



Measurement of Antioxidant Activity using PiCOEXPLORER (DPPH Radical Elimination Method)

The measurement data below was provided by the Department of Food Research Institute, Local Administrative Institution Tottori Institute of Industrial Technology

Overview

Because a variety of factors in the modern environment contribute to oxidative stress, the idea of alleviating or preventing such stress by supplementing antioxidants from food has currently gained widespread recognition. DPPH radical elimination method is widely regarded as one of the main means by which the anti-oxidative mechanism (antioxidant properties) of foods can be determined. DPPH is a stable radical, and changes to non-radical form in the presence of a substance possessing highly-antioxidant and radical-eliminating properties, with the result that the purple color of the DPPH solution fades to yellow. This property can be exploited to quantify antioxidants by using an absorbance meter. To start, Trolox was used as a standard antioxidant, and its concentration was measured using an absorbance meter (PiCOEXPLORER) to prepare a calibration curve. Using the calibration curve, the antioxidative efficacy of roasted and green (unroasted) herbal tea was evaluated (Experiment 1). Next, the correlation between PiCOEXPLORER and a microplate reader (Tecan, Infinite F200) was verified using samples of black tea, coffee, green tea, red wine and white wine (Experiment 2).

Procedure

Experiment 1 : Calibration curve preparation using PiCOEXPLORER and sample (herb tea) measurement

1. Use Trolox (Calbiochem 648471) as a standard antioxidant, and prepare adjusted concentration samples (0, 0.031, 0.062, 0.125, 0.250, 0.5 μ mole TE/ml) using a buffer solution (0.2 M MES, pH 6.0, Dojindo 314-01621), a dilute solution (50% ethanol, Wako Pure Chemical Industries 054-07225), and an active enzyme generating source (1 mM DPPH, Wako Pure Chemical Industries 047-04051).
2. Measure the concentration of the adjusted samples prepared in set (1) using PiCOEXPLORER to prepare the calibration curve. (Measurement conditions: Measured volume, 200 μ l / measurement mode, Green* / LED output level, 10%). * *Maximum absorption wavelength: 530 nm / Wavelength range: 455-630 nm
3. Pour 100 ml of boiling water into 0.5 g each of green and roasted herbal tea leaves (original products of Tottori Institute of Industrial Technology's Food Development Research Laboratory), 250 μ l of each sample was drawn, and then 250 μ l of buffer solution and 500 μ l of dilute solution were added to make the samples.
4. Add 1 ml of buffer solution and DDPH to the specimen from step (3) to prepare the samples. Concentration and absorption were measured with PiCOEXPLORER (under the same measurement conditions as in step (2) above).

Experiment 2 : Measurement of 5 samples using PiCOEXPLORER and microplate reader

Calibration curve preparation was the same as for Experiment 1.

1. 25% solutions were prepared of black tea, coffee, green tea, red wine, and white wine by adding buffer solution and dilute solution. The remainder of the procedure was the same as in step (4) of Experiment 1.
2. The absorption values of samples from step (1) were measured using the microplate reader (Measurement conditions: Measured volume, 150 μ l / Measurement wavelength, 520 nm).

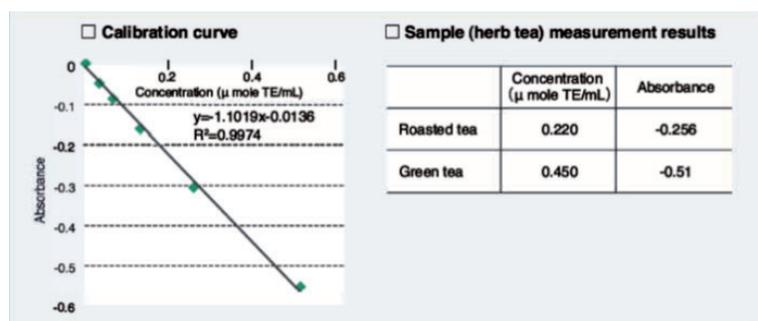


Measurement of Antioxidant Activity using PiCOEXPLORER (DPPH Radical Elimination Method) cont'd

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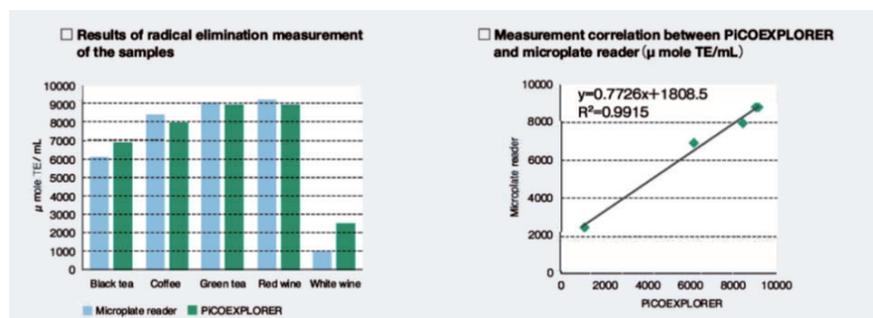
Result Experiment 1 : Calibration curve preparation using PiCOEXPLORER and sample (herb tea) measurement

A calibration curve with good linearity ($R^2 = 0.9974$) was obtained. The antioxidant properties of roasted tea and green tea were measured, yielding confirmation for the prevailing theory that the antioxidant properties of green tea are higher.



Experiment 2 : Measurement of 5 samples using PiCOEXPLORER and microplate reader

The antioxidant properties of black tea, coffee, green tea, red wine and white wine were measured and it was confirmed that there was a high degree of correlation between the results obtained by PiCOEXPLORER and the microplate reader ($R^2=0.9915$)



Comments

Yuko Nakamura/ The Department of Food Research Institute, (Local Independent Administrative Institution) Tottori Institute of Industrial Technology

Our lab supports food makers throughout the prefecture through analytical chemistry by providing technology support, joint research, and analysis services. We also conduct original research and development in our lab, such as functional evaluation development based on in-vitro testing. Food's antioxidant properties is a hot field, and recent years have witnessed a lot of research being directed at seeing the effects of foods after taken into the body (through measurement of in-vivo antioxidative activity). Besides individual food items, increasing attention is being paid to measurement of the antioxidant properties of food in general. Food makers in the prefecture who ask us for analysis services are saying that they would like to confirm the superiority of their products on their own. With regard to reagent kits, I get the feeling that many are being developed which are easy for on-site users and budding analysts to use, but the prices of measurement equipment remain high. Due to the high price, the microplate reader that our laboratory owns is utilized. From the results of this experiment, measurement results of antioxidant properties of each food sample were obtained using portable PiCOEXPLORER, and it was confirmed that there was a high degree of correlation between the results obtained by PiCOEXPLORER and the microplate reader.