



Physical Properties of Some Soy Powders and Functional and Sensory Properties of Milk Chocolates Prepared with These Powders

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ABSTRACT

Soybean is a nutritious crop commonly used for food enrichment due to its rich nutritional content and valuable functional characteristics. Soy products and also soymilk are potential ingredients 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. In this study, soymilk powder and soy protein isolate were added to milk chocolate to obtain a functional food product. Some chemical, physical and functional properties of powder ingredients were determined. 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. Functionality of chocolates was evaluated in terms of total phenolic content and total antioxidant capacity. The results were significantly higher than the literature data. Considering all results in terms of functionality, it can be stated that soymilk powder and soy protein isolate can be added to milk chocolate in order to obtain a functional food product.

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Introduction

Today, there is an increase in food products that respond to consumers' special diets and cultural or personal beliefs like organic, halal, kosher, vegetarian, vegan, etc. Due to its vegan nature and healthy content, soymilk is becoming more popular as consumers become more health-conscious. Soymilk is a very suitable candidate for functional or vegan foods and a good alternative to dairy products. It contains more protein, iron, unsaturated fatty acids, niacin, and fewer 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 on grinding conditions, heat treatment, and other ingredients. The heating process affects principally the microbiological activity, nutritional quality of the proteins, and flavors of the soymilk (Huang et al., 2006). Soymilk powder is also mentioned as a very good source of nutrients (low-cost protein and polyphenols) for a malnourished population in developing countries. It can fortificate foods with vitamin A, vitamin B-complex, iron, and zinc (Mazumder and Hongsprabhas, 2016) and can be used as an alternative to cow's milk powder. Soybean and its products contain some

anti-nutritional compounds other than nutritional compounds. Among these, isoflavones, lipoxygenase enzymes and trypsin inhibitors are the most important anti-nutritional compounds that should be detected before the production of food containing soy ingredients.

Chocolate, a popular food product, is one of the most consumed snacks by consumers of all ages worldwide. It is a semisolid suspension of solid cocoa particles in a continual fat phase (Afoakwa, 2016). According to the Turkish Standards Institution (TSE), the types of chocolate are determined depending on the percentages of dry cocoa solids, solid milk particles, milk fat, and cocoa butter. Chocolate types are bitter, couverture bitter, milk, couverture milk, extra milky, skim milk, creamy, vermicelli, chocolate flakes (bitter or milk), and white chocolate (Turkish Standards Institution, 2010). Chocolate has many functional properties. It comprises monomeric (epicatechin, catechin) and oligomeric (procyanidin) polyphenols that have some benefits on body cells counteracting the deterioration caused by free radicals (Beckett, 2009). 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). The antioxidant character of chocolate comes from the cocoa ingredient. As a result of that, milk chocolate has lower antioxidant character than bitter chocolate (Cooper et al., 2008). 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 have 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).

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).

In this study, some physical, chemical and functional properties (proximate composition, nitrogen solubility and protein dispersibility indices, lipoxigenase and trypsin inhibitor activities, density, solubility, wettability, emulsion capacity and stability) of soy powders, which were soymilk powder and soy protein isolates were investigated. Moreover, functionality of chocolates was evaluated in terms of total phenolic content and total

antioxidant capacity. Lastly, sensory analysis was performed for the chocolates. As far as it was known, this was the first research work that all of these were investigated in one study.

Materials and Methods

Soybean (Nazlıcan cv.) samples were kindly provided by The Eastern Mediterranean Agricultural Research Institute in Adana, Türkiye. Soymilk and soymilk powder (SoMP) were produced under laboratory conditions. Soy milk was produced by Cornell method (Abagoshu et al., 2017) and soy milk powder was obtained by freeze-drying (Christ 2B, Osterode am Harz, Germany). Cocoa liquor, cocoa butter and chocolate seeds were provided by Barry Callebout (Eskisehir, Türkiye) and powdered sugar (Kenton, Istanbul, Türkiye) was bought from local markets. Whole milk powder (WMP) (Ulker, Ankara, Türkiye) and skimmed milk powder (SMP) (Slava, Karaman, Türkiye) were used to produce milk chocolate. Soy protein isolate (SPI) (Alfasol, Türkiye) and SoMP were used as a substitute of milk powder.

