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Phenolic Compounds and Antioxidant Activity of Cocoa and Chocolate Products

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Abstract: Cocoa is the fruit of the tree *Theobroma cacao* L., growing in tropical regions of Africa and South America. Prefermented and fried cocoa serves as a basic raw material for the preparation of chocolate food products. Cocoa is extremely rich in antioxidants, which are responsible for the overall health of the humans. These antioxidants include polyphenols and catechins. This paper presents the investigation of total phenolic contents and antioxidant activity of extracts obtained from cocoa powder and different types of chocolate. The total content of phenolic compounds was determined by spectrophotometric Folin-Ciocalteu method and it varied from 0.046 ± 0.013 to 0.376 ± 0.022 mg GAE/g. The antioxidant activity of the extracts was tested using total antioxidant capacity method. The IC_{50} value was in the range of 1.968 ± 0.076 mg/ml to 42.200 ± 1.737 mg/ml. Cocoa powder and chocolate with a high content of cocoa contain relatively high amount of total phenolics, as well as high antioxidant capacity.

INTRODUCTION

Plants are natural factories for the production of chemical compounds, many of which are used to promote health and fight diseases and some of them are marketed as food or herbal medicines. Phenolics are compounds possessing one or more aromatic rings with one or more hydroxyl groups. They are broadly distributed in the plant kingdom and are the most abundant secondary metabolites of plants, with more than 8,000 phenolic structures currently known, ranging from simple molecules such as phenolic acids to highly polymerized substances such as tannins (Dai and Mumper, 2010; Cheynier, 2012). It is known that the antioxidant activity of the phenolics is primarily a result of their ability to be donors and hydrogen atoms, such as radicals are removed by the formation of less reactive phenoxyl radicals.

Antioxidants are defined as compounds that can delay, inhibit, or prevent the oxidation of oxidizable materials by scavenging free radicals and diminishing oxidative stress. Oxidative stress is an imbalanced state where excessive quantities of reactive oxygen and/or nitrogen species (ROS/RNS, e.g., superoxide anion, hydrogen peroxide, hydroxyl radical, peroxynitrite) overcome endogenous antioxidant capacity, leading to oxidation of a varieties of biomacromolecules, such as enzymes, proteins, DNA and lipids. Oxidative stress is important in the development of chronic degenerative diseases including coronary heart disease, cancer and aging (Ames *et al*., 1993).

Chocolate/cocoa has been known for its good taste and proposed health effects for centuries. Earlier, chocolate used to be criticized for its fat content and its consumption was a sin rather than a remedy, associated with acne, caries, obesity, high blood pressure, coronary artery disease and diabetes. However, the discovery of biologically active phenolic compounds in cocoa has changed this perception and stimulated research on its effects on ageing, oxidative stress, blood pressure regulation, and atherosclerosis.

Cocoa is the mature fruit of the cacao tree (*Theobroma cacao* L.), growing in tropical regions of Africa and South America. The fruit of the cocoa tree has length of about 20 cm and a weight of about 0.5 kg (Komes, 2008). Raw cocoa beans are one of the most nutritious foods in the world, and protects the body from the impact of free radicals, reduces stress and depression, protect against heart disease and blood vessels, protects against many types of cancer, is an excellent source of iron, regulates blood sugar and cholesterol levels, promotes better memory and concentration, reduces the risk of heart attack, and helps regulate blood pressure (Latif, 2013).
Polyphenols in cocoa beans are found in the pigment cells of the cotyledons. Its polyphenolic composition depends on many factors, such as type, geographical origin and growing conditions, and maturity of cocoa fruit (Katz *et al*., 2011), as well as fermentation and food processing (Goldoni, 2004; Nazaruddin *et al*., 2006).
This paper presents the investigation of total phenolic content in the extracts isolated from cocoa powder and different types of chocolate, as well as determination of antioxidant activity of isolated extracts.

