GIDA
THE JOURNAL OF FOOD
E-ISSN 1309-6273, ISSN 1300-3070

Research/ Araştırma
GIDA (2020) 45(5) 850-860
doi: 10.15237/gida.GD20034

DEVELOPMENT OF DARK CHOCOLATE ENRICHED WITH MATCHA GREEN TEA (*CAMELLIA SINENSIS*)

Mine Gültekin-Özgüven[1*], Ijlal Berktaş[1], Özlem Gönül[2], Beraat Özçelik[1,3]

[1]Department of Food Engineering, Faculty of Chemical and Metallurgical Engineering, Istanbul Technical University, Maslak, Istanbul, Turkey

[2]Department of Food Hygiene and Technology, Faculty of Veterinary, Istanbul University, Avcilar, Istanbul, Turkey

[3]BIOACTIVE Research & Innovation Food Manufac. Indust. Trade Ltd., Katar Street, Teknokent ARI-3, B110, Sarıyer, Istanbul, Turkey

Received/ Geliş: 25.02.2020; *Accepted*/ Kabul: 20.07.2020; *Published online*/ Online baskı: 15.08.2020

Gültekin-Özgüven, M., Berktaş, I., Gönül, Ö., Özçelik, B. (2020). Development of dark chocolate enriched with matcha green tea (Camellia sinensis). GIDA (2020) 45(5) 850-860 doi: 10.15237/gida.GD20034

Gültekin-Özgüven, M., Berktaş, I., Gönül, Ö., Özçelik, B. (2020). Maça yeşil çayı ile zenginleştirilmiş bitter çikolata geliştirilmesi. *GIDA* (2020) 45(5) 850-860 doi: 10.15237/gida.GD20034

ABSTRACT

One of the most consumed and popular food product among consumers across the globe is chocolate and consumers are becoming evermore demanding of their chocolate all over the world. The aim of this study was to design a dark chocolate enriched with matcha green tea (2%, 3%, 4%) to enhance its antioxidant activity. The most suitable matcha addition step (mixing, refining, conching, tempering) during chocolate production process was investigated by using total phenolics and flavonoids, and antioxidant activity (ORAC and DPPH assays) since phenolics may be destructed during the production process. As expected, increase in matcha percentage in chocolate formulation resulted in an increase of phenolics and antioxidant activity. However, addition of more than 3% matcha to the chocolate formulation reduced consumer acceptability.

Keywords: Dark chocolate; matcha green tea; total polyphenols; antioxidant activity; sensory evaluation

MAÇA YEŞİL ÇAYI İLE ZENGİNLEŞTİRİLMİŞ BİTTER ÇİKOLATA GELİŞTİRİLMESİ

ÖZ

Dünya genelinde tüketiciler arasında en çok popüler olarak tüketilen gıda ürünlerinden biri çikolatadır ve tüketicilerin çikolatadan beklentileri giderek artmaktadır. Bu çalışmanın amacı, antioksidan aktivitesini arttırmak için maça yeşil çayı (%2, %3, %4) ile zenginleştirilmiş bir bitter çikolata tasarlamaktır. Fenoliklerin üretim işlemi sırasında tahrip olabileceğinden dolayı çikolata üretim prosesi sırasında en uygun maça çayı ekleme aşaması (karıştırma, inceltme, konçlama veya temperleme), toplam fenolikler ve flavonoidler ve antioksidan aktivite (ORAC ve DPPH yöntemleri ile) kullanılarak araştırılmıştır. Beklenildiği gibi formülasyonda bulunan maça çayı yüzdesindeki artış, fenolik içeriğin ve antioksidan aktivitenin artmasına neden olmuştur. Ancak, % 3'ten fazla maça ilavesi tüketici beğenilirliğini ve kabul edilebilirliğini azaltmıştır.

Anahtar kelimeler: Bitter çikolata; maça yeşil çay; toplam polifenol; antioksidan aktivite; duyusal analiz

[*]*Corresponding author* / Yazışmalardan sorumlu yazar:
✉: gultekinmi@itu.edu.tr ✆ +90 212 285 7319 🖷: +90 212 285 7333

Mine Gültekin-Özgüven ORCID ID: 0000-0002-2073-8075
Ijlal Berktaş ORCID ID: 0000-0001-9836-5881
Özlem Gönül ORCID ID: 0000-0002-4669-607X
Beraat Özçelik ORCID ID: 0000-0002-1810-8154

INTRODUCTION

Chocolate is a popular and widely consumed product around the world. The main ingredients in chocolate are cocoa liquor, sugar and other sweeteners, cocoa butter, milk products, flavours and emulsifiers (Tanabe and Hofberger, 2005). Chocolate processing steps consist of mixing, refining, conching and tempering. The main ingredients are blended in mixing step. Then, particle size of the mass is reduced and smooth texture is obtained in refining step (Tanabe and Hofberger, 2005). In conching step, different time and temperature treatments are applied until all ingredients are homogenously dispersed in the continuous fat phase. Consequently, crystallization of triacylglycerols in cocoa butter is provided by shearing chocolate mass at controlled temperatures in tempering step where small stable cocoa butter crystals are occurred. As the final product, chocolate has a glossy appearance, a good texture and bloom resistance (Tanabe and Hofberger, 2005; Afoakwa et al., 2008).

