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Optimization of the spectroscopic method using potassium peroxymonosulfate for determination of antioxidant capacity

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Abstract: In this study, we were testing possible usage of commercial tablets for dental prosthesis, containing potassium peroxymonosulfate as a reagent for determination of antioxidant capacity *in vitro.* Our aim was to develop fast, simple and cheap method for determination of antioxidant capacity that will be suitable for laboratories with modest resources. This method was previously proposed, but we have chosen somewhat different approach. Because of the quite narrow linear range, when ascorbic acid was used as standard, for the preparation of the calibration curve, we have used a catechin in the concentration range from 0.125 mg/mL to 12.5 mg/mL. Obtained calibration curve was linear with correlation coefficient of R^2=0.992 and it was used for further determination of antioxidant capacity of selected samples. In order to test the possibility of this method for determination of antioxidant capacity of real samples, we have used five samples which antioxidant capacity was proven in previous testing with different *in vitro* and *in vivo* methods.

INTRODUCTION

Reactive oxygen species (ROS) are byproducts of normal cellular metabolism. Uncontrolled ROS production leads to their accumulation in cells, where they can cause oxidative damage, due to oxidative stress. Endogenic antioxidant repair systems are not enough to prevent disruption of normal cellular homeostasis, which requires substitution with exogenic antioxidants (Kunwar and Priyadarsin, 2011). For epidemiologic purposes it would be useful to determine total antioxidant capacity (TAC) of different classes and types of foods. So far many *in vitro* and *in vivo* methods for determination of TAC have been developed. In this study we were testing possible usage of commercial tablets for dental prosthesis, containing potassium peroxymonosulfate as a reagent for determination of antioxidant capacity *in vitro*. Our aim was to develop fast, simple and cheap method for determination of antioxidant capacity that will be suitable for laboratories with modest resources. In order to test our method, antioxidant capacity of selected samples was determined and compared with the result obtained using classical FRAP method.

EXPERIMENTAL

For determination of antioxidant capacity of selected food products, effervescent tablets for cleaning dental prosthesis, containing potassium peroxymonosulfate were used. This method was originally proposed by Al-Shahrani, Zaman and Amanullah (2013), but in this study method was modified and improved. In order to test this spectrophotometric method five samples available at the market in Bosnia and Herzegovina were analyzed: the Noni juice (*Morinda citrifolia L., Rubiaceae*), semi sweet red wine from Herzegovina, dry white wine from Herzegovina, 100% natural tomato juice (*Lycopersicum esculentum Mill., Solanaceae*) and Goji berries (*Lycium barbarum L., Solanaceae*).

Goji berries (5 g of homogenized sample) were extracted with 25 mL of boiled water. After 10 minutes, sample was filtered, and obtained infusion was used for further analysis. All the other samples were analyzed without prior preparation

For each sample 1 mL was mixed with 10 mL of reagent solution. All the spectroscopic measurements (Spectronic Genesis 2 UV/VIS spectrometer) were performed at 616 nm, with reagent solution as blank.

Reagent solution was prepared according to the previously described method by dissolving one effervescent tablet in 80 mL of saline solution. After adding a sample, the change of colour or the change in the blue color intensity indicates the presence of antioxidants. After 25 minutes this change can be quantified by measuring the absorbance at 616 nm. If the antioxidants are not present in the samples, blue colour wanish, while in the case of positive reaction (presence of antioxidant) intensity of blue colour is increased (Al-Shahrani, *et al*., 2013). The most imortant ingredients of effervescent tablets for cleaning dental prosthesis (available at the market in BiH) are potassium peroxymonosulfate ($2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$), tetraacetylethylenediamine (TAED), sodium perborate monohydrate ($BNaO_3xH_2O$), and indicators indigotin and brilliant blue.

Potassium peroxymonosulfate is a component of a triple salt that acts as oxidant. Sodium perborate from the tablet is soluble in water and releases hydrogen peroxide in the solution.

