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CHOCOLATE – A BITTERSWEET ANTIOXIDANT

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Abstract

We studied the positive health effect of different chocolates (antioxidative effects on stable free radicals, reactive oxygen species (ROS) and for prevention of lipid peroxidation). The results show that all chocolates successfully remove $^{\bullet}OH$ radicals, but only chocolates with high cocoa content are also effective for of $^{\bullet}O_2^-$. The capabilities for chocolate samples to reduce organic radicals are shown to be positive for hydrophobic DPPH which was not the case for hydrophilic Tempone. Only the chocolate samples with high cocoa content were shown to prevent lipid peroxidation induced by Fenton reaction. Obtained results showed that chocolates have diverse antioxidative effects which are not only dependant on the content of cocoa. The other chocolate constituents like: sugar, polyphenols, cocoa butter, emulsifier and other substances should also be considered for determining the positive health effect of chocolates.

Introduction

Reactive oxygen species (ROS) have important roles in many biological processes and are regularly produced during normal aerobic metabolism. However, ROS could also damage cell membranes and biological molecules. The ROS include hydroxyl radical ($^{\bullet}OH$), superoxide anion ($^{\bullet}O_2^-$), hydrogen peroxide (H_2O_2), hypochloric acid (HOCl), singlet oxygen ($^1O_2$) and others. [1], [2] There is a close relationship between antioxidants in diet and immune functions. Antioxidant assays have shown that chocolate have significant antioxidant activities primarily due to the content of polyphenols, in particular catechins and proanthocyanidins. [3], [4] However, the content of cocoa is usually underlined as the main substance responsible for such property. This study had aim to explore the antioxidative capabilities of different chocolates and compare it with their composition. EPR is one of the most efficient techniques for detection of various radical species. EPR spin-trapping technique is based on a reaction in which the transient radical species reacts with specific nitrone or nitroso spin-traps to yield more persistent nitroxide spin adducts. In this study we used spin-trap DEPMPO which can, by forming high yield of distinct and stabile adducts, simultaneously trap different free radicals and trace them to their origin. [5], [6] Spin-probing technique with different types of stable organic free radicals (DPPH and Tempone) have also been used. Reactive oxygen species can induce lipid peroxidation which can break down membrane integrity. Membrane fluidity is in correlation with increased lipid peroxidation, and was measured using the membrane spin probe 7-DS.

Sample preparation

In this experiment, we used chocolates which had been purchased from local market. Chocolates used for the experiments were: Alpenmilch Chocolate (Milka), White Chocolate (Milka), Dorina (milk chocolate, sugar free with sweeteners), Sensations-Côte d´Or (dark chocolate- 86% of cocoa), Guylian (dark chocolate- 70% of cocoa), Najlepše želje (milk chocolate), Najlepše želje (dark chocolate- 60% of cocoa). Chocolate solution

suitable for EPR measurements was made by scrapping and dissolving 10 mg of chocolates in 1 ml of 18MΩ bidistillated deionized water (avoiding additional presence of metal ions). Spin-trap DEPMPO (5-diethoxyphosphoryl-5-methyl-1-pyrroline-N-oxide) was purchased from Alexis Biochemical (Lausen, Switzerland). DPPH (1,1- diphenyl- 2- picrylhidrazyl) was purchased from Sigma chemical company (St. Louis, MO, USA). Tempone (4-Oxo-2,2,6,6-tetramethylpiperidine-1-oxyl) was purchased from Alexis biochemicals, Lausen, Switzerland. 7-DS (2-(5-carboxypentyl)-2-undecyl-4,4-dimethyloxazolidine-3-oxyl) was purchased from Molecular Probes, (Junction City, OR, USA). Hydroxyl radicals were generated by Fenton reaction system [5] and superoxide radicals by Hypoxanthine/Xanthine-oxidase reaction (XA/XO) [6].

EPR spectroscopy

EPR spectra were recorded at a room temperature using Varian E104-A EPR spectrometer operating at X-band (9.45 GHz) with following settings: modulation amplitude, 2 G; modulation frequency, 100 kHz; microwave power, 10 mW; scan range, 100 or 200 G. Spectra were recorded and analyzed using EW software (Scientific Software).