The cocoa (*Theobroma cacao*), raw material of cocoa liquor and cocoa butter, presents a potentially rich dietary source of flavonoids such as flavonol monomers (-)-epicatechin (EC) and (+)-catechin (C), and oligomers of these monomeric base units, which are known as the procyanidins (Andres-Lacueva et al., 2008; Mathur et al., 2002; Mursu et al., 2004). Polyphenols have gained much attention due to their possible benefits in human health such as protective effects against cardiovascular diseases, cancers and other age-related diseases arising from antioxidant activities of these compounds (Nishitani and Sagesaka, 2004; Wollgast and Anklam, 2000). However; processing parameters such as degree of alkalization treatment, temperature and time affect the flavonoid content of chocolates. Whereas, significant amounts of flavonoids can be remained in chocolate by applying appropriate processing conditions (Aachary et al., 2012). Still, consumers are becoming evermore demanding of their chocolate all over the world. Therefore, manufacturers are willing to produce value-added chocolates. For this reason, organic chocolate, chocolate containing cocoa (70%, 85%, 90%), probiotic chocolate, prebiotic chocolate, sugar-free chocolate, etc (Özgüven, 2014) have been produced until now. In the concept of enhancing flavonoids of chocolate, dark and white chocolates enriched with matcha tea are commercially available now in the international markets. Matcha tea is finely milled or fine powdered form of green tea (*Camellia sinensis*). Unlike green tea, matcha is grown avoiding direct sunlight since sunlight affects the composition and amount of catechins in the tea leaves (Weiss and Anderton, 2003). After harvesting, leaves are chopped, rolled and heated to inactivate polyphenol oxidase enzyme. Hence, significant amount of catechins in green tea is preserved (Komes et al., 2010). The major phenolics present in tea leaves are flavan-3-ols (catechins), which compose to 30% of their dry weight (Rusak et al., 2008). The important naturally occurring catechins in tea leaves are (-)-epigallocatechingallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechingallate (ECG), (-)-epicatechin (EC), (+)-gallocatechin (GC) and (+)-catechin (C) (Manian et al., 2008, Nishitani & Sagesaka, 2004). Although catechins are dominant phenolic compounds, tea leaves also contain up to 4% various flavonols and a trace of flavones (Rusak et al., 2008).

In this study, by combining dark chocolate and matcha (2%, 3% and 4%), a chocolate presenting high phenolic content, was developed. The aims of this study were to determine the most suitable chocolate production step (mixing, refining, conching and tempering) for matcha addition, to investigate the effect of chocolate production steps on phenolics and antioxidant activity, and to optimize a recipe for consumer acceptance due to possible astringent flavor of tea. This study has a great potential to serve as the model for a new product development as a dark chocolate enriched with high polyphenol content.

MATERIALS AND METHODS

Materials

Folin-Ciocalteu phenol reagent, Catechin, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azobis [2-methylpropionamidine], dihydrochloride (AAPH) and fluorescein were

purchased from Sigma-Aldrich Chemical (St. Louis, MO). Matcha was obtained from Aiya Inc., America. Chocolate samples were produced by Pelit Cikolata ve Gida Sanayi Inc., Turkey.

Production of dark chocolates with and without matcha

The recipe of bitter chocolate consisting of cocoa liquor, refined sugar, cocoa butter, soy lecithin, vanillin and matcha tea is given in Table 1. Matcha (2%, 3% and 4%) was added by replacing refined sugar instead of cocoa components in the formulation in order to prevent decreasing phenolic content that comes from cocoa. First, matcha (2%) was added in the formulation in different production steps (mixing, refining, conching and tempering) to determine the least destructive step on phenolics without disrupting textural properties of the chocolate product. Then, chocolate samples containing 2%, 3%, 4% matcha and control chocolate samples without matcha were produced. 5 kg sample batches were produced for each run. Ingredients were mixed in a Mixer (Model UMC-5, Stephan Machinery GmbH, Hameln, Germany) and refined to 18±2 micron particle size using a 3-roll mill refiner (Bühler SDY-200, Wohl Associates, Inc, Bohemia, Czech Republic). After conching in a Conche (Bühler, Richard Frisse GmbH ELK-02, Bad Salzuflen, Germany) at 77±3 °C for 12 hours, tempering was performed using a laboratory scale mini-temperer (Model TOP, Selmi srl, Via Statale, Santa Vittoria d'Alba, Italy). Tempered chocolate was then moulded and cooled in a cooling tunnel (Model TUN, Selmi srl, Via Statale, Santa Vittoria d'Alba, Italy).

Table 1. Recipe of bitter chocolate samples

Ingredients	Control chocolate	Chocolate containing 2% matcha	Chocolate containing 3% matcha	Chocolate containing 4% matcha
Cocoa liquor (%)	58.90	58.90	58.90	58.90
Refined sugar (%)	29.60	27.60	26.60	25.60
Cocoa butter (%)	11.10	11.10	11.10	11.10
Soy lecithin (%)	0.39	0.39	0.39	0.39
Vanillin (%)	0.01	0.01	0.01	0.01
Matcha tea (%)	0	2	3	4

Extraction of phenolic compounds

The extraction of phenolic compounds was performed according to the method described by Gültekin-Özgüven et al. (2016). The lipid phase was removed from 10 g of the ground chocolates by extracting three times with 45 ml of hexane. Defatted sample of 1.0 g was extracted with 10 ml of 80% methanol in water for 10 minutes at 30°C in the ultrasonic bath (USC900TH, VWR ultrasonic cleaner, Radnor, PA, USA). The extracts of the samples were obtained after centrifugation at 4000 rpm for 10 min at 4°C.