Chemicals were bought from Merck (Germany) and Sigma Aldrich (USA). Hexane, NaOH, HCl, potassium sulfate, petroleum ether, sodium tetraborate, borax, 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, glacial acetic acid, ethanol, potassium persulfate, copper (II) chloride, sodium acetate and gallic acid were bought from Merck.

Proximate Composition of Soymilk Powder

Proximate analysis was performed to specify the contribution of the nutritional value of soymilk powder on soy chocolates. The moisture content was determined gravimetrically according to AACC Method 82-21.01 (1999). The protein content was measured by AACC 46-12 Kjeldahl method containing digestion, distillation and titration steps (1999). Fat content was determined by AACC 30-10.01 method (1999). AACC 08-16.01 method was conducted for measurement of the ash content (1999). Carbohydrate content was calculated after protein, fat, ash and moisture content were specified. The pH of the soymilk powder sample was measured from its suspension (1:100, w/v) by using a pH-meter (PL-700AL, Türkiye).

Nitrogen Solubility Index (NSI) and Protein Dispersibility Index (PDI)

NSI was determined by the method AACC 46-23.01 (1999) and PDI was measured by the method of AACC 46-24.01 (1999).

Lipoxigenase Activity

The lipoxigenase (LOX) enzyme activity assay was carried out after soymilk powder was defatted by hexane and sieved to 212 µm pore size. Heat treatment was applied to sample for different times to observe the activity of lipoxigenase enzyme. Soymilk samples were subjected to a temperature of 98-100°C for 0, 5, 10, 15 and 20 minutes and then freeze-dried 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).

Trypsin Inhibitor Activity

The trypsin inhibitor activity of soymilk powder sample was specified spectrophotometrically by using the AACC method 22-40.01 (1999). 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.

Physical Analysis of Powder Ingredients

Bulk density, tapped bulk density, solubility, wettability values for skimmed milk powder (SMP), whole milk powder (WMP), soymilk powder (SoMP) and soy protein isolate (SPI) were specified and then, Carr Index and Hausner Ratio values were calculated.

The bulk density (g/ml) values of powder ingredients were measured according to the method of Jinapong et al. (2008). The tapped bulk density (g/ml) values were determined by the method of Dirim and Caliskan (2012).

The wettability and the solubility values were specified by the method of Nguyen et al. (2018).

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 to the tapped bulk density (Equation 1). This value was used to evaluate the flowability of products (Nguyen et al., 2018).

$$HR = \frac{\rho_{\text{tapped}}}{\rho_{\text{bulk}}} \quad (1)$$

The Carr index (CI) is another description obtained by density values. The Carr index was calculated according to equation 2, which represents the compressibility of the product (Nguyehn et al., 2018).

$$CI = \frac{\rho_{\text{tapped}} - \rho_{\text{bulk}}}{\rho_{\text{bulk}}} \quad (2)$$

The color values of the ingredient are important since they affect the color of the final product. Color values of the powder ingredients were determined by a colorimeter (Minolta Spectrophotometer CM-3600j, Japan) at room temperature. Results were expressed according to CIELAB system scale, where L^* is the brightness, a^* is green to red color scale and b^* is blue to yellow color scale. Hue angle (h°) for color tone and Chroma (C^*) value for saturation were calculated by using the following equations 3 and 4.

$$\text{hue angle } (h^\circ) = \arctan (b^*/a^*) \quad (3)$$

$$\text{Chroma } (C^*) = [(a^*)^2 + (b^*)^2]^{0.5} \quad (4)$$

Functional Properties of Soy Powders

Emulsion capacity (EC) and emulsion stability (ES) of soymilk powder and soy protein isolate were determined according to the method of Johnson et al., 1981). For EC measurements, suspension containing 1% powder ingredient (w/v) were prepared and 2.5 ml of this suspension was transferred into the 50 ml centrifuge tubes. They were homogenized for 2 minutes using 50 % power in an ultrasonic homogenizer (Bandelin Sonoplus HD 2070, Germany). Then, 2.5 ml corn oil was added and it

was homogenized again with 50 % power for 2,5 minutes. Emulsions were centrifuged at 5000 rpm for 3 minutes and final emulsion volumes were measured. EC (%) was measured as the ratio of the height of the emulsified layer to the height of the tube content. To determine ES, 2.5 ml of 1 % (w/v) suspension and 2.5 ml corn oil were emulsified using an ultrasonic homogenizer for 30 minutes in an 80°C water bath. Then, it was brought to room temperature and centrifuged at 5000 rpm for 3 minutes. ES (%) was measured as the ratio of the content of the emulsified layer to the height of the tube content.

