Measurement of total antioxidant capacity of selected infusions using ABTS method

Equipment/Material:

- Spectorphotometer
- quartz cuvets
- 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS)
- tea or caffee sample
- Trolox standard (external standard; MW= 250,29 g/mol)
- graduated cylinders
- Automatic pipets
- 10 ml, 100 mL volumetric flasks
- waste container
- potassium persulfate ($K_2S_2O_8$),
- methanol

Procedure:

1. ABTS solution : 7 mmol/L ABTS (360,2 mg/100 mL) and 2.45 mmol/l (66,22 mg/100 mL) potassium persulfate ($K_2S_2O_8$) prepare 24 h earlier

2. **Preparation of caffeine standard solutions:**

   A stock standard of Trolox prepare by dissolving about 16 mg of Trolox in 5 mL methanol in a volumetric flask (5 mL). Working standards prepare by pipetting the right amount of the stock standard solution into separate volumetric flasks (10 mL) to produce concentrations of 0, 0.01, 0.04, 0.08, 0.12, 0.016 and 0.2 µmol/10 mL, respectively standard solution. For each flask add 300 µL ABTS and dilute to volume with H_2O. The absorbance of each solution measure at absorption maximum of 725 nm using 10 mm quartz cuvette after 6 min of incubation at room temperature in the dark.. The absorbance values then plotte against concentrations to generate a standard calibration curve.

3. **Sample coffee or tea preparation**:

   100 mL of boiling water add to 250 mL beaker containing 0.2 g of coffee or tea respectively. The coffee or tea preparations stir and let it brew for 20 minutes. Then filter through a paper filter.

   To aliquot (200 µL) of the drink sample drawn with a pipette and place into a 10 mL volumetric add 300 µL ABTS and dilute to volume with H_2O. The absorbance of each solution measure at absorption maximum of 725 nm using 10 mm quartz cuvette after 6 min of incubation at room temperature in the dark.

4. **Calculation:**

   Antioxidant capacity expresse as percentage of ABTS scavenging relative to control use the following equation:

   $$\textbf{ABTS scavenging capacity (\%)} = ((A_c - A_s)/A_c)x100\%$$

   were: A_c – absorbance of control

   A_s – absorbance of sample

   Trolox use as a standard for the calibration curve. The ABTS scavenging activity present as Trolox equivalents use expresse as mg Trolox/ g tea or caffe.