1,91
34,6	0,54	5,77	0,09	0,940	1,91
35,0	2,18	5,77	0,38	0,769	1,91
35,8	-1,02	5,77	-0,18	0,888	1,91
36,4	1,37	5,77	0,24	0,851	1,91
36,5	4,01	4,17	0,96	0,513	1,48
37,5	9,25	5,77	1,60	0,355	1,91
40,0	4,58	5,77	0,79	0,573	1,91
40,2	5,54	5,77	0,96	0,513	1,91
43,1	3,42	5,77	0,59	0,659	1,91
43,8	2,49	5,77	0,43	0,741	1,91
58,4	-2,74	5,77	-0,48	0,718	1,91

Table C.5 General Linear Model: Hershcel-Bulkley yield versus D90; SSA

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
D90	22	560,31	25,47	0,44	0,853
Error	1	57,68	57,68		
Total	23	617,99			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
7,59503	90,67%	0,00%	*

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	20,91	1,57	13,35	0,048	
D90					
24,2	1,68	7,42	0,23	0,858	1,91
24,4	0,74	7,42	0,10	0,937	1,91
24,5	-1,29	7,42	-0,17	0,891	1,91
26,3	0,74	7,42	0,10	0,937	1,91
26,6	-1,46	7,42	-0,20	0,876	1,91
26,7	-8,30	7,42	-1,12	0,464	1,91
27,8	0,71	7,42	0,10	0,939	1,91
31,0	0,45	7,42	0,06	0,962	1,91
31,5	-8,71	7,42	-1,17	0,449	1,91
32,2	1,23	7,42	0,17	0,895	1,91
33,4	0,16	7,42	0,02	0,986	1,91
34,6	1,51	7,42	0,20	0,873	1,91
35,0	-9,39	7,42	-1,26	0,426	1,91
35,8	-2,99	7,42	-0,40	0,756	1,91
36,4	-9,99	7,42	-1,35	0,407	1,91
36,5	2,70	5,37	0,50	0,703	1,48
37,5	8,19	7,42	1,10	0,469	1,91
40,0	6,23	7,42	0,84	0,556	1,91
40,2	6,63	7,42	0,89	0,536	1,91
43,1	4,65	7,42	0,63	0,644	1,91
43,8	4,19	7,42	0,56	0,673	1,91
58,4	0,65	7,42	0,09	0,944	1,91

Table C.6 General Linear Model: Bingham yield versus D90; SSA

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
D90	22	910,1	41,37	0,34	0,899
Error	1	121,2	121,17		
Total	23	1031,2			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
11,0075	88,25%	0,00%	*

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	24,36	2,27	10,73	0,059	
D90					
24,2	0,7	10,8	0,06	0,961	1,91
24,4	1,3	10,8	0,12	0,924	1,91
24,5	-0,8	10,8	-0,08	0,952	1,91
26,3	-2,9	10,8	-0,27	0,834	1,91
26,6	-0,5	10,8	-0,05	0,970	1,91
26,7	-10,4	10,8	-0,97	0,511	1,91
27,8	-2,8	10,8	-0,26	0,838	1,91
31,0	-10,1	10,8	-0,94	0,520	1,91
31,5	-10,9	10,8	-1,01	0,496	1,91
32,2	-0,4	10,8	-0,04	0,978	1,91
33,4	-10,1	10,8	-0,94	0,520	1,91
34,6	0,2	10,8	0,02	0,988	1,91
35,0	1,8	10,8	0,17	0,894	1,91
35,8	-2,1	10,8	-0,19	0,877	1,91
36,4	1,6	10,8	0,15	0,905	1,91
36,5	6,40	7,78	0,82	0,561	1,48
37,5	14,7	10,8	1,37	0,401	1,91
40,0	6,6	10,8	0,61	0,650	1,91
40,2	7,3	10,8	0,68	0,621	1,91
43,1	5,3	10,8	0,49	0,709	1,91
43,8	5,2	10,8	0,48	0,714	1,91
58,4	0,1	10,8	0,01	0,992	1,91

Table C.7 General Linear Model: ABTS versus Ingredient;process time

Factor Information

Factor	Type	Levels	Values
Ingredient	Fixed	4	SMC; SoMC; SPC; WMC
Process time	Fixed	3	2; 4; 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Ingredient	3	2,59	0,86318	19,97	0
Process time	2	1,292	0,64605	14,95	0
Ingredient*Process time	6	0,192	0,03207	0,74	0,621
Error	24	1,037	0,04323		
Total	35	5,112			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,2079	79,70%	70,40%	54,33%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	4,1	0,035	118,08	0	
Ingredient					
SMC	-0	0,06	-2,32	0,029	1,5
SoMC	0,5	0,06	7,59	0	1,5
SPC	-0	0,06	-1,39	0,177	1,5
Process time					
2	-0	0,049	-4,71	0	1,33
4	0,2	0,049	4,76	0	1,33
Ingredient*Process time					
SMC 2	0,1	0,085	1,1	0,284	2
SMC 4	0	0,085	0,56	0,581	2
SoMC 2	-0	0,085	-1,14	0,265	2
SoMC 4	0	0,085	0,4	0,693	2
SPC 2	0	0,085	0,03	0,979	2
SPC 4	0	0,085	0,18	0,857	2