The water holding capacity (WHC) was determined according to the method of Nguyen et al. (2018) for 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 minutes. The remaining liquid was then filtered through filter paper and the remaining weight was measured. The WHC(g water/g dry matter) expressed as the amount of water bound in the dry matter per g sample, was calculated as shown in the following equation;

$$WHC = \frac{\text{last weight-tare-sample weight}}{\text{sample weight}} \times 100 \quad (5)$$

The oil binding capacity (OBC) analysis for soymilk powder and soy protein isolate samples was done according to the method of Nguyen et al. (2018). 10 ml of corn oil was added to 1 g sample and it was vortexed. The samples were kept at room temperature for 30 minutes and they were vortexed at every 5 minutes. The resulting suspension was centrifuged at 10000 rpm for 10 minutes. After centrifugation, the oil fraction was taken, the remaining part was filtered with filter paper and it was weighed. The OBC(g water/g dry matter) was calculated as shown in the following equation;

$$OBC = \frac{\text{last weight-tare-sample weight}}{\text{sample weight}} \times 100 \quad (6)$$

Preparation of Chocolates

Four different chocolates were formulated, which were whole milk (WMC), skimmed milk (SMC), soymilk (SoMC) and soy protein chocolates (SPC). Firstly, cocoa butter (24%) was melted at $45 \pm 2^\circ\text{C}$ by bain marie style and cocoa liquor (27.5%) was added after all the butter melted. Then, sugar (30%) and the powder ingredient (18%) were added after sieving to avoid the agglomeration of the particles. Lastly, lecithin (0.5%) was added and the mixture was transferred to the chocolate melanger (Premier, USA) for refining and conching steps were carried out simultaneously at room temperature without heating for 2, 4 and 6 hours.

Tempering step was conducted by a tempering machine (Chocovision R2, USA). Initially, temperature was raised to $45 \pm 0.1^\circ\text{C}$ to ensure that no crystal was left and decreased gradually to $27.4 \pm 0.1^\circ\text{C}$ to produce the Form V crystal type. Then, samples were heated again to 30°C to melt the unstable crystal types. Tempered chocolates were molded ($7 \text{ cm} \times 4.8 \text{ cm} \times 0.4 \text{ cm}$) and tapped slightly to remove bubbles left inside before they were left to cool at room temperature.

Functional Properties of Chocolates

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

Total Phenolic Content (TPC)

The TPC analysis was done according to the method described by Dogan (2015) with some modifications. Sodium carbonate solution (75 g/L) was prepared before the analysis. Folin-Ciocalteu Phenol Reagent (2 N) was diluted to 1:10 (v:v) by distilled water to obtain 0.2 N. This solution was kept in dark since it was sensitive to light.

1.25 ml Folin reagent (0.2 N) was added to 10 ml of diluted solid sample. The mixture was vortexed and kept in dark for 5 minutes. 1 ml of sodium carbonate solution was added to the mixture and it was vortexed. This last mixture was put in a shaker (Edmund Bühler GmbH, Germany) for 1 hour in the dark. Then the absorbance was determined in a spectrophotometer at 760 nm wavelength. A calibration curve of gallic acid for different concentration was prepared. The results were expressed as mg GAE/g dry matter (GAE: gallic acid equivalent).

Total Antioxidant Capacity (TAC)

DPPH Assay

This analysis was done according to the method described by Serpen et al. (2012). 40 mg/L DPPH stock solution was prepared by dissolving the DPPH reagent in ethanol:water (1:1, v:v) mixture. This solution was kept in dark.

10 mg diluted solid sample was mixed with 10 ml of DPPH stock solution and put in a shaker for 2 hours in the dark. Then, the mixture was centrifuged at 10000 rpm for 2 minutes. The supernatant was filtered with a 0.45 µm microfilter and the absorbance was determined in a spectrophotometer at 525 nm wavelength. A Trolox calibration curve was generated for different concentrations. The results (TAC_{DPPH}) were expressed as mM Trolox equivalent/g dry sample.