Results and Discussion

The ability of chocolate samples to remove chosen radicals is presented as antioxidative activity ($AA = (I_{control} - I_{sample})/ I_{control}$), performed by comparing the intensities of characteristic EPR peaks (Fig.1) of spin-adducts (or spin-probes) of chocolate and control samples. The ability of chocolate to prevent lipid peroxidation was determined by calculating the order parameter (S). Samples of pure cocoa and sugar were also taken into account.

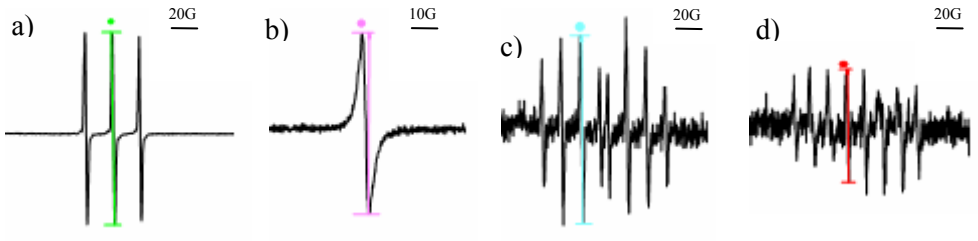


Fig. 1. Characteristic EPR spectra obtained using chocolate Guylian: a) Tempone; b) DPPH; c) DEPMPO/OH adducts (Fenton system); d) DEPMPO/OOH adducts (HX/XO) reaction. Measured EPR peaks are marked with filled circles,

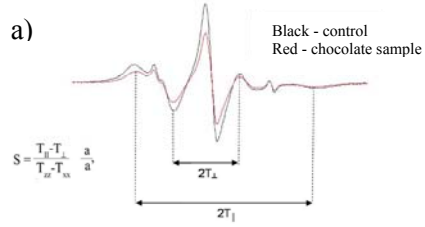
Reduction of Tempone and DPPH and removing of $^{\bullet}OH$ and $^{\bullet}O_2^-$ radicals. Results (Table 1) indicate that some chocolates (Sensations > Najlepše želje > Najlepše želje with 60% of cocoa > Guylian) reduce DPPH, but show poor results for reduction of Tempone. Generally, chocolates with higher cocoa content showed to be effective for removing $^{\bullet}OH$ radicals (in addition, dark chocolates-Guylian and Sensations showed better results than pure cocoa). Only two chocolates, Sensations and Najlepše želje (60% of cocoa) gave good results for removing $^{\bullet}O_2^-$ radicals.

Table 1: Antioxidative activities of different chocolate samples.

Sample	AA (%) Tempone	AA (%) DPPH	AA (%) ($^{\cdot}OH$)	AA (%) ($^{\cdot}O_2^-$)
Sugar	0.93	4.45	48.56	0
Cocoa	10.04	82.37	91.70	55.93
Alpenmilch chocolate	5.90	23.81	86.42	3.39
Milka white	4.13	13.39	83.09	35.59
Dorina (for diabetics)	7.29	61.47	62.30	20.34
Guylian, 70%	13.24	61.89	92.75	27.12
Côte d`Or- Sensations, 86%	12.46	75.69	91.92	67.80
Najlepše želje	14.19	71.45	88.35	0
Najlepše želje, 60%	7.13	69.48	84.83	54.24

Lipid peroxidation preventing capabilities. The ability of chocolate to prevent lipid peroxidation was tested using Fenton system. Order parameter (S), reciprocally proportional to the membrane fluidity, was measured by inserting EPR spin label 7-DS into liposomes (Fig. 2).

a)



b)

Sample type	Liposomes (control sample)	Alpenmilch Chocolate	Guylian Chocolate
Order parameter (S) for untreated samples ± S.D.	0.588±0.003	0.568±0.007	0.576±0.002
Order parameter (S) for OH^- treated samples ± S.D.	0.606±0.001	0.611±0.004	0.571±0.005

Fig. 2. a) EPR spectra of 7-DS (treated and untreated sample) and determination of order parameter S; **b)** Calculated order parameters (S).

Table shows that Guylian chocolate prevent lipid peroxidation which was not the case with Alpenmilch sample.

Conclusions

Obtained results show that different chocolates have diverse antioxidative effects. Among cocoa, the amount of sugar, polyphenols, cocoa butter, emulsifier and other substances clearly show to contribute to antioxidative ability of chocolate.

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