Table C.8 General Linear Model: DPPH versus Ingredient;process time

Factor Information

Factor	Type	Levels	Values
Ingredient	Fixed	4	SMC; SoMC; SPC; WMC
Process time	Fixed	3	2; 4; 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Ingredient	3	2,9232	0,9744	34,28	0
Process time	2	1,1221	0,56106	19,74	0
Ingredient*Process time	6	0,1418	0,02363	0,83	0,558
Error	24	0,6821	0,02842		
Total	35	4,8692			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,16859	85,99%	79,57%	68,48%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	3,9872	0,0281	141,9	0	
Ingredient					
SMC	-0,1097	0,0487	-2,25	0,034	1,5
SoMC	0,4704	0,0487	9,67	0	1,5
SPC	-0,0656	0,0487	-1,35	0,191	1,5
Process time					
2	-0,1865	0,0397	-4,69	0	1,33
4	0,237	0,0397	5,96	0	1,33
Ingredient*Process time					
SMC 2	0,0812	0,0688	1,18	0,249	2
SMC 4	0,0524	0,0688	0,76	0,454	2
SoMC 2	-0,0665	0,0688	-0,97	0,343	2
SoMC 4	-0,002	0,0688	-0,03	0,977	2
SPC 2	-0,0282	0,0688	-0,41	0,686	2
SPC 4	0,0243	0,0688	0,35	0,727	2

Table C.9 General Linear Model: CUPRAC versus Ingredient; process time

Factor Information

Factor	Type	Levels	Values
Ingredient	Fixed	4	SMC; SoMC; SPC; WMC
Process time	Fixed	3	2; 4; 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Ingredient	3	0,06203	0,020677	24,81	0
Process time	2	1,10295	0,551475	661,77	0
Ingredient*Process time	6	0,56869	0,094782	113,74	0
Error	24	0,02	0,000833		
Total	35	1,75368			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,028868	98,86%	98,34%	97,43%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	5,36583	0,00481	1115,27	0	
Ingredient					
SMC	-0,0325	0,00833	-3,9	0,001	1,5
SoMC	0,06083	0,00833	7,3	0	1,5
SPC	-0,04361	0,00833	-5,23	0	1,5
Process time					
2	-0,1725	0,0068	-25,35	0	1,33
4	0,24	0,0068	35,27	0	1,33
Ingredient*Process time					
SMC 2	0,0225	0,0118	1,91	0,068	2
SMC 4	0,1367	0,0118	11,6	0	2
SoMC 2	0,1725	0,0118	14,64	0	2
SoMC 4	-0,24	0,0118	-20,36	0	2
SPC 2	-0,1164	0,0118	-9,88	0	2
SPC 4	0,1378	0,0118	11,69	0	2

Table C.10 General Linear Model: Total Phenolic versus Ingredient;process time

Factor Information

Factor	Type	Levels	Values
Ingredient	Fixed	4	SMC; SoMC; SPC; WMC
Process time	Fixed	3	2; 4; 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Ingredient	3	5197	1732,49	36,3	0
Process time	2	1259	629,59	13,19	0
Ingredient*Process time	6	2081	346,82	7,27	0
Error	24	1145	47,72		
Total	35	9683			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
6,90803	88,17%	82,75%	73,39%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	107,98	1,15	93,79	0	
Ingredient					
SMC	10,08	1,99	5,06	0	1,5
SoMC	3,49	1,99	1,75	0,092	1,5
SPC	-20,42	1,99	-10,24	0	1,5
Process time					
2	8,16	1,63	5,01	0	1,33
4	-5,68	1,63	-3,49	0,002	1,33
Ingredient*Process time					
SMC 2	9,74	2,82	3,45	0,002	2
SMC 4	-4,79	2,82	-1,7	0,102	2
SoMC 2	-10,67	2,82	-3,78	0,001	2
SoMC 4	13,73	2,82	4,87	0	2
SPC 2	4,47	2,82	1,59	0,126	2
SPC 4	-1,02	2,82	-0,36	0,719	2