ABTS Assay

ABTS solution was prepared according to Serpen et al. (2012). The ABTS reagent was dissolved in ethanol and then the same amount of water was added. Then, potassium persulfate was added and mixed. The final solution contained 7 nM ABTS and 2.45 mM potassium persulfate. This solution was kept in dark for 12-16 hours and was available for reaction for the next 24 hours.

For the analysis, 10 ml ABTS solution was added to the solid sample diluted with 10 mg cellulose and mixed in the dark for 26 minutes. Then, mixture was centrifuged at 10000 rpm for 2 minutes. The supernatant was filtered through 0.45 µm microfilter and the absorbance was determined in a spectrophotometer at 734 nm wavelength. A Trolox calibration curve was generated for different concentrations. The results (TAC_{ABTS}) were expressed as mM Trolox equivalent/g dry sample.

CUPRAC Assay

This analysis was done according to Tufan et al. (2013). Firstly, 0.2 M copper (II) chloride solution, 1 M and pH 7.0 ammonium acetate solution and 7.5×10^{-3} M neocuproin solution were prepared.

1 ml copper (II) chloride solution, 1 ml neocuproin solution, 1 ml ammonium acetate solution and 1.1 ml water: ethanol (1:1, v: v) mixture were mixed with 10 ml diluted solid sample and this was vortexed for 1 minute. The mixture was put in a shaker for 30 minutes in the dark. After the reaction, the mixture was centrifuged at 10000 rpm for 2 minutes. The supernatant was filtered through 0.45 µm microfilter and the absorbance was determined in a spectrophotometer at 450 nm wavelength. A Trolox calibration curve was generated for different concentrations. The results (TAC_{ABTS}) were expressed as mM Trolox equivalent/g dry sample.

Sensory Properties of Chocolates

Sensory analysis was performed with 10 untrained panelists. The scale used in the sensory analysis was explained to the panelists before the evaluation. The samples were coded with three random digits numbers and given to panelists in random order. Bottled water was offered to panelists to clean their mouth between evaluating different chocolate samples. Appearance, texture, flavor, odor and after taste attributes were chosen to evaluate the sensory properties of the chocolates. The assessment was conducted using a 5-point-hedonic scale. The chocolate samples were served at room temperature and under fluorescent lighting.

Statistical Analysis

After 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 $p \leq 0.05$.

Results and Discussion

Proximate Analysis of Soymilk Powder

Moisture content of all powder ingredients used in chocolate production was determined on wet basis. Moisture content values were found as 5.5 % in SMP, 2.8 % in WMP, 3.4 % in SoMP and 5.9 % in SPI samples. The highest value was obtained from SPI sample and the lowest value was given by WMP sample. The moisture content of non-fat samples was higher than fat-containing samples due to their free hydrophilic sites. The proximate composition of SoMP was shown in Table 1.

Table 1. Proximate composition of soymilk powder

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 ash content was 6.06%, the fat content was 18.14%, the protein content was 44.43%, and the carbohydrate content was 27.96%. Obatoye and Ogunwolu (2014) produced soymilk by Illonis method and dried the milk at 60°C for 14 hours in an 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. Another study previously reported that soymilk powder, which was obtained by spray-drying method, had 19.9% fat content (Ishiwu et al., 2014). The fat content in soy milk plays a crucial role for physical characterization such as texture and sensorial properties. Jinapong et al. (2008) specified the proximate composition of spray dried soymilk powder as 48.79% protein, 28.77% fat, 4.97% ash and 17.47% carbohydrate content on dry basis.

The pH of 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 powder suspension had a pH of 6.90 and the soy protein isolate suspension had a pH of 7.85. In previous studies, it was stated that the 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).

The 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. PDI is highly influenced by the pH and the process conditions.

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 value. Additionally, increasing temperature caused the NSI values to decrease. Spray-dried powders had 6% NSI (Van Burren 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.

