GIDA (2016) 41 (5): 311-316
doi: 10.15237/gida.GD16029

Research / Araştırma

ENRICHMENT OF FUNCTIONAL PROPERTIES OF WHITE CHOCOLATES WITH CORNELIAN CHERRY, SPINACH AND POLLEN POWDERS

İnci Cerit, Sara Şenkaya, Berna Tulukoğlu, Merve Kurtuluş, Ü. Rida Seçilmişoğlu, Omca Demirkol*

Department of Food Engineering, Sakarya University, Esentepe / Sakarya, Turkey

Received / Geliş Tarihi: 24.02.2016
Received in revised form / Düzeltilerek Geliş Tarihi 24.03.2016
Accepted / Kabul Tarihi 29.03.2016

Abstract

Nowadays, there is an increasing demand to reduce the chemical additives and enrich the functional properties of food with using natural ingredients such as fruit and vegetables. In this study, the objective was to determine the influence of addition of cornelian cherry, spinach and bee pollen powders on the total phenolic content and antioxidant capacity of white chocolate. The Folin-Ciocalteu reagent was used to evaluate the total phenolic content and the antioxidant capacity of samples was measured by two different assays which were DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity and FRAP (ferric reducing antioxidant power) tests. The obtained results showed that plain white chocolate exhibited no phenolic compound. In addition, antioxidant activity of white chocolate was found so low. However, the addition of cornelian cherry and spinach to chocolates enhanced the total phenolic content and antioxidant capacity and contribution of pollen powder to functional property of white chocolate was found as highest among all powders used.

Keywords: White chocolate, antioxidant capacity, cornelian cherry, spinach, pollen.

KIZILCIK, ISPANAK VE POLEN TOZLARI KULLANILARAK BEYAZ ÇİKOLATALARIN FONKSİYONEL ÖZELLİKLERİNİN ZENGİNLEŞTİRİLMESİ

Özet

Günümüzde, meyve ve sebzeler gibi doğal bileşenler kullanılarak gıdaların fonksiyonel özelliklerinin zenginleştirilmesi ve kimyasal katkıların azaltılmasına gösterilen talep artmaktadır. Bu çalışmada amaç, beyaz çikolataya eklenen kızılcık, ıspanak ve arı poleni tozlarının toplam fenolik madde ve antioksidan kapasiteye etkisini belirlemektir. Örneklerin toplam fenolik maddesini belirlemek için Folin-Ciocalteu reaktifi, antioksidan kapasitesini belirlemek için ise DPPH (2,2-difenil-1-pikrilhidrazil) süpürme aktivitesi ve FRAP (demir indirgeme antioksidan gücü) testleri kullanılmıştır. Elde edilen sonuçlar sade beyaz çikolatada fenolik madde bulunmadığını göstermiştir. Ayrıca, beyaz çikolatanın antioksidan aktivitesi de çok düşük bulunmuştur. Ancak, çikolatalara eklenen kızılcık ve ıspanak tozu toplam fenolik madde ve antioksidan kapasiteyi geliştirmiş ve beyaz çikolatanın fonksiyonel özelliklerine katkısı çalışmada kullanılan tozlar arasında en yüksek polen tozunda sağlanmıştır.

Anahtar kelimeler: Beyaz çikolata, antioksidan kapasite, kızılcık, ıspanak, polen.

* Corresponding author /Yazışmalardan sorumlu yazar;
omcad@sakarya.edu.tr, (+90) 264 295 5921, (+90) 264 295 5601