APPENDIX D

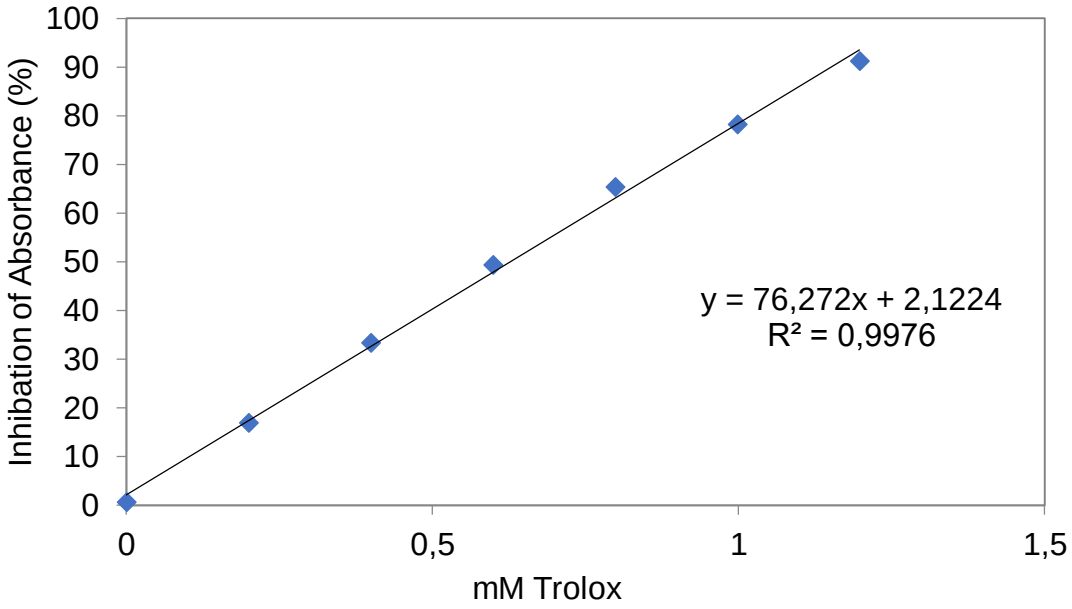

Figure A.1 Trolox calibration curve of ABTS method at 734 nm

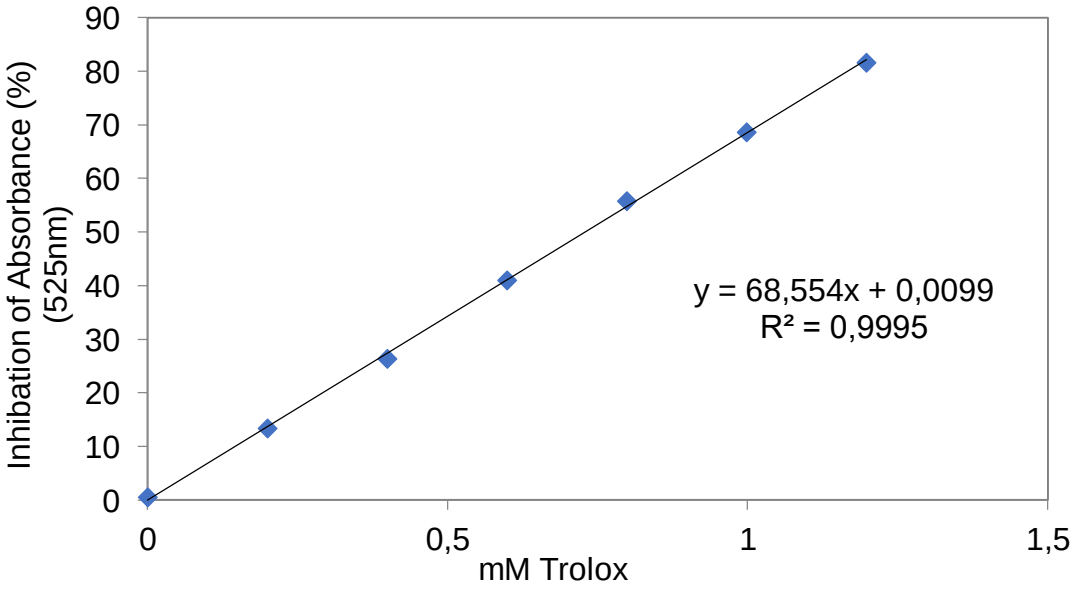

Figure A.2 Trolox calibration curve of DPPH method at 525 nm

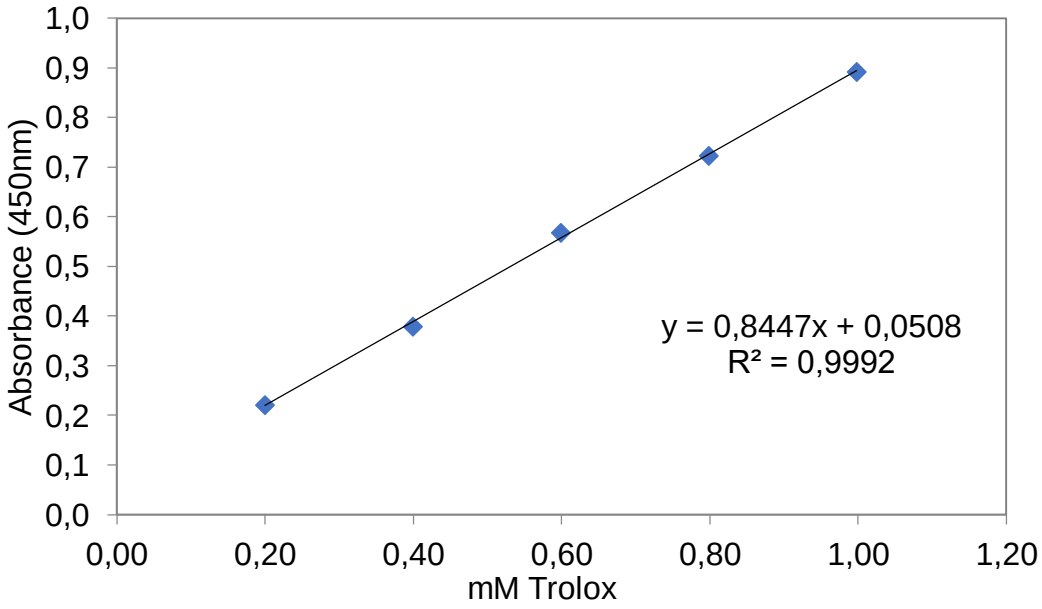

Figure A.3 Trolox calibration curve of CUPRAC method at 450 nm

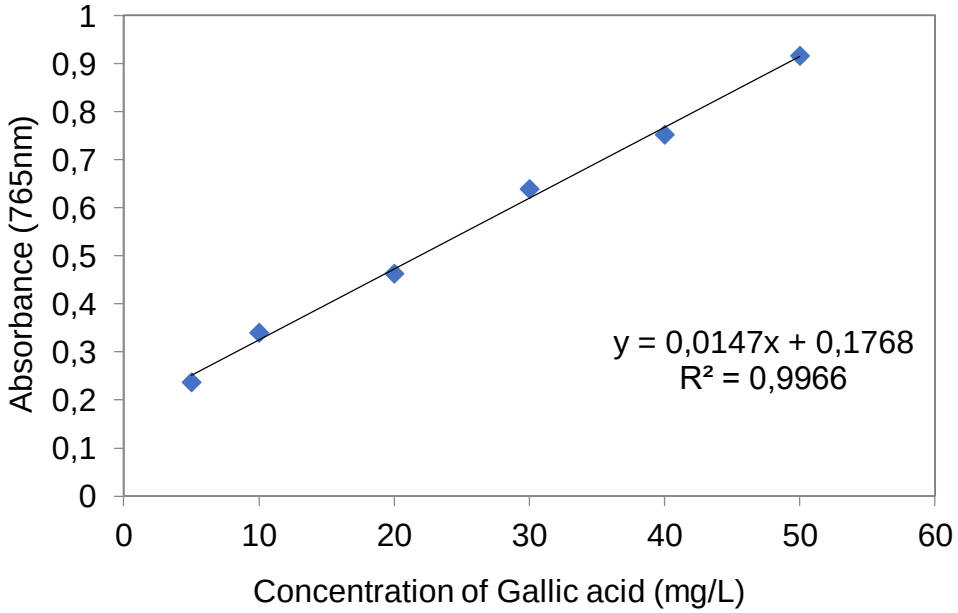

Figure A.4 Gallic acid calibration curve for TPC method at 765 nm

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THESIS/~~DISSERTATION~~ ORIGINALITY REPORT

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TO THE DEPARTMENT OF FOOD ENGNEERING

Date: 03/07/2019

Thesis Title : EFFECTS OF SOME SOY PRODUCTS ON RHEOLOGICAL, FUNCTIONAL AND SENSORY PROPERTIES OF MILK CHOCOLATE

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PUBLICATIONS FROM M.Sc. THESIS

-

ORAL PRESENTATIONS

Meclis I.C, Yolacaner E, Oztop M.H., A study on the effects of some soy products on rheological and textural properties of milk chocolate, *ISEKI Food Congress 2018 At: Hohenheim Stuttgart, Germany,* 3-5 July 2018*.*