Lipoxygenase Activity

High temperature application in soymilk production leads to reducing functionality and forming 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 and 20 minutes), raw soybean and soy protein isolate were shown in Table 2. 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 lower after the heat treatment. In this case, the time and temperature (20 minutes at 98-100°C) determined to ensure the inactivation of the lipoxygenase enzyme was

sufficient. According to Zhu et al. (1996), 30 minutes at 98.9°C heat treatment resulted in 4% LOX-1 and 0% LOX-3 residual content. The lower value of our results might be caused by the overnight soaking of soybeans and hot-grinding stage before the soymilk production.

Trypsin Inhibitors

The trypsin inhibitor activity (TIU/mg) change of the soymilk samples exposed to heat treatment for different time periods (5, 10, 15 and 20 minutes) were shown in Figure 1. Johnson et al. (1981) stated that 99°C heat treatment at 6.7 pH for almost 20 minutes resulted in approximately 10 % residual trypsin inhibitors. The results in this study were more satisfying (98.5 % inactivation) than this value which was ensured by soaking and hot grinding steps.

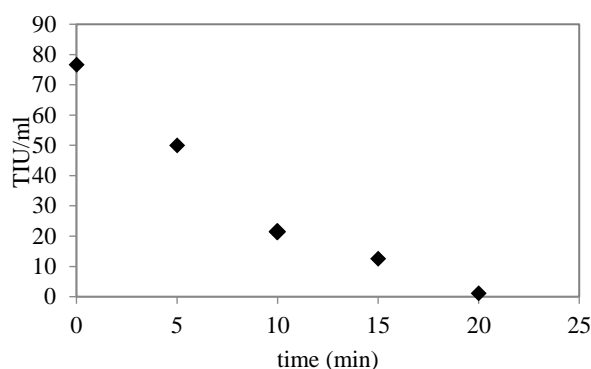


Figure 1. Trypsin inhibitor activity change of soymilk with time

Physical Properties of Powder Ingredients

The physical properties of powder ingredients are shown in Table 3. The bulk densities were found as 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. The tapped bulk densities were found as 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 bulk density values of spray-dried soymilk powders were changed between 359-470 kg/m³ while tapped bulk density values were changed between 532-640 kg/m³ (Nguyen et al., 2018). Spray-dried powders had non-homogeneous particle dispersion that was the cavity of big particles that was filled in small particles resulting in high bulk density. The density values were determined to understand the flow characteristics and cohesiveness of the powder ingredients.

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 the particle to absorb water on its surface. Wettability was measured visually and results were expressed as time of the particle absorb water. Long wetting time means less wettability of the powder ingredient. Particle size is the primary factor affecting the 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.

Table 2. Lipoxygenase residue in soybean, SoMP and SPI

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'	07.2 ± 0.13	4.6 ± 1.31
20'	00.9 ± 0.02	-
SPI	03.9 ± 0.58	2.9 ± 0.61

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

Table 3. Physical properties of powder ingredients

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

The nature of the particle surface also affects the wettability. For instance, the wettability reduces in the presence of free fat on the surface (Santana et al., 2017). According to the wettability values given in Table 3, SPI samples had the lowest wettability due to its protein character. Previous studies showed that the powder ingredients obtained by freeze-drying method had more porous structure (Caparino et al., 2012). Thus, SoMP had a higher wettability than other powder ingredients as expected. Wettability also affects the solubility character of the powders. Poor wettability causes poor solubility. Solubility character of the powder is affected by particle size and heat treatments. Lower the particle size, lower the solubility and the flowability of the powders (Koç et al., 2014). Generally, the porous systems are expected to have high solubility (Rogers et al., 2008). However, SoMP 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 powder. Although soymilk powder production process was different in our study, the results were similar. This is because of the surface area, surface charge, density, porosity and surface activity affecting the solubility (Koç et al., 2014). Caparino et al. (2012) reported that the solubility of freeze-dried mango powders was significantly lower than the 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. A previous study reported that soy protein isolate had 17.4% solubility, which is very close to our result (17.01%) (Kinsella, 1979). Statistically, wettability and solubility values of the samples were significantly different from each other ($P < 0.05$).

