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A novel, micro, rapid and direct assay to assess total antioxidant capacity of solid foods

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ABSTRACT

A novel, micro, rapid and direct procedure to measure the total antioxidant capacity of solid foods using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) (mR-QUENCHER-DPPH) was developed and validated. The mR-QUENCHER-DPPH assay was performed in semi-aqueous medium (methanol–Tris buffer) using very small sample amounts (below 3.6 μg), as estimated by a Bradford reagent-based chemical predictor, and it was completed in 10 min at room temperature. The total antioxidant capacity (TAC) of solid foods was expressed as scavenging capacity index (SCI, mmol DPPH scavenged per kg sample), a theoretical and stoichiometric parameter deduced in this study. SCI values measured by mR-QUENCHER-DPPH assay for cereals cous-cous (7.20 ± 0.35), amaranth (7.99 ± 0.35) and buckwheat (194.2 ± 6.72); Goji fruit (91.27 ± 3.98); lotus root (2402 ± 168); and spices turmeric (3767 ± 355), ginger (2493 ± 283), and cinnamon (10461 ± 2133) were further validated using Folin–Ciocalteau assay. Bland–Altman analysis showed that there were not statistically significant differences in TAC values as measured by both assays. In the same way, TAC values measured by mR-QUENCHER-DPPH were correlated with free ($r=0.8088$, $P=0.0151$), bound ($r=0.9668$, $P<0.0001$) and total ($r=0.9067$, $P=0.0019$) reducing capacity of extracts from solid foods as assessed by Folin–Ciocalteau assay. The mR-QUENCHER-DPPH assay allows to measure TAC values using micro-gram amounts in solid food samples with a wide content range of antioxidants (low, high and very high), and omitting the time-consuming dilution cellulose-step commonly employed in the traditional QUENCHER procedures.

1. Introduction

Some foods contain insoluble compounds such as bound phenolics, which exhibit antioxidant activity although they cannot be extracted by solvents [1,2]. QUENCHER approaches, based on well documented solid–liquid reaction, have been developed as direct assays, without a solvent extraction step, to assess total antioxidant activity of cereal products [3], raw and cooked meats [4], and grains and fruits [5] quantifying soluble and/or insoluble antioxidants [1,3]. QUENCHER assays have used common antioxidant activity procedures (ABTS, DPPH and CUPRAC) with a modification of the reaction medium composition to improve the solid–liquid reaction rate between antioxidants and radicals or oxidant reagents [3,6,7].

Despite the fact that QUENCHER assays have been successfully applied to solid foods, mainly cereals, some experimental questions deserve to be further improved. For instance, QUENCHER procedures require a solid dilution step using powdered cellulose for foods with medium and high total antioxidant capacity [1,3]. However, the dilution step is very time consuming and increases the sample heterogeneity and variability of the measurements [3,6,7]. Moreover, QUENCHER assays using either ABTS or DPPH radicals must be performed with different sample weights because a nonlinear concentration-curve response has been described [8]. Therefore, a single antioxidant concentration, as currently used in these assays, could be inappropriate for judging of antioxidant activities. In addition, it is well established that the steric accessibility to ABTS and DPPH radical has an influence on the reaction rate with antioxidant [7], which in turn affects the reaction time required. Thus, optimal reaction times for ABTS and DPPH-based QUENCHER assays have been established as 30 min and 120 min, respectively [6]. Furthermore, the dilution of sample with powdered cellulose can reduce the mass transfer coefficient, by increasing of solid to liquid ratio, and the reaction time in QUENCHER procedures is increased [9]. Although, steric issues have been recently overcome by use of metal copper ion as probe (CUPRAC-QUENCHER assay), it is only useful to measure the content of antioxidants with single electron transfer (SET) mechanism [7]. However, a DPPH-based QUENCHER assay is able to further quantify antioxidants, such as melatonin present in solid foods [10], with a hydrogen atom transfer (HAT) mechanism [11,12]

Recently, we have described a new microplate-adapted DPPH rapid assay, attractive from a cost-efficiency basis for routine

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measurements, which allows to assess the antioxidant capacity of pure compounds and liquid foods [13]. The assay was carried out in a buffered medium (methanol: 10 mmol L^{-1} Tris buffer pH 7.5; 1:1 v/v; MT buffer), the reaction was completed in 10 min or less and a theoretical antioxidant parameter was calculated to measure the antioxidant capacity of the samples [13]. The aim of the present study was to develop and validate a novel, micro, rapid and direct DPPH assay (mR-QUENCHER-DPPH) to measure the antioxidant capacity of solid foods using random and very low sample amounts (below 3.6 μg). The weight of the sample loaded was estimated using a chemical predictor based on Bradford reagent reaction. The scavenging capacity index (SCI), a theoretical and stoichiometric antioxidant parameter used to express the total antioxidant capacity of solid foods, was also deduced.

2. Experimental

2.1. Reagents

2, 2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid sodium carbonate, sodium hydroxide, and trizma® base were purchased from Sigma-Aldrich (Madrid, Spain). Folin–Ciocalteau phenol reagent, hydrochloric acid, methanol, and sulfuric acid were acquired from Merck (Barcelona, Spain). Bradford reagent was supplied by Bio-Rad (Bio-Rad, Spain). MilliQ ultrapure grade water (< 18.2 mΩ) was used for solution preparation.