INTRODUCTION

Cocoa and chocolate products have been one of the most popular foods for thousands of years. They have been consumed for both energy source and health benefits. Chocolates are mainly categorized as dark, milk and white that differs in contents of cocoa solids, milk fat and cocoa butter (1). White chocolate is formulated with sugar, cocoa butter, milk solids, lecithin and vanillin. It contains sugar and milk solids covered by a continuous fatty phase. If a chocolate is named as "white chocolate", it has to include whole milk powder and cocoa butter in the formulation (2, 3). Studies about the health benefits of cocoa and cocoa products are focused on especially degenerative diseases. Cocoa beans contain many different biologically active substances which are enzymes, vitamins, dietary fibers, minerals, xanthines (caffeine and theobromine) and polyphenols (4-6). The percentage of cocoa content determines the amount of polyphenols in chocolates. Although dark and milk chocolate contain cocoa mass, white chocolate does not contain, so it lacks in terms of phenolic subtances. Because white chocolate has no cocoa solid content, antioxidant effect of white chocolate is low. Therefore, enrichment of white chocolate with fruits and vegetables can enhance its antioxidant capacity.

Functional foods contain a considerable amount of bioactive substances. They provide health benefits and prevent from choronic diseases. Phytochemicals such as phenolic substances and antioxidants in fruits and vegetables play an important role in creating functional foods (7). Cornelian cherry grown in Asia and Europe (*Cornus mas* L.) has bright, red colour and sour taste. Antioxidant activity of cornelian cherry is high because it contains ascorbic acid and polyphenolic compounds. In Turkey, it is consumed as directly or jam, marmalade, fruit pulp, in syrup or dry form. In addition, because of high antioxidant capacity, cornelian cherry can be used as food additive (8, 9). Spinach (*Spinacia oleracea*), one of the most important vegetables is consumed as fresh in salads or cooked. It involves higher amount of phenolic compounds, chlorophyll and β-carotene than many vegetables. Up to now, spinach powder has been added in bread, noodles and cheese due to its colour and nutrients (10-12). Bee pollen includes carbohydrates, proteins, lipids, vitamins, minerals, micronutrients and polyphenolic substances which scavenge free radicals. Therefore, bee pollen can be used as additivite due to health benefits. (13, 14). In literature, there are some studies about the chocolate enriched with raspberry, red pepper, rosemary and dried fruits like prunes, papaya, cranberry (15-17). However, there is no study on functional chocolate using powder of cornelian cherry, spinach or bee pollen. In addition, there are studies about the antioxidant capacity of the cocoa and chocolate products which are milk and dark chocolate (18-21). However, the number of studies are limited on antioxidant activities of white chocolates (22-24).

The purpose of this study was to produce white chocolate with addition of cornelian cherry, spinach and bee pollen powders. Coloring of chocolates and increasing antioxidant activity were targeted by using powders. The antioxidant potential of chocolates was determined with total phenolic content, DPPH scavenging activity and FRAP analysis by spectrophotometrically.

MATERIALS AND METHODS

Materials

Methanol, Folin-Ciocalteu reagent, sodium carbonate, ferric chloride hexahydrate, sodium acetate trihydrate were provided from Merck™ (Darmstadt, Germany). DPPH (2,2-diphenyl-1-picrylhydrazyl), TPTZ (2,4,6-tris(2-pyridyl)-s-triazine), gallic acid (GAE) and $FeCl_3$ x $6H_2O$ were supplied from Sigma-Aldrich (St. Louis, MO, USA). Cornelian cherry, spinach, bee pollen and other ingredients used in chocolate were taken from local markets (Sakarya, Turkey).

Preparation of chocolate samples

Cornelian cherry and spinach were washed with tap water then freeze-dried with the freeze drier (Labconco Freezone 6, USA). Bee pollen was dried in oven (Jeio Tech ON-12G, Korea) at 40-50 °C for 2 hours. After drying, all samples were ground into powder by using grinder (Fakir, Germany). Ingredients of white chocolate are listed in Table 1. Cocoa butter was melted by bain-marie method, ingredients in powder form were added and mixed with a mixer (Fakir-mezza plus, Germany).

Table 1. Ingredients of white and dark chocolate. Powdered sugar was used instead of cornelian cherry/spinach/bee pollen powder in control samples.