In order to investigate the influence of oxidants and reductants on spectral characteristics of indicator present in reagent solution, H_2O_2 and sodium dithionite were used. For each probe one effervescent tablet was dissolved in 80 mL of saline solution and one was used as blank, while in the other two 1 mL of H_2O_2 or 0.1 g of solid sodium dithionite were added, respectively (Figure 1). After 25 minutes, the spectra of these solutions were recorded in the 200-1100 nm wavelength range.

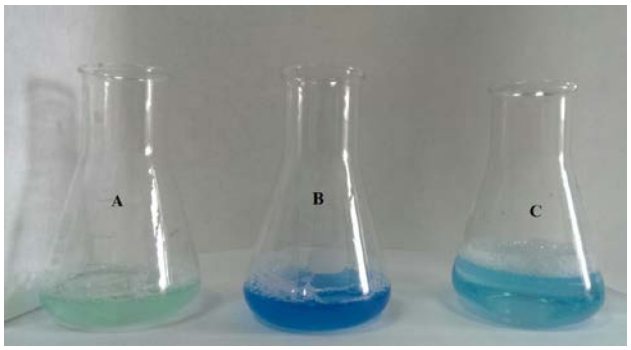

Figure 1: Solution of effervesent tablet after adding sodium dithionite (A), native solution (B), after adding H_2O_2 (C)

In order to quantify amount of antioxidant capacity of the selected samples calibration curve of methanolic catechin solution was used. The concentration range was from 0.125 mg/mL to 12.5 mg/mL. Antioxidant capacity of 10 mg of catechin was used as one EAPK (equivalent of antioxidative potential of catechin) unit. After measuring the absobance for each selected sample antioxidant capacity was expressed in EAPK units.

Ferric Ion Reducing Antioxidant Power Assay (FRAP assay)

This method was described by Benzie and Straine (1996) and has been used since then with small adjustments (Gorjanović, Alvarez-Suarez, Novaković, *et al*., 2013). Complex Fe(III)-TPTZ (2,4,6-tri(2-pyridil)-s-triazine) is reduced to Fe(II)-TPTZ (intense blue complex) in the presence of antioxidant and its absorbance can be measured at 593 nm. Results are expressed in FRAP (mmol/L Fe^{+2}) and relatively to ascorbic acid as pure antioxidant (Gorjanović, *et al*., 2013).

To each investigated sample (50 µL) before measurement (at 593 nm) 1.5 mL of FRAP reagents is added. Abosrbance is measured in 0 and 4 minute, and mean value was used for further calculation. In order to prepare calibration curve, solution of Fe(II) sulphate $x7H_2O$ was dilute in concentration range of 100 - 4000 µM. Results are expressed as FRAP (mmol/L Fe^{2+}).

FRAP reagent was prepared briefly before measurement by mixing in 10:1:1 ratio of 300 mM acetate buffer (pH 3.6), 10 mM solution of TPTZ (in 40 mM HCl) and 20 mM solution of $FeCl_3$ x 6 H_2O, respectively.

RESULTS AND DISCUSSION

According to Apak, Gorinstein, Böhm, *et al*., (2013) the basis of most of the antioxidant based assays is either electron transfer or hydrogen atom transfer. In the first type of assays measuring the capacity of an antioxidant is based on reduction of an oxidant, which further changes colour of inidicator. The degree of colour change correlates with the concentration of antioxidant present in sample. On the other hand, reactions based on hydrogen atom transfer apply different reaction scheme, in which antioxidant quench free radicals by hydrogen donation (Apak, *et al*., 2013).

TAED from the tablet reacts with hydrogen peroxide, and partly removes it, and the presumption is that a dominant mechanism of reaction is based on electron transfer, where antioxidants pottentialy present in samples react with potassium peroxymonosulfate which acts as oxidant.

So the possible mechanism of this reaction can be presented with equation 1.

$$HSO_5^- + 2H^+ + 2e^- \longrightarrow HSO_4^- + H_2O \qquad (1)$$

The change of colour of indigotin present in tablet during the reaction can be measured spectrophotometrically. Indigotin, (2-(1,3-dihydro-3-oxo-2H-indol-2-ylidene)-1,2-dihydro-3H-indol-3-one) is a dark blue crystalline powder, incorporated in effervescent tablet along with other indicator, brilliant blue. In oxidized form, indigotin is insoluble in water, so it has to be reduced to its leuco form, which is water soluble. During this reduction dye changes colour from blue to yellow-green. On the other hand, oxidation of indigotin leads to decreasing the intensity of colour, which vanishes in the end, because of the total oxidative degradation of indigotin (dehydroindigo) (Polette – Niewold, Manciu, Torres, *et al*., 2007).