Determination of total phenolics

The amount of total phenolics (TP) in the matcha and chocolate extracts was determined using the Folin–Ciocalteu method described by Wollgast (2004). 300 µl of diluted extracts were mixed with 1.5 ml of 10-fold-diluted Folin–Ciocalteu reagent and 1.2 ml of 7.5 g/100g of sodium carbonate. The mixture was allowed to stand for 30 minutes at room temperature before the absorbance was measured at 765 nm. Results were expressed as mg catechin equivalents (CE) per g defatted chocolate. Samples were analyzed in triplicate.

Determination of total flavonoids

Determination of flavonoids in the matcha and chocolate extracts was performed according to the colorimetric assay of Lee et al. (2003). 1 ml of diluted extracts were mixed with 300 µl of $NaNO_2$ (5%). Then at 5th min, 300 µl of $AlCl_3$ (10%) and at 6th min, 1 ml of 1 mol/L NaOH were added. After addition of 2.4 ml of water, the absorbance of the mixtures were determined at 510 nm. Results were expressed as mg CE per g defatted chocolate sample. Samples were analyzed in triplicate.

Determination of phenolic profile of matcha
Phenolic profile of matcha was determined using HPLC according to the method described by Dragović-Uzelac et al. (2005). The liquid chromatographic system consisted of a Waters 2695 Seperation module and Waters 2996 PDA dedector fitted with a Waters Supelcosil C18 column (5 μm, 250 x 4.6 mm i.d.). The injection system with 20 μl sample loop was used. Detection was done at wavelength of 280 nm (210-360 nm). Elution was carried out at a flow rate of 1 mL/min under a linear gradient of 3% acetic acid in water (solvent A) and 3% acetic acid:25% acetonitrile:72% H_2O (solvent B) from 100% A to 30% A in 40 min and then to 20% A in 45 min, to 15% A in 55 min, to 10% A in 57 min, finally 10% A in 57-75 min. Fine matcha powder was extracted using 80% methanol in water in an ultrasonic bath at 30°C for 10 min and 20 μl of the extract was injected into the HPLC. Individual phenolic compounds were identified and quantified using curves of phenolic standards. Results were expressed as mg per g tea sample.

DPPH radical scavenging activity assay
DPPH assay described by Brand-Williams et al. (1995) was slightly modified and employed in this study (Sanchez-Moreno et al., 1998). The extract stock solutions were diluted with water at different ratios changing between 1/20 and 1/50. Then, an aliquot of chocolate extracts (0.1 ml) for each dilution was added to 2.9 ml of $6x10^{-5}$ M of methanol solutions of DPPH. The reaction was allowed to take place in the dark at room temperature for 30 minutes. The absorbance values were measured at 517 nm. The percentage of remaining DPPH ($DPPH_R$%) was calculated by using the equation (1).

$$DPPH_R\% = [(DPPH_T)/DPPH_{T=0}] \times 100 \quad \text{(Equation 1)}$$

where $DPPH_{T=0}$ is the concentration of the DPPH solution at the time of zero and $DPPH_T$ is the concentration of the DPPH after 30 min. The percentage of remaining DPPH against the sample concentration was plotted to calculate the EC_{50} values (the amount of antioxidant necessary to decrease by 50% the initial DPPH concentration). Samples were analyzed in triplicate.

Oxygen radical absorbance capacity (ORAC) assay
The ORAC assay was performed according to method described by Huang et al. (2002). AAPH (153 mmol/L) and fluorescein stock solutions (4 x 10^{-3} mmol/L) were prepared with 75 mmol/L phosphate buffer (pH 7.4). Fluorescein working solution (8.16 x10^{-5} mmol/L) was made by diluting stock solution with 75 mmol/L phosphate buffer (pH 7.4). 25 μl of diluted chocolate extracts were mixed with 150 μl of fluorescein working solution in wells. 75 mM phosphate buffer was used as a blank. Then, the plates were allowed to equilibrate by incubating for 15 minutes in the Synergy™ HT Multi-Detection Microplate Reader (BioTek Instruments, Winooski, VT) at 37°C. Reactions were initiated by the addition of 25 μl of AAPH solution. The fluorescence was then monitored kinetically for 3 hours with data taken every minute. ORAC values were calculated according to Cao and Prior (1999). The area under curve (AUC) of the samples were determined using equations 2 and 3 respectively.

$$AUC = 0.5 + f1/f0 + ... fi /f0 + ... + f34/f0 + 0.5(f35/f0) \quad \text{(Equation 2)}$$

where f0: initial fluorescence reading at 0 min and fi: fluorescence reading at time i.

$$\text{Net AUC} = \text{AUCsample} - \text{AUCblank} \quad \text{(Equation 3)}$$

The regression equation between net AUC and Trolox concentration was determined. ORAC values were expressed as μmol Trolox equivalents (TE)/g sample using the prepared standard curve. Samples were analyzed in triplicate.

