Application Note:
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An Easy Determination of the Antioxidant Power of Beverage Samples with Thermo Scientific Multiskan FC Microplate Photometer

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Summary

This paper shows how antioxidant power of different beverage samples can be easily and rapidly tested with a photometric assay using Thermo Scientific Multiskan FC microplate photometer. With Multiskan® FC reader it is possible to measure these antioxidants with high sensitivity and assay robustness. The assay is also very fast to perform, the total time for the complete assay is around 5 - 10 min.

Introduction

Oxidative stress has been demonstrated to be involved in many diseases including atherochlerosis, Parkinson disease and cancer. Oxidative stress is caused by an imbalance between the production of reactive oxygen and a biological system's ability to detoxify the reactive intermediates, or easily repair the resulting damages. The most common forms of reactive oxygen are free radicals like superoxide anions, hydroxy radicals and alkoxy or peroxy radicals, which start chain reactions that damage cells.

Antioxidants are molecules capable of slowing or preventing the oxidation of other molecules, therefore protecting cells from the oxidation damages. Antioxidants can remove free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. Antioxidants are often reducing agents such as thiols or polyphenols. Commonly known examples of antioxidants are vitamins E and C, uric acid, carotenes and glutathione.

This paper describes how the total antioxidant capacity of the unknown samples can be measured with the photometric assay. The assay is based on the reduction of bivalent copper ion, Cu^{2}+, to Cu^{+} by the antioxidants present on the sample. This reduced form of copper is then selectively bound to chromogenic compound to form a complex that has strong absorbance at 490 nm. The assay is calibrated by using a known concentration series of standard antioxidant.

Materials and Methods

The beverage samples used to measure antioxidant power were 100% natural orange and apple juices as well as green, black and white tea. Antioxidant power was determined using photometric Total Antioxidant Power kit (Cat. no. TA 01) from Oxford Biomedical Research Inc. (Oxford, MI, USA) and clear flat bottom 96-well Thermo Scientific Immulon 1B microplates (Cat. no. 3355). The assay was performed according to the kit instructions except that vitamin B analogue Trolox™ ((±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid. Sigma-Aldrich, Cat. no. 56510) was used as a standard for calibrating the assay.

The Trolox calibration series with seven calibrators was prepared to cover the concentration range of 0.78 – 50 µM. Beverage samples were tested with three dilutions: undiluted, 1:5 and 1:10 dilutions. A 200 µl aliquot of each calibrator and sample dilution was added as duplicate into a 96-well microplate. Then, the background absorbance of the plate was measured with 492 nm with Multiskan FC controlled by Thermo Scientific SkanIt Software version 2.5. After that, 50 µl of the Copper Solution was added. The assay plate was then incubated for three minutes at room temperature and the reaction was stopped by adding 50 µl of the Stop Solution. The absorbance of the plate was then read again at 492 nm immediately.

The results were calculated according to kit instructions. Background absorbance of each well before the addition of the Copper Solution was subtracted from the final absorbance values and a linear calibration curve was prepared based on these subtracted

absorbances. The antioxidant power of each unknown sample was then resolved from the calibration curve as Trolox equivalents (Trolox concentration that has equivalent antioxidant efficiency as the unknown sample).

Results and Discussion

All results of this Antioxidant Power assay were calculated as instructed in the kit insert. First, 492 nm absorbance values from the background measurement at the beginning were subtracted from the result obtained after the reagent additions using automatic pre-calculation feature of the SkanIt® Software. Then the linear calibration curve was drawn with curve fit function of the software. The resulting calibration curve is shown in Figure 1. Assay robustness was analyzed using Z-prime calculation and resulting values are shown in figure 2.

Antioxidant power of unknown beverage samples was claculated automatically by SkanIt software curve fit module. The module calculates the concentration of samples based on the calibration curve and then calculates the final result value by taking the dilution factors into account. With this assay, extrapolation of the calibrator curve data was allowed so that result values higher than the highest calibrator could also be calculated. These results are shown in Table 1.

When we analyze the result data from the measurements, one striking observation is the excellent precision of the reader with low level absorbances. The background values measured before the dye addition have clearly below 0.001 absorbance unit standard deviation in these blank values (n = 46). This exceptional low level precision improves the assay sensitivity because absorbance values very close to background become statistically relevant and can be used for the real measurements.