EXPERIMENTAL

Isolation

Ten grams of grinded samples (cocoa powder and chocolates) were mixed with solvent and extracted using ultrasonic bath for 30 minutes. Each chocolate sample was subjected for two different extraction procedures, extraction with etanol, with/without initial defatting with *n*-hexane.

Determination of phenolics

Total phenolic content was measured using Folin-Ciocalteu spectrophotometric method (Singleton, and Rossi, 1965), using gallic acid for calibration curve. All tests were performed in triplicates, and results are presented as gallic acid equivalents.

Antioxidant activity

Antioxidant activity of isolated extracts was tested using total antioxidant capacity spectrophotometric method (Prieto *et al*., 1999). The method is based on the ability of potent antioxidant reduce molybdenum ions. All tests were performed in triplicates, and results are presented as IC_{50} values that indicate the concentration of extracts that reduces the 50% of molybdenum. Catechin was as standard probe.

RESULTS AND DISCUSSION

Isolation of phenolic compounds from cocoa powder and chocolates was performed using ultrasonic extraction. Yields of isolated extracts are presented in Table 1, and they do not show high variation for direct extraction from ethanol, while previously defatted extracts do. The percentage from cocoa was taken from declaration of the product.

Table 1: Yields of isolated extracts

Extraction	Sample	Yield (%)
without initial defatting with *n*-hexane	1a	0.36
	2a	0.23
	3a	0.25
	4a	0.25
	5a	0.33
with initial defatting with *n*-hexane	1b	0.36
	2b	0.34
	3b	0.15
	4b	0.41
	5b	2.10

1 – cocoa powder (100% cocoa), 2 – chocolate powder (55% cocoa); 3 – baking chocolate (43% cocoa); 4 – milk chocolate (29% cocoa); 5 – chocolate bar with creamy filling (35% cocoa).

Results from spectrophotometric determination of total phenolic content in isolated extracts are summarized in Table 2 as mg of gallic acid equivalent per gram of extract, and as content of phenolic compounds in extract. Values are represented as the mean taking into account the standard deviation.

Table 2: The phenolic content of extracts of cocoa and chocolates

Sample	Total Phenolics	
	GAE mg/g	(%)
1a	0.376 ± 0.022	10.46 ± 0.60
2a	0.147 ± 0.032	6.74 ± 0.39
3a	0.135 ± 0.006	5.89 ± 0.27
4a	0.097 ± 0.026	3.30 ± 0.10
5a	0.041 ± 0.013	2.05 ± 0.13
1b	0.184 ± 0.014	5.12 ± 0.38
2b	0.051 ± 0.017	3.62 ± 0.27
3b	0.070 ± 0.005	1.85 ± 0.07
4b	0.064 ± 0.009	0.99 ± 0.00
5b	0.046 ± 0.013	0.34 ± 0.03

Total content of phenolic compounds vary from 0.376 ± 0.022 to 0.041 ± 0.013 mg GAE/g, and from 0.184 ± 0.014 to 0.046 ± 0.013 mg GAE/g for non-defatted and defatted extracts, respectively. Presented results are in agreement with those found in the literature (Waterhouse *et al*., 1996; Ortega *et al*., 2008; Miller *et al*., 2009).
Comparing the results obtained for the hexane samples with previously published data by Martos *et al*. (2011), who also used the Folin-Ciocalteu method, it was found that their values are slightly higher. Despite the fact that the samples were defatted before determining polyphenols, cocoa butter interaction with other components of chocolate, during the production of chocolate, may contribute to a different final result, i.e. to increase in the total phenolic content (Jolic *et al*., 2011). The main limitation of the Folin Ciocalteu method is the lack of specificity, due to interference of other oxidation products during the reaction, and causing variation of the true content of polyphenols (Ainsworth & Gillespie, 2007).

Results of determination of total antioxidant activity using molybdenum reduction method are shown in Table 3, where the IC_{50} present the concentration of extract to reduce 50% of molybdenum cation. Values are represented as the mean taking into account the standard deviation.