Ingredients	Percentage
Cocoa butter	39.00%
Powdered sugar	33.50%
Milk powder	17.00%
Whey powder	7.60%
Lecithin	0.50%
Butter	0.40%
Cornelian cherry/spinach/bee pollen powder	2.00%

The mixture was ground in melanger (Santha, USA) to gain required particle size for 6 hours. After counching, lecithin (emulgator) was added to the mixture. Chocolate mass was cooled to 27 °C, then heated to 32 °C and tempering was completed. The samples were stored in 4 °C until analysed.

Preparation of chocolate extracts

Chocolate extracts were prepared according to Wojdylo (25) with some modifications. 1 g of each chocolate sample was extracted with 10 ml of aqueous methanol (70ml/100ml) for 15 min in an ultrasonic water bath (Bandelin Sonorex, Germany). Then, each sample was centrifuged in centrifuge (Hettich Univarsal 320R, England) for 10 min at 13,130 x g. The supernatants of samples were seperated in tubes to use in analysis. Step of lipid elimination from samples was not applied due to possibility of loss in powders (cornelian cherry, spinach and bee pollen) during process. Although lipids from chocolates have been removed in most of the studies, there are some studies in the literature which did not eliminate lipids from chocolates (18).

Determination of total phenolic content

Total phenolic content of chocolate extracts was determined according to Singleton et al. (26) by spectrophotometrically. One hundred microliter of extract was mixed with 2 ml of distilled water and 1 ml Folin-Ciocalteu reagent was added to the mixture. After 3 min, 1 ml of sodium carbonate solution (20%) was added and incubated for 1 hour. Absorbances of samples were measured at 765 nm in UV-VIS spectrophotometer (Shimadzu UVmini-1240, North America). Total phenolic content was expressed as mg GAE/100 g chocolate.

Antioxidant activity assays

DPPH assay

DPPH assay was applied according to the procedure of Brand and Williams (27) with some modifications. Two hundred microliter of chocolate extract was added to 3 ml of 0.051 mM DPPH in methanol and incubated at room temperature for 30 min. The DPPH scavenging capacity was evaluated by measuring the decrease in absorbance at 517 nm. Antioxidant capacity was calculated by using following equation:

$$\%\ DPPH\ scavenging\ activity = \frac{A(control) - A(sample)}{A(control)} \times 100$$

FRAP assay

The ferric ion reducing antioxidant power assay was done according to modified method of Benzie and Strain (28). Three hundred milimolar acetate buffer (pH 3.6), 10 mM TPTZ, 20 mM $FeCl_3 \times 6H_2O$ were prepared as stock solutions. Then, FRAP reagent was made by mixing 25 ml of acetate buffer, 2.5 ml of TPTZ and 2.5 ml of $FeCl_3 \times 6H_2O$ solutions. One hundred microliter of chocolate extract and 1.2 ml of distilled water were added to 1.8 ml of FRAP reagent. Absorbance was measured at 593 nm after incubation at 37 °C for 15 min. For calibration curves, aqueous solutions of $FeSO_4 \times 7H_2O$ were used and the results were expressed as mmol Fe(II)/L.

Statistical analysis

Analysis were applied in three independent samples for each chocolate product and all determinations were performed in triplicate in each chocolate extract. Statistical analyses were done with the SPSS program (Version 20, Chicago, IL, USA) and the differences between chocolate samples were determined using Duncan's multiple range test. Results were expressed as the average ± standard deviation.

RESULTS AND DISCUSSION

Total phenolic content

Total phenolic content of white chocolate samples are shown in Table 2. The total phenolic content in control sample was not detected which is not suprising because white chocolate contains no cocoa mass. According to literature, there is high positive correlation between amount of cocoa solid and phenolic compounds (18, 21).