Sensory Evaluation
Sensory attributes of dark chocolate with matcha and control without matcha were evaluated to determine bitter taste, mouthfeel, melting, brittleness, and acceptable matcha taste using a 5-point hedonic scale (1=dislike extremely,

3=moderate, 5=like extremely) by 25 panelists selected from post-graduate students and staff of the Food Engineering Department of Istanbul Technical University. Selected panelists were trained in 3 different sessions to carry out sensory analysis. They worked under the direction of the panel leader to develop the glossary of terms and their definitions and scores for references. In sensory analysis, the samples were served monadically, and the serving order of the samples was randomized. Sensory tests were carried out in individual air-conditioned booths. Crackers and taste-free water were provided for palate cleansing.

Statistical Analysis

All analyses were repeated at least three times using triplicate samples for each chocolate sample. The data are presented as mean ± standard deviation. The significance ($p<0.05$) of the differences between the means was determined using one-way analysis of variance (ANOVA) with Duncan's post-hoc test. The statistical analyses were performed with the SPSS v.15 statistical programme (SPSS Inc., Chicago, USA). The correlation between phenolic content, flavonoid content and ORAC were performed using the Microsoft Excel Data Analysis (Microsoft Corp., Redmond, Wash., USA).

RESULTS AND DISCUSSION

Characterization of matcha

Total phenolic and flavonoid content of matcha tea extract was found to be 156.6 mg CE/g and 136.2 mg CE/g, respectively. The reverse phase HPLC analysis was used for the separation and identification of flavan-3-ols in matcha tea powder. According to the results, the most abundant bioactive constituents of green tea were EGCG (63 mg/g) and EGC (40 mg/g) followed by EC (11 mg/g) and GC (3 mg/g). The results of catechins in the matcha are comply with the study of Weiss and Anderton (2003), who determined EGCG (57.4 mg/g), EGC (12.6 mg/g), ECG (12.8 mg/g), EC (4.0 mg/g) and C (0.83 mg/g). Antioxidant activity of the matcha extract was determined to be 2116 μmol TE/g and 0.82 mg/ml, respectively in terms of ORAC and EC_{50} values, which means that matcha green tea showed a strong antioxidant activity due to its high phenolic content. In this case, a low EC_{50} value (0.82 mg/ml) is indicative of strong antioxidant activity.

Incorporation of matcha into dark chocolate

The most suitable chocolate production step for addition of matcha (2%) without destroying phenolics was determined considering total phenolics, flavonoids and antioxidant activity (Figure 1A, 1B, 1C and 1D). According to the results of chocolates containing 2% matcha, addition of matcha in mixing, refining or conching steps was not statistically significant ($P > 0.05$) in terms of TPC, TFC and antioxidant activity (ORAC and DPPH assays). On the contrary, a significant increase in phenolic content of chocolates was observed after addition of matcha in tempering step. As presented in Figure 1C and 1D, antioxidant activity results of dark chocolate with matcha (2%) were in agreement with those of TPC and TFC. Even though addition of matcha in tempering step was found to be more preservative method for phenolics, it was not suitable for chocolate production since matcha could not be combined with chocolate homogeneously. For these reasons, matcha was incorporated into chocolate in mixing step to ensure homogeneous chocolate samples were obtained. Therefore, chocolates with 2%, 3% and 4% matcha were produced by adding matcha in the mixing step. So, chocolate samples containing 2%, 3% and 4% matcha were produced to increase phenolic content without deteriorating sensorial properties of the products. Due to bitter and astringent flavour of phenolic compounds (Streit et al., 2007), 4% was selected as the upper limit for matcha addition in chocolate recipe.

TPC, TFC and antioxidant activity of chocolate samples

After the decision to add matcha in the mixing step, all chocolate samples containing matcha (2%, 3% and 4%) were produced in this way. The final chocolate products were obtained after tempering step. Total phenolic and flavonoid content of control chocolate sample without matcha and chocolate samples containing matcha (2%, 3% and 4%) was shown in Figure 2A and

2B. In the final products, phenolic content of control and chocolate samples containing matcha (2%, 3% and 4%) was significantly different from each other ($P < 0.05$). As expected, chocolate samples with 4% matcha exhibited the highest TPC (21.23 mg CE/g) and TFC (12.21 mg CE/g) while control chocolates contained the lowest TPC (16.28 mg CE/g) and TFC (9.49 mg CE/g). Compared to the control chocolate, TPC increased 12.32%, 23.68%, 30.41% and TFC increased 7.76%, 17.32%, 28.59% in chocolates with 2%, 3% and 4% matcha, respectively. In fact, total phenolic and flavonoid content of chocolate samples was comply with the study of Pimentel et al. (2010) who determined TPC 62.9 µmol CE/g (18.26 mg CE/g) and TFC 21.6 µmol CE/g (6.27 mg CE/g) in dark chocolate containing 71% cocoa, which was similar to the results of our control chocolate but lower than the chocolate samples with matcha. Meanwhile, there was a positive and significant correlation between total phenolics and total flavonoids of the chocolate samples ($r^2 = 0.952$).