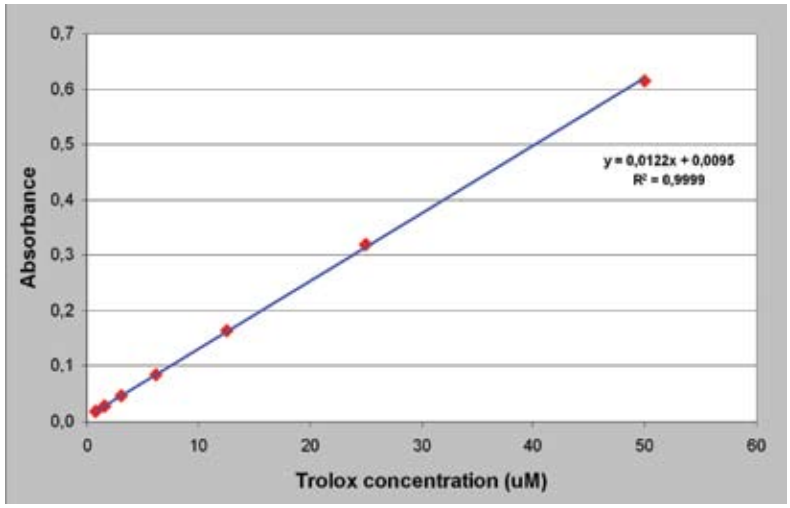

Figure 1. Trolox calibration curve of antioxidant power assay. Calibration curve show the perfect correlation between amount of known antioxidant and resulting absorbance. Calculated R^2 value was 0.9999.

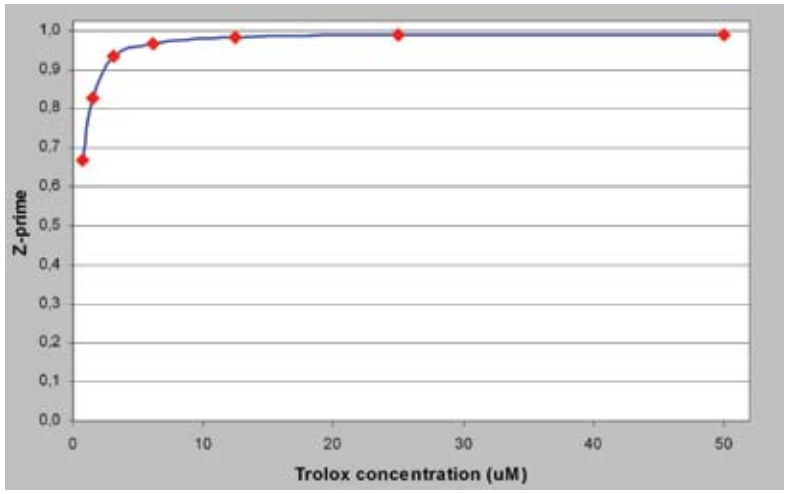

Figure 2. Z-prime values of antioxidant power assay. The Z-prime value is well over 0.6 over whole calibration range which proves the excellent robustness of the assay.

Both calibration curve analysis and Z-prime analysis show that this antioxidant power assay is performed perfectly with the Multiskan FC reader. The calibration curve shows complete linarity over whole concentration range and Z-prime values prove that the assay could be done even below the concentration range tested in this work.

When results of unknown beverage samples are analyzed, first thing to be noticed is that undiluted samples clearly contain some component disturbing the assay. This is seen from the calculated Trolox equivalents that were always remarkably lower when an undiluted sample was measured compared to diluted samples. Instead, two samples with different dilution factors behave as expected so that they gave identical results when dilutions are taken into account. This shows that it is recommended to always test such heterogenous samples with the dilution series instead of only one sample concentration.
Green tea was shown to be the most powerful antioxidant

Sample	Measured abs.	Calculated conc.	Dilution factor	Trolox equivalent
Apple juice, undiluted	0.427	34.3	1	34.3
Apple juice, dilution 1:5	0.116	8.77	5	43.8
Apple juice, dilution 1:10	0.067	4.74	10	47.4
Orange juice, undiluted	1.067**	86.8	1	86.8
Orange juice, dilution 1:5	0.405	32.4	5	162
Orange juice, dilution 1:10	0.226	17.7	10	177
Black tea, undiluted	0.961**	78.0	1	78.0
Black tea, dilution 1:5	0.480	38.6	5	193
Black tea, dilution 1:10	0.249	19.6	10	196
Green tea, undiluted	0.918**	74.5	1	74.5
Green tea, dilution 1:5	1.123**	91.4	5	457
Green tea, dilution 1:10	0.549	44.2	10	442
White tea, undiluted	0.880**	71.4	1	71.4
White tea, dilution 1:5	0.650**	52.5	5	263
White tea, dilution 1:10	0.334	26.6	10	266
***Extrapolated value*				

Table 1. Antioxidant power of analyzed beverage samples given as Trolox equivalents.

power in this assay. This was to be expected because for already some time, green tea has been well known to be quite a strong antioxidant. White tea was also a rather powerful antioxidant in this assay when orange and apple juices were the two least powerful beverages. These values are quite typical for the beverages analyzed here. Large variations in the antioxidative power can anyhow be observed when other types of green, black or white teas, or juices, would have been used.

Further Information

For further information about Multiskan FC, please refer to the following web pages:

www.thermo.com/readingroom

www.thermo.com/mpi

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