Tabernero et al (19) determined the total phenolic content of milk and dark chocolates as 13.10 and 18.16 mg GAE/g, respectively. Miller et al. (20) also reported that total phenolic content of dark chocolate was 11.73-14.88 mg GAE/g. These values are high according to this study due to difference in chocolate variety. When the white chocolate samples were compared, as can be seen from the data in Table 2, cornelian cherry, spinach and pollen enhanced the phenolic content of white chocolate. Pollen had the highest effect on total phenolic compounds (205.15 mg GAE/100 g) because it contains significant amount of polyphenolic substances (13). In literature, there are different results about phenolic contents of chocolates enriched with fruits and vegetables. In the study of Komes et al. (17), the effect of dried fruits (dried prunes, dried papaya, dried apricots, dried raisins, dried cranberries) on sensory properties and bioactive content was examined in samples. It was shown that the prunes increased the total phenolic content of dark chocolate and cranberry enhanced the phenolic content of milk chocolate. In contrast, Todorovic et al. (15) reported the total phenolic content of dark chocolate and dark chocolate with raspberry as 11.99 ve 11.56 mg GAE/g, respectively. As a result of the study, raspberry could not have significant influence on the dark chocolates. Similarly, Cervellati et al. (16) added the red pepper and rosemary in chocolate samples, however, there was no change on phenolic content. In the literature, studies have been done with different chocolates, fruits or spices and amount of ingredients are also different. Therefore, results may not be similar with each other.

Antioxidant activity

DPPH and FRAP analysis, which are most frequently used to determine the antioxidant capacity of foods, were applied chocolate samples in this research. As can be seen from Figure 1, DPPH scaveging activity of plain white chocolate were found as 9.72%. Antioxidant activity of white chcocolate was found low because the majority of antioxidant capacity in chocolate is due to the non fat cocoa solid content which contains important phenolic substances. When the contribution of powders to DPPH value of chocolates was examined, cornelian cherry and pollen powders provided increase in antioxidant activity of white chocolates. However, there was no significant difference between control of white chocolates and white chocolate with spinach. Todorovic et al. (15) reported that DPPH scavenging activity of dark chocolate was higher than the milk chocolate and addition of raspberry could not affect the activity of dark samples. Similarly, Komes et al. (17) explained that the antioxidant capacities of dark chocolates with dried fruits were higher than the dried fruits as opposed to total phenolic content. These

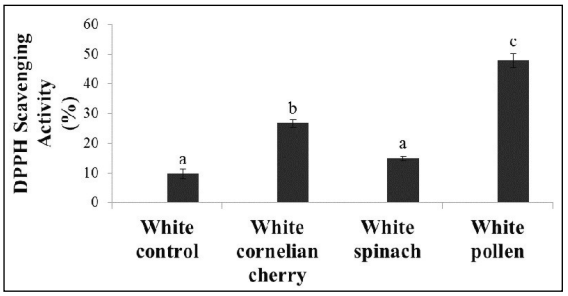

Figure 1. DPPH (2,2-dyphenil 1-picrylhydrazyl) content of chocolate samples. Values are mean± SD, (n=3). Results followed by the same letter (a–c) are not statistically different at $P<0.05$.

Table 2. Total phenolic content of chocolate samples.

Chocolates	TPC (mg GAE/100 g)	FRAP (mmol Fe(II)/L)
White chocolate control	ND	3,20±0,42a
White chocolate with cornelian cherry	16.03±3.34a	8,14±1,54b
White chocolate with spinach	78.48±14.13b	7,15±0,77b
White chocolate with pollen	205.15±5.67c	18,55±2,01c

Values are expressed as means ± SD (n = 3). Results followed by the same letter (a–c) are not statistically different at $P<0.05$. ND: Not detected.

results lead to conclusion that cocoa polyphenols are major contributor to the antioxidant capacity instead of polyphenol content of dried fruits.