These colour changes are additionally proven, by investigation of spectral characteristics of indigotin, which is present is effervescent tablet. It is observed that change from indigotin to reduced leuco form does not lead to notable shift of absorption maximum, and the effects of colour change are obvious through absorption intensity change. During measurement of absorbance, spectra for solution with reducing agent (sodium dithionite), native solution and with added oxidant (hydrogen peroxide) are recorded (Figures 2-4).

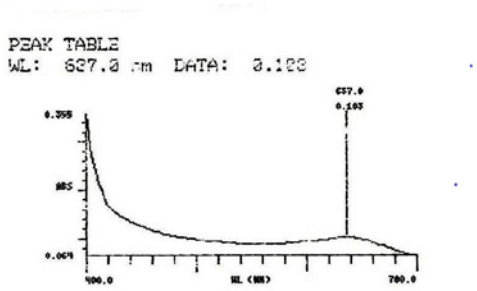

Figure 2: Spectrum of reduced indigotin from effervescent tablet after adding sodium dithionite

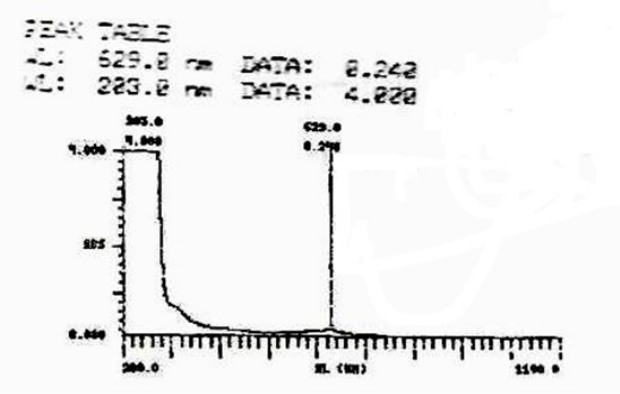

Figure 3: Spectrum of indigotin from effervescent tablet

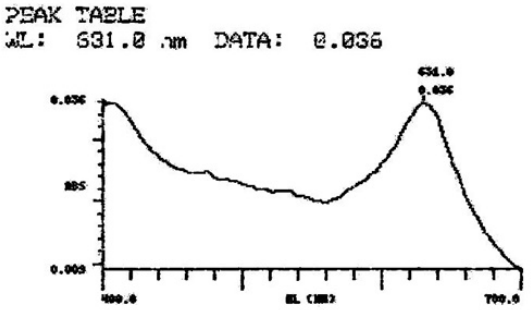

Figure 4: Spectrum of oxidized indigotin from effervescent tablet after adding H_2O_2

In this work, somewhat different approach was chosen comparing to originally proposed method (Al-Shahrani, *et al.*, 2013). For determination of antioxidant capacity calibration curve was made with the standard solution of ascorbic acid, within the 10 mg/80 mL (0.125 mg/mL) to 1000 mg/80 mL (12.5 mg/mL) concentration range. Obtained calibration curve was linear for concentrations from 10 mg/80 mL to 100 mg/80 mL, following the decrease of absorbances (for the concentration range from 100 mg/80 mL to 500 mg/80 mL), with no further change of the intensity of the absorption for the concentration range from 500 mg/80 mL to 1000 mg/80 mL. Because of this quite narrow linear range, we have investigated other option, which was to use catechin standard instead of ascorbic acid for preparation of the calibration curve. The same concentration range was chosen (0.125 mg/mL to 12.5 mg/mL). In this case obtained calibration curve was linear with correlation coefficient of R^2=0.992. This curve was used for further determination of antioxidant capacity of selected samples.

