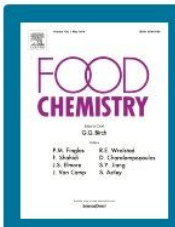

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FOOD CHEMISTRY

Aims and Scope

Food Chemistry publishes original research papers dealing with the chemistry and biochemistry of foods and raw materials covering the entire food chain from *'farm to fork.'* Topics include:

- Chemistry relating to major and minor components of food, their nutritional, physiological, sensory, flavour and microbiological aspects;
- Bioactive constituents of foods, including antioxidants, phytochemicals, and botanicals. Data must accompany sufficient discussion to demonstrate their relevance to food and/or food chemistry;
- Chemical and biochemical composition and structure changes in molecules induced by processing, distribution and domestic conditions;
- Effects of processing on the composition, quality and safety of foods, other bio-based materials, by-products, and processing wastes;
- Chemistry of food additives, contaminants, and other agro-chemicals, together with their metabolism, toxicology and food fate.

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Analytical papers related to the microbiological, sensory, nutritional, physiological, authenticity and origin aspects of food. Papers should be primarily concerned with new or novel methods (especially instrumental or rapid) provided adequate validation is described including sufficient data from real samples to demonstrate robustness. Papers dealing with significant improvements to existing methods, or data from application of existing methods to new foods, or commodities produced in unreported geographical areas, will also be considered.

- Methods for the determination of both major and minor components of food especially nutrients and non-nutrient bioactive compounds (with putative health benefits) will be considered.
- Results of method inter-comparison studies and development of food reference materials for use in the assay of food components;
- Methods concerned with the chemical forms in food, nutrient bioavailability and nutritional status;
- General authentication and origin [e.g. Country of Origin Labelling (COOL), Protected Designation of Origin (PDO), Protected Geographical Indication (PGI), Certificate of Specific Character (CSC)] determination of foods (both geographical and production including commodity substitution, and verification of *organic, biological* and *ecological* labelling) providing sufficient data from authentic samples should be included to ensure that interpretations are meaningful.

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Analytical Methods

A novel high throughput method based on the DPPH dry reagent array for determination of antioxidant activity

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ABSTRACT

A stable chromogenic radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) is commonly used for the determination of antioxidant activity. In this paper, DPPH was dried into 96 well microplate to produce DPPH dry reagent array plate, based on which the highly sensitive and high throughput determination of antioxidant activities was achieved. The spectrophotometric characterization of the microplate containing dried or fresh DPPH free radicals was reported. The response of the DPPH dry reagent array towards different standard antioxidants was studied. The reaction for DPPH in fresh or dry reagent array with Trolox was reported and compared. The DPPH dry reagent array was used to study the antioxidant activity of banana, green tea, pink guava, and honeydew and the results were compared to the samples reacted with freshly prepared DPPH. The proposed method is comparable to the classical DPPH method, more convenient, simple to operate with minimal solvent required and excellent sensitivity.

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1. Introduction

Oxidation is one of the most important processes of food deterioration because it may affect food safety, colour, flavour and texture. There is an increasing interest in antioxidants, particularly in those intended to prevent the presumed deleterious effects of free radicals in the human body, and to prevent the deterioration of fats and other constituents of foodstuffs (Molyneux, 2004).

A number of assays have been developed to determine the antioxidant activity based on different chemical and biological mechanisms. These include; Trolox equivalent antioxidant capacity (TEAC; Miller, Rice-Evans, Davies, Gopinathan, & Milner, 1993), oxygen radical absorption capacity (ORAC; Cao, Alessio, & Cutler, 1993), ferric reducing antioxidant power (FRAP; Benzie & Strain, 1999), and free radical scavenging (DPPH; Brand-Williams, Cuvelier, & Berset, 1995). Among these assays that measure radical scavenging capacity, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) is one of the most widely employed. The capacity to scavenge the stable DPPH free radical can be expressed as a measure of antioxidant activity. During this assay, the purple chromogen radical is reduced by antioxidant/reducing compounds to the corresponding pale yellow hydrazine. The reduction of the purple chromogen radical by hydrogen-donating antioxidants is monitored by the decrease of optical density at long wavelengths (515–520 nm). The reaction is shown in Fig. 1, where AH is donor molecule, and A**.** is free radical produced.

DPPH assay is simple, does not require any special preparation (Arnao, 2000), sensitive, independent of sample polarity and does not require sophisticated equipment such as HPLC or GCMS (Ozcelik, Lee, & Min, 2003). The DPPH assay requires mild experimental conditions, which is an advantage compared to other commonly used methods that require preliminary sample treatment such as high temperatures and or oxygen supply (Koleva, van Beek, Linssen, de Groot, & Evstatieva, 2002).

However, the DPPH assay is influenced by factors such as the type and amount of solvent used, water content, and hydrogen or metal ion concentration (Dawidowicz, Wianowska, & Olszowy, 2012). The strong absorption of some pigments, such as anthocyanins, at the same wavelength as DPPH (500–550 nm) is a limitation of the assay, which may result in undetected changes by colorimetric methods. To overcome this problem, Sun et al. (2012) used HPLC–DAD to determine changes in rabbit eye blueberry (*Vaccinium virgatum*). However, the use of HPLC–DAD makes the determination of the antioxidant activity more complicated and time consuming.

In the DPPH assay, a large volume of freshly prepared DPPH solution (2.9–3.9 ml) is required daily (Rufino et al., 2011; Villano, Fernandez-Pachon, Moya, Troncoso, & Garcıa-Parrilla, 2007). To avoid this limitation, storage of the DPPH stock solution at low temperature has been reported (Shian, Abdullah, Musa, Maskat, & Ghani, 2012). However, DPPH prepared and stored at low temperatures undergoes rapid decomposition (Deng, Cheng, & Yang,

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