

Aim of the Experiment:**DETERMINATION OF ANTIOXIDANT ACTIVITY BY DPPH METHOD****Introduction**

Oxidative stress refers to elevated intracellular levels of oxygen radicals that cause damage to lipids, proteins, and DNA. Reactive oxygen species (ROS), including superoxide anion radicals, hydroxyl radicals, and hydrogen peroxide, are one of the highly active oxygen free radicals. During evolution, most living organisms possess enzymatic defenses (superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR)), non-enzymatic antioxidant defenses (glutathione, thioredoxin, Vitamin C, Vitamin E), and repair systems to protect them against oxidative stress. However, these native antioxidant systems are generally not enough to prevent living organisms from oxidative damage. Antioxidant additives using substances that delay or prevent the oxidation of cellular substrates have demonstrated the capacity to protect the human body against oxidative damage. Although several synthetic antioxidants, including butylated hydroxyanisole and butylated hydroxytoluene, have been widely used in retarding lipid oxidation, their safety has recently been questioned due to liver damage and carcinogenicity. Therefore, in recent years, finding safer and natural antioxidants from bio-resources to replace synthetic antioxidants has received a great deal of attention. In vitro and in vivo studies indicate that probiotics exhibit antioxidant potential. In this view, consumption of probiotics alone or foods supplemented with probiotics may reduce oxidative damage, free radical scavenging rate, and modification in activity of crucial antioxidative enzymes in human cells. The total antioxidant activity of probiotic candidates can be investigated by means of three potential antioxidant methods; 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) free radical scavenging, ferric reducing ability of plasma (FRAP) assays and 2,2-diphenyl-2-picrylhydrazyl (DPPH).

Materials**A. Reagents****a) DPPH (2, 2 Diphenyl-1- picryl hydrazyl) Methanolic solution (1mM):**

19.72 mg of DPPH was taken in 50 mL amber colored reagent bottle and dissolved in 25 mL methanol by stirring over magnetic stirrer overnight at 40°C. The final volume was adjusted with methanol to 50 mL using volumetric flask and the stock solution was stored at -20°C.

b) Preparation of DPPH working solution (0.1 mM)

1 mL of stock solution was diluted with methanol (HPLC grade) to 10 mL in a volumetric flask. Working solution was prepared freshly prior to analysis and kept in amber glass bottle.

c) Trolox solution (5 mM / L):

12.5 mg of Trolox [6-hydroxy, 2,5,7,8 tetra methyl chroman-2- carboxylic acid] was dissolved in 10mL of ethanol. It was diluted with distilled water to 100-1000 μ M concentration.

Procedure

1. Working solution of DPPH (60 μ M in methanol) was prepared by diluting stock solution (10 mM) with Milli Q water in order to avoid precipitation of proteins by methanol.
2. Briefly, 100 μ L of fermented supernatants was pipetted into 96 wells micro-plate and mixed with 100 μ L/well of working DPPH solution and incubated at 37°C for 2 hrs. after wrapping in aluminum foil and absorbance was measured at 517 nm in Tecan-Infinite F200 PRO plate reader.
3. The readings were compared with the control, which contained 100 μ L of water instead of fermented supernatants.

Calculation:

The results were expressed as:

$$\% \text{DPPH scavenging activity} = (A_{\text{Control}} - A_{\text{Test}}) / (A_{\text{Control}}) \times 100.$$