Ferric reducing antioxidant power (FRAP) is a method which has been developed to measure the reduction ability of a compound from Fe^{+3} to Fe^{+2} by Benzie and Strain (25). FRAP value of plain white chocolate was determined as 3.20 mmol Fe(II)/L (Table 2). In addition, FRAP values of enriched chocolates changed between 7.15 and 18.55 mmol Fe(II)/L. As it can be understood from the values, cornelian cherry, spinach and pollen powders increased the antioxidant capacity of white chocolates due to high phenolic contents. Belscak et al. (21) determined the antioxidant capacity of chocolates with different cocoa solid content. They found that FRAP value of dark chocolate (with 88% cocoa solid) was 24.93 mmol Fe(II)/L. In the study of Komes et al. (17), FRAP value of milk and dark chocolates were determined as 4.00 and 8.06 mmol Fe(II)/L. Although all of powders increased the FRAP values of white chocolate, the major contributor was bee pollen as in the DPPH activity assay. Bee pollen contains important micronutrients and phenolic subtances which increase the antioxidant capacity of foods.

CONCLUSIONS

This study investigated the contribution of cornelian cherry, spinach and pollen powders to antioxidant capacity of white chocolate. It was determined that white chocolate had no phenolic substances. Therefore, the antioxidant capacity of white chocolate was low. However, addition of cornelian cherry, spinach and pollen powders to the chocolates increased the phenolic compounds. Pollen powders on white chocolate was the most efficient one among three powders used in this study. When the effect of powders on antioxidant activity of chocolates were compared, it was found that the highest antioxidant capacity also belong to pollen powder. Considering the limited researches on antioxidant capacity of enriched chocolates, the findings in current study may contribute to literature data. Further studies may be done with adding different powders of fruit and vegetables to chocolates.

Acknowledgements

The authors are grateful for the financial support provided by Sakarya University. We are indebted to Prof. Dr. Zehra Ayhan for pre-reviewing the manuscript. We also thank to R.A Ayse Sarıçam for her help in laboratory analysis.

REFERENCES

1. Afoakwa EO, Paterson A, Fowler M. 2007. Factors influencing rheological and textural qualities in chocolate - a review. *Trends Food Sci Technol*, 18: 290-298.

2. Vercet A. 2003. Browning of white chocolate during storage. *Food Chem*, 81 (3): 371-377.

3. Samsudin S, Rahim AA. 1996. Use of palm mid-fraction in white chocolate formulation. *J Sci Food Agr*, 71 (4): 483-490.

4. Jalil AMM, Ismail A. 2008. Polyphenols in cocoa and cocoa products: is there a link between antioxidant properties and health? *Molecules*, 13 (9): 2190-2219.

5. Gu L, House SE, Wu X, Ou B, Prior RL. 2006. Procyanidin and catechin contents and antioxidant capacity of cocoa and chocolate products. *J Agr Food Chem*, 54 (11): 4057-4061.

6. Yao LH, Jiang YM, Shi J, Tomas-Barberan FA, Datta N, Singanusong R, Chen SS. 2004. Flavonoids in food and their health benefits. *Plant Food Hum Nutr*, 59 (3): 113-122.

7. Liu RH. 2003. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *Am J Clin Nutr,* 78 (3): 517-520.

8. Tural S, Koca I. 2008. Physico-chemical and antioxidant properties of cornelian cherry fruits (*Cornus mas* L.) grown in Turkey. *Sci Hort,* 116 (4): 362-366.

9. Pantelidis GE, Vasilakakis M, Manganaris GA, Diamantidis G. 2007. Antioxidant capacity, phenol, anthocyanin and ascorbic acid contents in raspberries, blackberries, red currants, gooseberries and Cornelian cherries. *Food Chem*, 102 (3): 777-783.

10. Lee J, Lee S, Lee H, Park K, Choe E. 2002. Spinach (*Spinacia oleracea*) powder as a natural food-grade antioxidant in deep-fat-fried products. *J Agr Food Chem*, 50 (20): 5664-5669.

11. Howard LR, Pandjaitan N, Morelock T, Gil MI. 2002. Antioxidant capacity and phenolic content of spinach as affected by genetics and growing season. *J Agr Food Chem*, 50 (21): 5891-5896.