2.2. Samples

Total antioxidant capacities of commercial solid foods were evaluated by the developed mR-QUENCHER-DPPH and Folin–Ciocalteu (F–C) assays. The following samples were assayed: a) Cereals: cous-cous from spelt wheat (*Triticum spelta* L.) (Finestra Cielo, IT-BIO-014, Spain), amaranth (*Amaranthus caudatus* L.) (Biogra, Spain), and buckwheat (*Fagopyrum esculentum* Mönch) from Biotrigo Sarraceno (Biogra, Spain); b) Fruit: whole sun-dried Goji (*Lycium barbarum*) berry (Linwoods Group, Spain); c) Root: lotus (*Nelumbo nucifera*) powder (MIMASA, Barcelona, Spain, imported from Japan); and d) Spices: turmeric (*Curcuma longa* L.) powder (Hacendado, Spain), ginger (*Zingiber officinale* L.) powder (Soria Natural, Soria, Spain), and cinnamon (*Cinnamomum verum*) powder (Hacendado, Spain). Cous-cous, buckwheat and goji samples were ground with a commercial coffee blender and sieved through a 100 mm mesh (Endecotts Ltd., London, England). Powdered solid foods were packed in a sealed plastic container and stored at −20 °C until analysis.

2.3. mR-QUENCHER-DPPH assay to assess the total antioxidant capacity of solid foods

2.3.1. Sample loading and weight estimation

Powdered solid foods were dispensed into hermetic tubes with a semi-conic base using the sample loader, a plastic tip of a repetitive pipette (Fig. 1). This sample loader allowed to apply very small and random amounts of the samples within each tube, avoiding the time-consuming step -related to sample dilution with powdered cellulose- of traditional QUENCHER procedures [3,6,7].

The weight of loaded sample (*Ws*) was estimated using a chemical predictor based on the reaction of soluble proteins from samples with Coomassie blue reagent (Bradford reagent). Specific standard curves for each sample were prepared using 1, 2, 3, 4 and 5 mg, which were incubated with 1 mL of DPPH solution (60 μmol L^{-1} in MT buffer) under constant shaking at maximum speed in a "Movil Rod" (J.P. Selecta S.A, Barcelona, Spain) shaker at room temperature for 10 min. Thereafter, the sample was

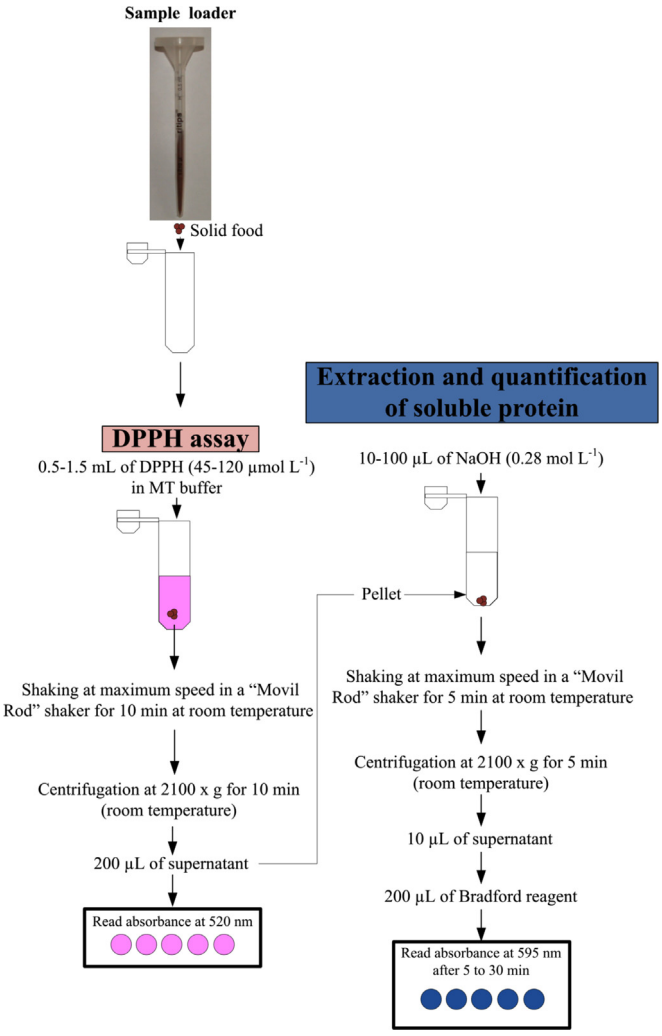

Fig. 1. The mR-QUENCHER-DPPH assay to assess the total antioxidant capacity of solid foods. MT buffer is a methanol–10 mmol L^{-1} Tris buffer (50:50) v/v pH 7.5. Different volume and DPPH concentration were used based on the total antioxidant capacity of the solid food. For the protein quantification, the volume of sodium hydroxide was adjusted according to the level of protein content and the amount of solid food loaded.

centrifuged at $2100 \times g$ for 10 min, the supernatant discarded and the pellet was dried by tube inversion at room temperature. For protein extraction from samples, 1000 μL or 10–100 μL of sodium hydroxide (0.28 mol L^{-1}) were used either for standard curves or for mR-QUENCHER-DPPH assay, respectively. Soluble proteins, extracted by shaking and centrifugation as described above, reacted with Bradford reagent using a microplate assay (Bio-Rad, Madrid, Spain) and the absorbance was read at 595 nm (As). The *Ws* in each tube was estimated by specific power equation, as follows:

$$W_s = V_S \times \left[\frac{A_S}{a}\right]^{\frac{1}{b}} \quad (1)$$

Where V_s is the volume in milliliter of 0.28 mol L^{-1} sodium hydroxide used for protein extraction; and *a*, *b* are specific parameters of Eq. (1) estimated by nonlinear regression analysis of absorbance at 595 nm *versus* sample concentration in mg mL^{-1}.

2.3.2. Kinetic of reaction

From a kinetic point of view, the reaction between antioxidants (pure or contained in liquid foods) and DPPH in a semi-aqueous