REVIEW

Detailed Development of DPPH method of antioxidant in Different Media

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Abstract α, α-diphenyl-β-picrylhydrazyl (DPPH) free radical scavenging method offers the first approach for evaluating the antioxidant potential of a compound, an extract or other biological sources. This is the simplest method, wherein the prospective compound or extract is mixed with DPPH solution and absorbance is recorded after a defined period. However, with the advancement and sophistication in instrumental techniques, the method has undergone various modifications to suit the requirements, even though the basic approach remains same in all of them. This article presents a critical review on various developments to the DPPH method.

Keywords DPPH · Absorbance · Free radicals · Antioxidant · SIA

Free radicals are inevitably produced in biological systems and also encountered exogenously, and are known to cause various degenerative disorders, like mutagenesis, carcinogenesis, cardiovascular disturbances and ageing (Singh and Singh 2008). Antioxidants are the compounds, which combat the free radicals by intervening at any one of the three major steps of the free radical mediated oxidative process, viz., initiation, propagation and termination (Cui et al. 2004). These antioxidants are also produced by biological system and occur naturally in many foods and the balance between oxidants and antioxidants decides the health and vigor (Halliwell 1996).

Methods for antioxidant activity determination

Thus it is important to know the antioxidant content and their efficacy in foods, for preservation or protection against oxidative damage, to avoid deleterious changes and loss of commercial and nutritional value (Halliwell 1997). This necessitates the development of a rapid method for determining the potential antioxidant capacity in various foods.

DPPH assay

This method was developed by Blois (1958) with the viewpoint to determine the antioxidant activity in a like manner by using a stable free radical α, α-diphenyl-β-picrylhydrazyl (DPPH; $C_{18}H_{12}N_5O_6$, M=394.33). The assay is based on the measurement of the scavenging capacity of antioxidants towards it. The odd electron of nitrogen atom in DPPH is reduced by receiving a hydrogen atom from antioxidants to the corresponding hydrazine (Contreras-Guzman and Srong 1982).

DPPH is characterized as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole (Fig. 1), so that the molecules do not dimerise, like most other free radicals. The delocalisation also gives rise to the deep violet colour, with an absorption in ethanol solution at around 520 nm. On mixing DPPH solution with a substance that can donate a hydrogen atom, it gives rise to the reduced form with the loss of violet colour. Representing the DPPH radical by Z• and the donor molecule by AH, the primary reaction is

$$Z^{\bullet} + AH = ZH + A^{\bullet} \quad (1)$$