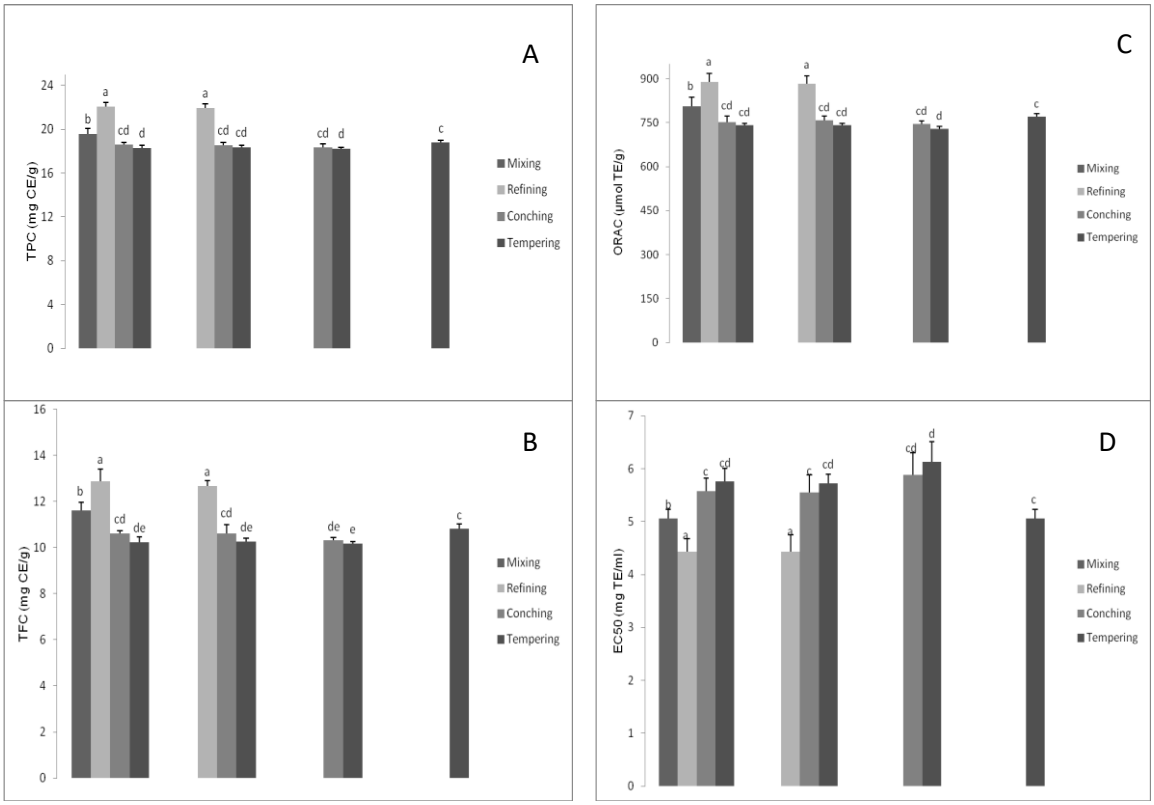

Figure 1. TPC (A), TFC (B), ORAC (C), EC_{50} (D) of chocolate with 2% matcha after addition of matcha in mixing, refining, conching and tempering steps.
Values are expressed as mean ± standard deviation.
a-e: Means with different letters are significantly different at the level of $P < 0.05$
TPC: Total Phenolic Content, TFC: Total Flavonoid Content

Meanwhile, the highest ORAC value was obtained in chocolates with 4% matcha (852 µmol TE/g), followed by chocolates with 3% matcha (808 µmol TE/g), chocolates with 2% matcha (741 µmol TE/g), and control chocolate (682 µmol TE/g) (Figure 2C). In comparison to control chocolate, ORAC values of chocolates enhanced by 8.65%, 18.48% and 24.93% after addition of 2%, 3% and 4% matcha, respectively. Gu et al. (2006), who determined ORAC values ranging from 516 to 444 µmol of TE/g in unsweetened chocolate with 49.5% of nonfat

cocoa solid content, which was lower than chocolate samples results in this study due to their higher nonfat cocoa solid (70%) and matcha green tea content. The EC_{50} value of the extract of control chocolate (6.24 mg/ml) was significantly higher than that of chocolate extracts containing 2% (5.76 mg/ml), 3% (5.24 mg/ml) and 4% (4.92 mg/ml) matcha (Figure 2D). The lower EC_{50} value indicates a higher antioxidant activity. In terms of DPPH assay, antioxidant activity increased 7.69%, 16.01% and 21.20% after addition of matcha to chocolate samples by 2%, 3% and 4%, respectively. Therefore, antioxidant activity results measured by ORAC and DPPH assays are in agreement.