In order to test the possibility of this method in determination of antioxidant capacity of real samples, we have used five samples which antioxidant capacity was proven in previous testing with different *in vitro* and *in vivo* methods (Wang, Lutfiyya, Weidenbacher-Hoper, *et al*., 2009; Bramorski, Cherem, Marmentini, *et a*l., 2010; West, Deng and Jensen, 2011; Iloki Assanga, Lewis Luján, Rivera-Castañeda, *et al*., 2013; Ionica, Nour and Trandafir, 2012; Tarko, Duda-Chodak, Satora, *et al*., 2013; Katalinić, Milos, Modun, *et al*., 2004).

Selected samples were also tested using FRAP assay. Results of measurement are expressed as FRAP units (mmol/L Fe^{+2}) and were calculated from the calibration curve that was previously constructed (R^2=0,998).

Results of testing antioxidant capacity of selected samples in EAPK and FRAP units are presented in Table 1.

Table 1: Antioxidant capacity of selected samples

Sample	Antioxidant capacity (EAPK)	Antioxidant capacity FRAP (mmol/L Fe^{+2})
Dry white wine	0,81±0,017	7,96±0,01
Semi sweet red wine	1,60±0,046	11,9±0,005
Noni juice	1,50±0,046	11,2±1,79
Goji berries	9,19±0,04	33,8±1,20
Tomato juice	2,30±0,08	13,2±2,01

Since proposed mechanism of action for the method tested in this study is electron transfer, results are compared with those obtained with FRAP assay that has similar mechanism. There is a strong, positive correlation ($p<0.05$) with correlation coefficient of R^2=0,997 (Figure 5).

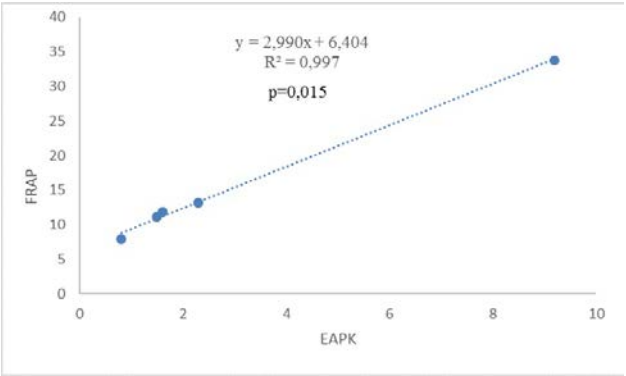

Figure 5: Correlation of results of our method and FRAP assay

CONCLUSION

Although very few samples were analysed with this method, obtained results show that potassium peroxymonosulfate can be used as a reagent for determination of antioxidant capacity *in vitro* in routine analysis. Advantages of this proposed method are cheap and available reagents that are used for the performing the analysis, and also a fact that there is no need for expensive equipement, which makes it suitable for laboratories with modest resources.

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Summary/Sažetak

U našem radu, ispitivana je mogućnost upotrebe komercijalnih tableta za čišćenje zubnih proteza, koje sadrže kalij peroksimonosulfat kao reagens za određivanje antioksidativnog kapaciteta *in vitro*. Cilj je bio razviti brzu, jednostavnu i jeftinu metodu za određivanje antioksidativnog kapaciteta, koja će se moći koristiti i u skromnije opremljenim laboratorijama. Ovaj metod je prethodno objavljen, međutim u našem radu korišten je različiti pristup uz modifikaciju originalne metode. Korištenjem askorbinske kiseline kao standardne supstance za izradu kalibracione krive dobivao se uzak raspon linearnosti, pa je iz tog razloga kao standardna supstanca korišten katehin u rasponu koncentracija od 0,125 mg/ml do 12,5 mg/ml. Dobivena kalibraciona kriva bila je linearna sa koeficijentom korelacije R^2=0,992 i ona je dalje korištena za određivanje antioksidativnog kapaciteta odabranih uzoraka. Kako bi se testirao predloženi metod u realnim uslovima, ispitano je pet uzoraka čiji je antioksidativni kapacitet potvrđen prethodnim testiranjima različitim *in vitro* i *in vivo* metodama.