The IC_{50} value is in the range of 1.968 ± 0.076 mg/ml to 42.200 ± 1.737 mg/ml that can be compared with antioxidant activity of catechin (2.171 ± 0.023 mg/ml) which was used as standard probe. In general, previously defatted extracts revealed higher antioxidant activity than extracts without initial defatting.

To the best of our knowledge, there are no previously published data concerning evaluation of antioxidant activity of cocoa products using this method.

Table 3: Antioxidant activity of extracts of Cocoa and Chocolate

Sample	IC_{50} (mg/ml)
1a	2.201 ± 0.021
2a	2.537 ± 0.233
3a	1.968 ± 0.076
4a	2.040 ± 0.150
5a	3.280 ± 0.309
1b	3.112 ± 0.050
2b	2.244 ± 0.038
3b	4.322 ± 0.051
4b	5.145 ± 0.218
5b	42.200 ± 1.737
Catechin	2.171 ± 0.023

However, even with different method used, presented results are comparable with those found in the literature. Kroyer and Molnar (2011) evaluated antioxidant activity of cocoa and chocolate products using DPPH radical scavenging activity method. They recorded the highest antioxidant activity for cocoa powder, following a dark chocolate with 85% cocoa content.

It seems that the antioxidant activity is correlated with the content of cocoa that is correlated with the content of phenolic compounds in investigated product (Miller *et al*., 2009).

This is in agreement with the fact that antioxidant activity is correlated with the content of phenolic compounds (Muselik *et al*., 2007; Lucena *et al*., 2010). Moreover, Serafini *et al*. (2003) and Halliwell (2003) suggested that milk may interfere with the absorption of antioxidants from chocolate *in vivo* and may therefore negate the potential health benefits that can be derived from eating moderate amounts of dark chocolate.

CONCLUSIONS

Interest in the biological activities of cocoa polyphenols is increasing steadily. In fact, the high polyphenol content of cocoa, coupled with its widespread presence in many food items, render this food of particular interest from the nutritional and "pharmacological" viewpoints.

In summary, the results reported here demonstrate the phenolic content and antioxidant activity of different cocoa and chocolate products. These results indicate that total phenolic content as well as antioxidant activity is dependent of the amount of cocoa in investigated products.

REFERENCES

Ainsworth E.A., & Gillespie K.M. (**2007**). Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. *Nature Protocols* 2 (4), 875-877.

Ames B.N., Shigenaga M.K. & Hagen, T.M. (**1993**). Oxidants, antioxidants, and the degenerative diseases of aging. *Proceedings of the National Academy of Sciences USA*, 90, 7915-7922.

Cheynier, V. (**2012**). Phenolic compounds: from plants to foods. *Phytochemistry Reviews*, 11, 152-177.

Dai J. & Mumper P. J. (**2010**). Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules*, 15, 7313-7352.

Goldoni L. (**2004**). Teh*nologija konditorskih proizvoda – I dio Kakao proizvodi i proizvodi slični čokoladi*. Kugler, Zagreb.

Halliwell, B. (**2003**). Plasma antioxidants (communication arising): Health benefits of eating chocolate? *Nature*, 426, 787-787.

Jolic S. M., Redovnikovic I. R., Markovic K., Sipusic D. I. & Delonga K. (**2011**). Changes of phenolic compounds and antioxidant capacity in cocoa beans processing. *International Journal of Food Science and Technology*, 46, 1793-1800.

Katz D. L., Doughty K. & Ali A. (**2011**). Cocoa and chocolate in human health and disease. *Antioxidants & Redox Signaling*, 15, 2779-2811.

Komes D. (**2008**). *Tehnologija konditorskih proizvoda*. Zavod za prehrambeno-tehnološko inženjerstvo, Zagreb.

Kroyer G. & Molnar T. (**2011**). Evaluation of polyphenols and antioxidative activity of cocoa and chocolate products. *Polish Journal of Food and Nutrition Science*, 61, 1230-0322.

Latif, R. (**2013**). Health benefits of cocoa. *Current Opinion in Clinical Nutrition and Metabolic Care*, 16, 669-674.