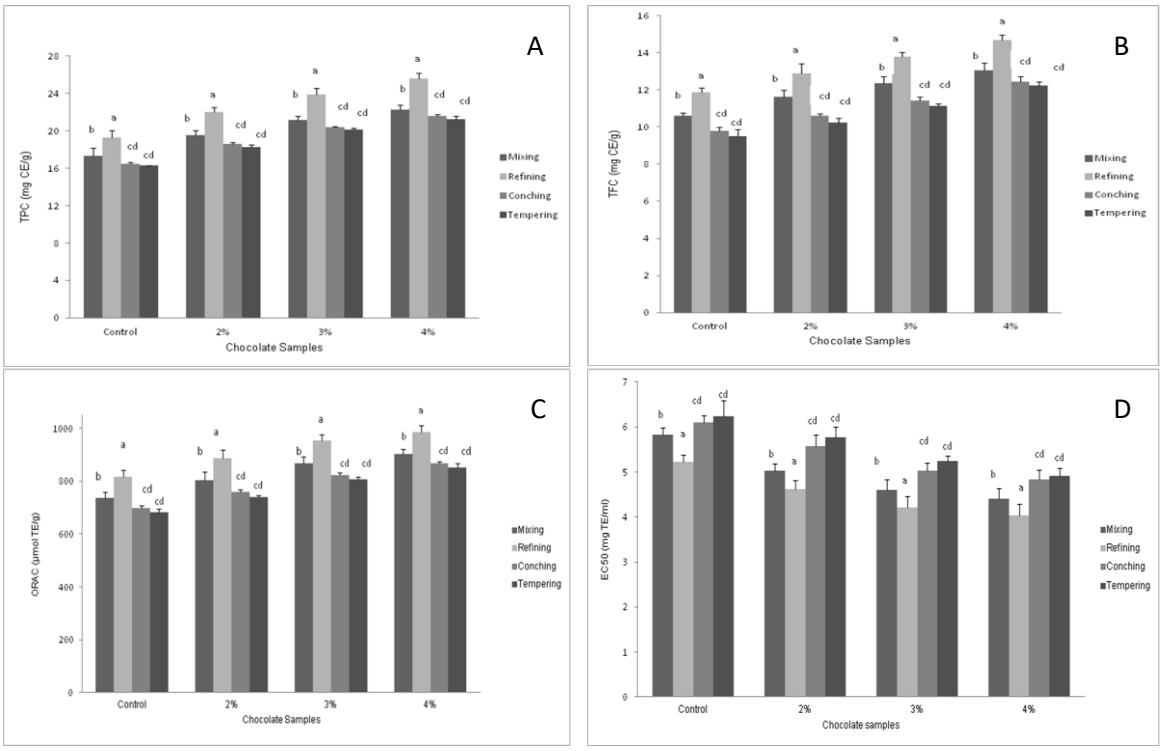

Figure 2. Changes in TPC (A) and TFC (B), ORAC (C) and EC_{50} (D) values of control and chocolates containing 2%, 3% and 4% matcha during chocolate production steps.

Values are expressed as mean ± standard deviation.

a-d: Means with different letters are significantly different in each chocolate sample at the level of $P < 0.05$

TPC: Total Phenolic Content, TFC: Total Flavonoid Content

Changes in TPC, TFC and antioxidant activity during chocolate production

Figure 2 (A, B, C and D) shows the effect of chocolate production process on TPC, TFC and antioxidant activity. A significant increase was observed in refining step while a significant reduction was detected in conching step in terms of TPC, TFC and antioxidant activity. This process effect is given in Table 2 using the average results of all chocolate samples including control chocolate, chocolate samples with 2%, 3% and 4% matcha in terms of TPC, TFC and antioxidant activity. According to the results, TPC and TFC of chocolate samples increased by 13.02% and 11.72% during refining step. However, conching caused a decrease of 15.37% in TPC and 16.94% in TFC of chocolates. Similarly, antioxidant activity measured by ORAC and DPPH assays showed an increase of 10.02% and 8.89% during refining step. In contrast, conching caused a reduction of 13.67% and 19.21% in antioxidant activity in terms of ORAC and DPPH assays. Therefore, it is possible to say that difference between the effect of chocolate production steps on phenolics was statistically significant ($P < 0.05$).

Table 2. The effect of refining and conching steps on the TPC, TFC, ORAC and EC_{50} values of control and chocolates containing matcha (2%, 3% and 4%).

	Percentage Increase by Refining (%)				
	Control[1]	Chocolate with 2% matcha	Chocolate with 3% matcha	Chocolate with 4% matcha	Average*
TPC	11.51	12.60	12.95	15.03	13.02
TFC	11.82	10.94	11.72	12.40	11.72
ORAC	10.43	10.26	9.84	9.54	10.02
EC50	10.23	8.27	8.35	8.69	8.89
	Percentage Decrease by Conching (%)				
	Control[1]	Chocolate with 2% matcha	Chocolate with 3% matcha	Chocolate with 4% matcha	Average*
TPC	14.92	15.79	14.87	15.90	15.37
TFC	17.69	17.65	17.14	15.27	16.94
ORAC	14.40	14.55	13.66	12.05	13.67
EC_{50}	16.83	20.56	19.24	20.20	19.21

[1]Control: Chocolate without matcha
TPC: Total phenolic content
TFC: Total flavonoid content
*Standard deviation (<10%) is omitted to simplify the result

Meanwhile, there was a high negative correlation between the ORAC and EC_{50} values (r^2=-0.942). Antioxidant activity of chocolate samples were in accordance with the TPC, which was confirmed by a strong correlation obtained between the results (r^2ORAC/TPC=0.979, r^2DPPH/TPC=-0.90). Similarly, a high correlation was observed between the antioxidant activity and flavonoid compounds of chocolates (r^2ORAC/TFC=0.985, r^2DPPH/TFC=-0.921) as well, which implies that the antioxidant potential of chocolate was directly related to their phenolic content.