The Hausner Ratio (HR) has a great potential to be a fingerprint criterion in assessing the behaviour of powder products. HR is calculated using bulk and tapped bulk densities and describes the mobility of the powder ingredient (Ortega-Rivas et al., 2006). HR also indicates the powder cohesiveness. The decreasing HR is regarded as a reduction of cohesiveness. The powders are divided into four different groups in order to evaluate the flowability with respect to HR values. The HR ranges defining the flowability of the powders are shown below;

- 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

HR values of powder ingredients are shown in Table 3. Results showed that SMP was better in flowability than WMP and SoMP, which had the most difficult flow behaviours, and SPI had the easiest one. This can be explained by the fact that soy-oil increases the cohesiveness and adhesiveness of powder ingredients 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 it diminishes the cohesiveness (Abdullah and Geldart, 1999). Fat-containing samples (WMP and SoMP) showed more viscous (cohesive) character. Spray-dried soymilk powders were reported to have HR values between 1.416-1.603 by Nguyen et al. (2018). Fat-containing samples used in our study had higher HR values than these values while SMP and SPI had lower values. Jinapong et al. (2008) found that HR value of spray-dried soymilk powder was 1.67. According to statistical tests, SoMP was similar to WMP and SPI samples. In order to define the cohesiveness levels, the HR 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 ingredients in our study had high cohesiveness level. WMP and SoMP samples were found to exhibit higher cohesiveness due to their fat content.

The Carr Index (CI) used for determining the flowability levels is also known as compressibility index. The flowability values are grouped as the following with respect to CI values (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

The CI values (%) of various soy powders are shown in Table 3. According to these values, there is no ingredient that can flow very good or good. SPI sample showed fair flow and SMP, WMP and SoMP samples showed bad flow character. 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. On the other hand, freeze-dried samples have porous structure with poor flow character. Both HR and CI values showed that SoMP had the poorest flow behaviour. All the samples were significantly different from each other with respect to CI value ($P < 0.05$).

The brightness (L^*), green to red color degree (a^*), blue to yellow color degree (b^*), chroma value (C^*) and hue angle (h°) values of powder ingredients are shown in Table 4. The L^* value of SoMP (84.6) 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 varied between 86.2-88.8 (Giri et al., 2017), which are higher than our results. According to the study of Johnson et al. (1981), L^* value of heat-treated soymilk decreased with increasing temperature. L^* , a^* and b^* values of soymilk are considered as an indicator of the 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. The highest b^* value was obtained from SoMP sample. SMP and WMP samples had lower b^* values. a^* and b^* values of each sample were found to be significantly different ($P < 0.05$).

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 a high degree of browning in soymilk (Kwok et al., 1999). h° value of raw soymilk was reported as 108° describing with yellow region (90° - 180°). It is also known that upon heating, the h° value of soymilk decreases towards 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 treatment ($P < 0.05$) while h° values of SoMP and SPI samples were not significantly different from each other.

Functional Properties of Soy Powders

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 functional properties, water holding capacity (WHC), oil binding capacity (OBC), emulsion capacity (EC) and emulsion stability (EB) values of SoMP and SPI are given in Table 5. WHC value of SoMP sample was found as 0.96 g water/g dry matter, while it was found as 3.52 g water/g dry matter for SPI sample. Generally, increasing fat content results in lower WHC values due to reducing available hydrophilic binding sites to hold by protein (Heywood et al., 2002). Nguyen et al. (2018) reported that the WHC value of spray-dried soymilk powder ranged from 0.7 to 1.1 g / g. Enders (2001) previously reported that SPI had a high-water binding capacity and can reach up to 400 %. The results found by the study of Yalcin (2011) are close to our results. They showed that the WHC value was 338 % (3.38 g/g) for infrared treated soy flour samples. It is known that the lyophilized samples have more open porous structure and improved reconstitution properties. The WHC 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 a good WHC value but SPI had greater capacity than SoMP.

Table 4. Color values of powder products

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

Table 5. Functional properties of SoMP and SPI ingredients

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

Results are given with mean values and standard deviations over three replicates. * g water/g dry matter ** g oil/g dry matter

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. Yalcin (2011) 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. 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 5). 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. The present study also concluded the same way. SoMP was more stable than SPI due to its fat content.

Functional Properties of Chocolates

Total Phenolic Content

The total phenolic content (TPC) results were found between 80.10 mg GAE/g dry weight (SPC6) and 129.33 mg GAE/g dry sample (SoMC2). Table 6 shows the total phenolic content of chocolates prepared by different ingredient type and conching times.

