

## 15.

### Antioxidant Activity - Spectrophotometric Method - DPPH

The hydrogen atom or electron donation abilities of extracts and pure compounds can be measured by the bleaching of purple colored methanol solution of DPPH. This spectrometric assay uses the stable radical, 2,2'-diphenylpicrylhydrazyl (DPPH), as a reagent.

#### Test

Add 50  $\mu$ l of various concentrations of extracts/pure compounds in methanol to 5 ml of a 0.004% methanol solution of DPPH. After a 30 min incubation period at room temperature, read the absorbance against blank at 517 nm. Calculate Inhibition of free radical DPPH in percent (%) in following way.

$$\% = \frac{(A_{\text{blank}} - A_{\text{sample}}) \times 100}{A_{\text{blank}}}$$

Where  $A_{\text{blank}}$  is the absorbance of the control reaction (containing all reagents except the test compound) and  $A_{\text{sample}}$  is the absorbance of the test compound. Extract concentration providing 50% inhibition ( $IC_{50}$ ) can be calculated from the graph plotting inhibition percentage against extract concentration. Tests should carry out in triplicate.

#### References:

- Tepe, B., Eminagaoglu, O., Akpulat, H.A. and Aydin, E. (2007) *Food Chemistry* 100, 985 -989.
- Burits, M. and Bucar, F. (2000) *Phytotherapy Research* 14, 323 - 328.
- Cuendet, M., Hostettmann, K. and Potterat, O. (1997) *Helvetica Chimica Acta*, 80, 1144-1152.

## The $\beta$ -Carotene Bleaching Method

Generally it is good to use two different types of assays to investigate the antioxidant activity of plants and their components. The early stage of lipid peroxidation can be monitored by the beta-carotene bleaching method. The DPPH assay is the most frequently used in antioxidant studies of natural products - it is simple and highly sensitive. The ABTS radical assay is widely used to evaluate AOA in foods and beverages because it is applicable in aqueous and lipid phases. The Ferric Reducing/Antioxidant Power (FRAP) Assay gives fast, reproducible results

Lipids such as linoleic acid form a peroxy radical ( $\text{LOO}^\cdot$ ) in the presence of ROS and  $\text{O}_2$ . The peroxy radical reacts with  $\beta$ -carotene to form a stable  $\beta$ -carotene radical. If an antioxidant is present in a testing solution, it reacts competitively with the peroxy radical. Anti-oxidant effects are monitored using a spectrophotometer at 470 nm, which is the absorbance of  $\beta$ -carotene.

### Procedure

Antioxidant activity of the catechin fractions,  $\alpha$ -tocopherol, and butylated hydroxyl anisole (BHA) can be determined according to the modified version of the  $\beta$ -carotene bleaching method of Gadov (1997).

- i. Dissolve  $\beta$ -Carotene (2.5 mg) in  $\text{CHCl}_3$  (25 ml), and add an aliquot (1 ml) of the solution to a r.b. flask (250 ml) along with linoleic acid (40 mg) and Tween 20 (400 mg). Evaporate to dryness under reduced pressure at a low temperature ( $< 30^\circ\text{C}$ ). Add oxygenated distilled water (100 ml) to the dried mixture and shake the mixture thoroughly to form an emulsion (A).
- ii. Prepare a second emulsion (B) with linoleic acid (40 mg), Tween 20 (400 mg) and oxygenated distilled water (100 ml).
- iii. Prepare a stock solution (C) consisting of  $1.3 \times 10^{-3}$  M antioxidant in MeOH. the reaction mixture.
- iv. Prepare blanks of the antioxidant by mixing B (200  $\mu\text{l}$ ) with A (5 ml) in test tubes.
- v. Mix water (200  $\mu\text{l}$ ) with A (5 ml) in test tubes to prepare the control mixture
- vi. Mix water (200  $\mu\text{l}$ ) with B (5 ml) and to zero the spectrophotometer

Take readings immediately (t=0) and at 30 minute intervals for 2h (t=120 min) on a spectrophotometer at 470 nm. Place the vials in a water bath at 50°C between measurements. All determinations were performed in triplicates. The antioxidant activity coefficient (AAC) was calculated from the data using the formula

$$AAC = \left[ \frac{A_{A(120)} - A_{C(120)}}{A_{C(0)} - A_{C(120)}} \right] \times 1000$$

Where  $A_{A(120)}$  is the absorbance of the antioxidant at t = 120 min,  $A_{C(120)}$  is the absorbance of control at t = 120,  $A_{C(0)}$  is the absorbance of the control at t = 0 min

**Reference:**

Gadow, A.V., Joubert, E., Hansman, C. (1997) *J. of Agric. Food Chem.* 45,632-638.