Sensory Evaluation

The chocolate samples were evaluated for bitterness, acceptable matcha taste, brittleness, melting rate and smooth mouthfeel attributes (Figure 3). For bitterness and acceptable matcha taste, there was no significant difference among chocolates with 2% matcha and control ($P > 0.05$). However, for acceptable taste of matcha, there was a significant difference between chocolates with 2% matcha and chocolates with 3% and 4% matcha. In relation to brittleness, the chocolates with 2% and 3% matcha and the control were scored the highest with no significant difference among them ($P > 0.05$). Furthermore, the melting rate and smooth mouthfeel of the chocolates significantly decreased due to addition of matcha ($P < 0.05$). This study indicates that maximum 3% matcha addition was acceptable for consumer, but adding matcha more than 3% in dark chocolates reduce the consumer acceptability.

CONCLUSIONS

In summary, matcha green tea powder may be used as a value-added ingredient besides being a beverage due to exhibiting a strong antioxidant activity. In dark chocolate product, the results were successful. As expected, a significant increase in phenolic content was obtained by adding 2%, 3% and 4% matcha ($P < 0.05$). However, according to sensory test results using matcha more than 3% causes undesirable taste in bitter chocolates. Adding matcha in tempering step causes less destruction on phenolics but matcha could not be mixed with chocolate homogeneously. Therefore, it was preferred to add matcha in mixing at the beginning of the process.

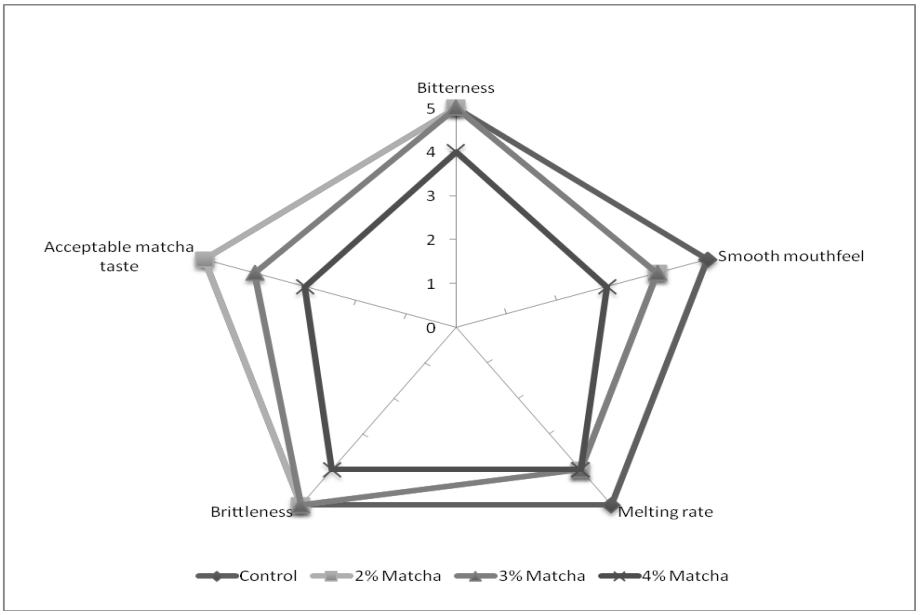

Figure 3. Spider diagram showing the sensory profile of control and chocolates with 2%, 3% and 4% matcha.

ACKNOWLEDGEMENTS

Authors would like to thank to TÜBİTAK (TEYDEB 1501, Project ID: 3090322) for their support on this project. We would also like to show our gratitude to Mr. Selahattin Ayan; Chairman of the Board of Pelit Chocolate Company for his inspiration and motivation during this research.

CONFLICT OF INTEREST

There is no possible conflict of interest among the authors.

AUTHOR CONTRIBUTION

Özlem Gönül was responsible for producing of bitter chocolate samples. Mine Gültekin-Özgüven and İjlal Berktaş performed the chemical analyses and wrote the article. Beraat Özçelik was responsible for experimental design, interpretation and discussion of the results.

REFERENCES

Aachary, A. A., Thiyam, U., Eskin, N. A. M. (2012). Chocolate and cocoa: A comprehensive review on cardiovascular benefits. In: *Functional foods and Cardiovascular Disease,* Moghadasian, M.H., Eskin N.A.M (eds.), CRC Press, USA, pp. 233–258.

Afoakwa, E. O., Paterson, A., Fowler, M., Vieira, J. (2008). Effects of tempering and fat crystallization behaviour on microstructure, mechanical properties and appearance in dark chocolate systems. *J Food Eng*, 89:128–136, doi: 10.1016/j.jfoodeng.2008.04.021.

Andres-Lacueva, C., Monagas, M., Khan, N., Izquierdo-Pulido, M., Urpi-Sarda, M., Permanyer, J., Lamuela-Raventos, R. M. (2008). Flavanol and flavonol contents of cocoa powder products: Influence of the manufacturing process. *J Agr Food Chem*, 56: 3111–3117, doi: 10.1021/jf0728754.

Brand-Williams, W., Cuvelier, M.E., Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Food Sci Technol*, 28: 25-30.

Cao, G., Prior, R. (1999). Measurement of oxygen radical absorbance capacity in biological samples. oxidants and antioxidants. *Methods Enzymol*, 299: 50-62, doi: 10.1016/S0076-6879(99)99008-0.