12. Ismail A, Marjan ZM, Foong CW. 2004. Total antioxidant activity and phenolic content in selected vegetables. *Food Chem*, 87 (4): 581-586.

13. Feás X, Vázquez-Tato MP, Estevinho L, Seijas JA, Iglesias A. 2012. Organic bee pollen: botanical origin, nutritional value, bioactive compounds, antioxidant activity and microbiological quality. *Molecules*, 17 (7): 8359-8377.

14. Carpes ST, Begnini R, Alencar SMD, Masson ML. 2007. Study of preparations of bee pollen extracts, antioxidant and antibacterial activity. *Ciênc Agrotec*, 31 (6): 1818-1825.

15. Todorovic V, Redovnikovic IR, Todorovic Z, Jankovic G, Dodevska M, Sobajic S. 2015. Polyphenols, methylxanthines, and antioxidant capacity of chocolates produced in Serbia. *J Food Comp Anal*, 41: 137-143.

16. Cervellati R, Greco E, Costa S, Guerra MC, Speroni E. 2008. A comparison of antioxidant properties between artisan-made and factory-produced chocolate. *Int J Food Sci Tech*, 43 (10): 1866-1870.

17. Komes D, Belscak-Cvitanovic A, Skrabal S, Vojvodic A, Busic A. 2013. The influence of dried fruits enrichment on sensory properties of bitter and milk chocolates and bioactive content of their extracts affected by different solvents. *LWT-Food Sci and Technol*, 53 (1): 360-369.

18. Vertuani S, Scalambra E, Vittorio T, Bino A, Malisardi G, Baldisserotto A, Manfredini S. 2014. Evaluation of antiradical activity of different cocoa and chocolate products: relation with lipid and protein composition. *J Med Food*, 17 (4): 512-516.

19. Tabernero M, Serrano J, Saura-Calixto F. 2006. The antioxidant capacity of cocoa products: Contribution to the Spanish diet. *Int J Food Sci Tech*, 41 (1): 28-32.

20. Miller KB, Stuart DA, Smith NL, Lee CY, McHale NL, Flanagan JA, Hurst WJ. 2006. Antioxidant activity and polyphenol and procyanidin contents of selected commercially available cocoa-containing and chocolate products in the United States. *J Agr Food Chem*, 54 (11): 4062-4068.

21. Belscak A, Komes D, Horzic D, Ganic KK, Karlovic D. 2009. Comparative study of commercially available cocoa products in terms of their bioactive composition. *Food Res Int*, 42 (5): 707-716.

22. Meng CC, Jalil AMM, Ismail A. 2009. Phenolic and theobromine contents of commercial dark, milk and white chocolates on the Malaysian market. *Molecules*, 14(1): 200-209.

23. Carlsen MH, Halvorsen BL, Holte K, Bøhn SK, Dragland S, Sampson L, Willey C, Senoo H, Umezono Y, Sanada C, Barikmo I, Berhe N, Willet W, Philips KM, Jacobs DR, Blomhoff R. 2010. The total antioxidant content of more than 3100 foods, beverages, spices, herbs and supplements used worldwide. *Nutr J*, 9(1): 1-11.

24. Pimentel FA, Nitzke JA, Klipel CB, de Jong EV. 2010. Chocolate and red wine–A comparison between flavonoids content. *Food Chem*, 120 (1): 109-112.

25. Wojdylo A, Oszmianski J, Czemerys R. 2007. Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chem*, 105 (3): 940-949.

26. Singleton VL, Orthofer R, Lamuela-Raventos RM. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Method Enzymol*, 299: 152-178.

27. Brand-Williams W, Cuvelier M, Berset C. 1995. Use of a free radical method to evaluate antioxidant activity. *LWT- Food Sci and Technol,* 28 (1): 25-30.

28. Benzie IFF, Strain J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "Antioxidant power": The FRAP assay. *Anal Biochem,* 239: 70-76.