Lucena A. P. S., Nascimento R. J. B., Maciel J. A. C. & Tavares J. X. (**2010**). Antioxidant activity and phenolic content of selected Brazilian wines. *Journal of Food Composition and Analysis*, 23, 30-36.

Martos M.V., Mohamady M.A., Fernández-Lòpez J., Abd ElRazik K.A & Omer E.A.(**2011**). *In vitro* antioxidant and antibacterial activities of essential oils obtained from Egyptian aromatic plants. *Food Control*, 22, 1715-1722.

Miller K. B., Hurst W. Y., Flannigan N., Ou B., C. Lee Y., Smith N., Stuart D. A. (**2009**). Survey of commercially available chocolate- and cocoa-containing products in the United States. 2. Comparison of flavan-3-ol content with nonfat cocoa solids, total polyphenols, and percent cacao. *Journal of Agricultural and Food Chemistry*, 57, 9169-9180.

Muselik J., Garcio-Alonso M., Martin-Lopez M. P., Ţemlicka M. & Rivas-Gonzalo J. C. (**2007**). Measurement of antioxidant activity of wine catechins, procyanidins, anthocyanins and pyranoanthocyanins. *International Journal of Molecular Sciences*, 8, 797-809.

Nazaruddin R., Seng L. K., Hassan O. & Said M. (**2006**). Effect of pulp preconditioning on the content of polyphenols in cocoa beans (*Theobroma cacao*) during fermentation. *Industrial Crops and Products*, 24, 87-94.

Ortega N., Romero M. P., Macia A., Reguant J., Angles N., Morello J. R. & Motilva M. J. (**2008**). Obtention and characterization of phenolic extracts from different cocoa sources. *Journal of Agricultural and Food Chemistry*, 56, 9621-9627.

Prieto P., Pineda M. & Agular M. (**1999**). Spectrophotometric quantitation of antioxidant capacitythrough the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E1. *Analytical Biochemistry*, 269, 337-341.

Serafini M., Bugianesi R., Maiani G., Valtuena S., De Santis S., Crozier A. (2003). Plasma antioxidants from chocolate. *Nature*, 424, 1013-1013.

Singleton V. L. & Rossi A. (**1965**). Colorimetry of total phenolics with phospitomolybolic-phosphotungstic acid reagents. *American Journal of Enology and Viciculture*, 16, 144-158.

Waterhouse A. L., Teissedre P. L., Frankel E. N., Peleg, H. & German, J. B. (**1996**). Inhibition of in vitro human LDL oxidation by phenolic antioxidants from grapes and wines. *Journal of the Science of Food and Agriculture*, 70, 55-61.

Summary/Sažetak

Kakao je zreli plod drveta kakaovca koji u sebi sadrži sjemenke u nizovima, a raste u tropskim predjelima Afrike i Južne Amerike. Prethodno fermentiran, a zatim prepržen kakao služi kao temeljna sirovina za dobivanje čokolade. Po svom sastavu, kakao je iznimno bogat antioksidansima koji su odgovorni za ukupno zdravlje organizma. Ti antioksidanti uključuju polifenole i katehine. Ovaj rad predstavlja određivanje sadržaja ukupnih fenola i antioksidativne aktivnosti u ekstraktima dobivenih iz kakaa u prahu i različitih vrsta čokolade, koristeći ultrazvučnu ekstrakciju. Ukupan sadržaj fenolskih spojeva određen je spektrofotometrijskom Folin-Ciocalteu metodom i on varira od 0.046 ± 0.013 do 0.376 ± 0.022 mg GAE/g. Antioksidativna aktivnost ekstrakata ispitana je pomoću metode redukcije molibdena. Vrijednost IC_{50} se nalazi u intervalu od 1.968 ± 0.076 mg/ml do 42.200 ± 1.737 mg/ml. Kakao u prahu i čokolade sa visokim procentom kakaa, sadrže visok sadržaj ukupnih fenola i imaju visoku antioksidativnu sposobnost.