Dragovic-Uzelac, V., Delonga, K., Levaj, B., Djarkovic, S., Pospisil, J. (2005). Phenolic profiles

of raw apricots, pumkins, and their purees in the evaluation of apricot nectar and jam authencity. *J Agr Food Chem*, 53: 4836-43, doi: 10.1021/jf040494+.

Gu, L., House, S. E., Wu, X., Ou, B., Prior, R. L. (2006). Procyanidin and catechin contents and antioxidant capacity of cocoa and chocolate products. *J Agr Food Chem,* 54: 4057−4061, doi: 10.1021/jf060360r.

Gültekin-Özgüven, M., Berktaş, İ., Özçelik, B. (2016). Influence of processing conditions on procyanidin profiles and antioxidant capacity of chocolates: Optimization of dark chocolate manufacturing by response surface methodology. *Food Sci Technol*, 66: 252-259.

Huang, D., Ou, B., Hampsch-Woodill, M., Flanagan, J., Prior, R. (2002). High-throughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate fluorescence reader in 96-well format. *J Agr Food Chem*, 50: 4437-4444, doi: 10.1021/jf0201529.

Komes, D., Horzic, D., Belščak, A., Ganic, K. K., Vulic, I. (2010). Green tea preparation and its influence on the content of bioactive compounds. *Food Res Int*, 43: 167–176, doi: 10.1016/j.foodres.2009.09.022.

Lee, K. W., Kim, Y. J., Lee, H. J., Lee, C. Y. (2003). Cocoa has more phenolic phytochemicals and a higher antioxidant capacity than teas and red wine. *J Agr Food Chem*, 51: 7292−7295, doi: 10.1021/jf0344385.

Manian, R., Anusuya, N., Siddhuraju, P., Manian, S. (2008). The antioxidant activity and free radical scavenging potential of two different solvent extracts of *Camellia sinensis* (L.) O. Kuntz, *Ficus bengalensis* L. and *Ficus racemosa* L. *Food Chem*, 107: 1000–1007, doi: 10.1016/j.foodchem. 2007.09.008.

Mathur, S., Devaraj, S., Grundy, S. M., Jialal, I. (2002). Cocoa products decrease low density lipoprotein oxidative susceptibility but do not affect biomarkers of inflammation in humans. *J Nutr*, 132: 3663-3667, doi: 10.1093/ jn/132.12.3663.

Mursu, J., Voutilainen, S., Nurmi, T., Rissanen, T. H., Virtanen, J. K., Kaikkonen, J., Nyyssonen K., Salonen, J. T. (2004). Dark chocolate consumption increases HDL cholesterol concentration and chocolate fatty acids may inhibit lipid peroxidation in healthy humans. *Free Radic Biol Med*, 37(9): 1351–1359, doi:10.1016/j.freeradbiomed.2004.06.002.

Nishitani, E., Sagesaka, Y. M. (2004). Simultaneous determination of catechins, caffeine and other phenolic compounds in tea using new HPLC method. *J Food Compost and Anal*, 17: 675–685, doi: 10.1016/j.jfca.2003.09.009.

Özgüven, M. (2014). Functional chocolate development: enrichment of dark chocolate with nano-liposome encapsulated antioxidants, and/or pro- and pre-biotics. PhD Dissertation, Istanbul Technical University, Istanbul, Turkey, 144 p.

Pimentel, F. A., Nitzke J. A., Klipel, C. B., Jong, E. V. (2010). Chocolate and red wine – A comparison between flavonoids content. *Food Chem*, 120: 109–112, doi: 10.1016/j.foodchem. 2009.09.078.

Rusak, G., Komes, D., Likic, S., Horzic, D., Kovac, M. (2008). Phenolic content and antioxidative capacity of green and white tea extracts depending on extraction conditions and the solvent used. *Food Chem*, 110: 852–858, doi: 10.1016/j.foodchem.2008.02.072.

Sanchez-Moreno, C., Larrauri, J., Saura-Calixto, F.A. (1998). Procedure to measure the antiradical efficiency of polyphenols. *J Agr Food Chem*, 76: 270-276.

Streit, N. M., Hecktheuer L. H. R., Canto, M. W., Mallmann, C. A., Streck, L., Vey Parodi, T., Canterle, L.P. (2007). Relation among taste-related compounds (phenolics and caffeine) and sensory profile of erva-mate (*Ilex paraguariensis*). *Food Chem*, 102: 560–564, doi: 10.1016/j.foodchem.2006.05.028.

Tanabe, N. A., Hofberger, R. (2005). Chocolate. In: *Handbook of Food Science, Technology, and Engineering*, Hui, Y.H. (chief ed.), Volume 4, CRC Press, USA, pp. 1-11.

Weiss, D. J., Anderton, C. R. (2003). Determination of catechins in matcha green tea by micellarelectrokinetic chromatography. *J Chromatogr A*, 1011: 173–180, doi: 10.1016/S0021-9673(03)01133-6.

Wollgast, J., Anklam, E. (2000). Polyphenols in chocolate: is there a contribution to human health? *Food Res Int*, 33: 449-459, doi: 10.1016/S0963-9969(00)00069-7.

Wollgast, J. (2004). The contents and effects of polyphenols in chocolate. PhD Dissertation, University of Gießen, Gießen, Germany, 284 p.