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.

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 7. 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).

Table 6. Total phenolic contents of chocolates (mg GAE / g dry sample)

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

Table 7. Total antioxidant capacity of chocolates (mM Trolox / g dry sample)

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

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 isoflavone 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 the 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.

Sensory Properties of Chocolates

Sensory analysis is one of the most important analyzes to determine the consumability of products. Ingredient type is a major parameter affecting the sensory perception of chocolate. In this study, sensory evaluation was conducted only to evaluate the different ingredient types in order to compare the consumability of soy-included products with milk chocolates. Sensory analysis of chocolates was evaluated in terms of aftertaste, appearance, flavor, odor and odor parameters and scores of samples are shown in Figure 2.

A previous study investigated the difference in taste between conched and unconched 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 D₉₀ was correlated with the sensory character and PSD properties have a high impact on flow and sensory properties. Therefore only 6 hours conched chocolates 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 characteristics. 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 appearance 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 considerably. 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.

Conclusion

Chocolate is the most famous snack in the world that has consumer in any age. 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 isoflavone 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 functionality of chocolate.

Soymilk powder and soy protein isolate were used as a substituent in chocolate and skimmed milk powder and whole milk powder was used to produce control chocolates. Physical analyses 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. Functionality of chocolates was evaluated in terms of total phenolic content and total antioxidant capacity. 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.

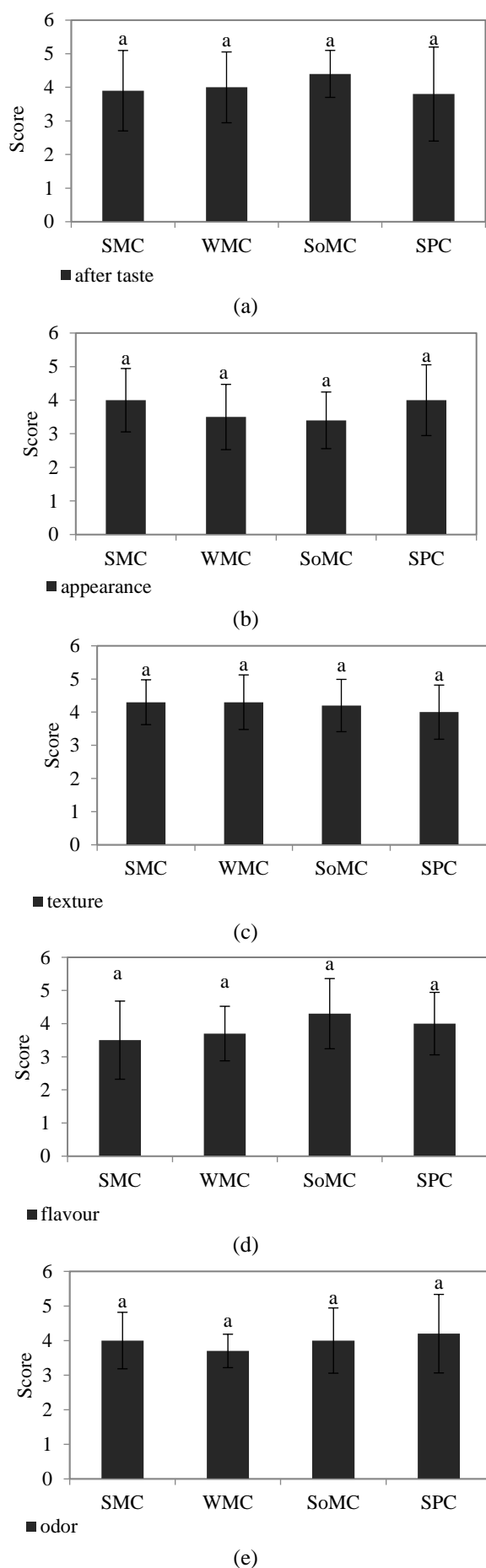


Figure 2. Sensory analysis scores a) after taste, b) appearance, c) texture, d) flavour, e) odor

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. SPC samples showed the lowest antioxidant activity and total phenolic content.

Consequently, the soymilk powder used in this study affected the chocolate with greater protein content and higher antioxidant capacity levels than milk powder. This study contributed to understanding 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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