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Original article

Modes of handling Oxygen Radical Absorbance Capacity (ORAC) data and reporting values in product labelling

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ABSTRACT

Antioxidant measurement assays are widely used and should be chosen based on their being fit for purpose. Likewise, the mode of reporting antioxidant measurements should also be fit for purpose. The Oxygen Radical Absorbance Capacity (ORAC) assay is widely used internationally for measuring the antioxidant capacity of commodities using the peroxyl radical. However, the current mode of reporting of the ORAC values is not obvious, especially for the consumer groups. In this mode, reporting of the ORAC values is the unit of micromoles of vitamin E analogue (VEA), known commercially as Trolox Equivalents per kilogram or per litre (μM T.E./kg or L). Unlike mass units, molar units are not widely used in nutrition information panels (NIP). This paper presents a simple mathematical model for conversion of ORAC values to mass units to facilitate better understanding of the antioxidant capacity quoted. Additionally, mass values are in keeping with current labelling practice in Australia. Unless legislation is passed for the regulation of ORAC data use in labelling and product marketing, mass units should be considered as a mode of reporting, limiting sensationalism of antioxidant capacity and keeping with current labelling practice.

1. Introduction

Naturally occurring radical species arise from various metabolic processes, dietary and environmental factors and exposure to different radiation sources (Huang et al., 2005; Cao et al., 1993; Sies, 1997) and are recognised as having a role in cell damage, disease and cancer (Thaipong et al., 2006; Adom and Liu, 2002). Given the potential benefits of antioxidants in human health and disease prevention, measurement of antioxidant capacity in foods is a significant area of international study (George et al., 2005).

Currently, one of the most widely used antioxidant assays is the Oxygen Radical Absorbance Capacity (ORAC) assay (Bisby et al., 2008; Thaipong et al., 2006; Huang et al., 2002a; Alarcón et al., 2008; Ou et al., 2001) and antioxidant capacity is reported by comparison with an antioxidant standard which is a water soluble vitamin E analogue (VEA) known commercially as Trolox (Gomes et al., 2005).

The final ORAC measurement, known as the Total Antioxidant Capacity ($ORAC_{TAC}$), is given as a sum of the individual ORAC lipophilic and hydrophilic fractions and is reported in micromoles of Trolox Equivalents per litre or per kilogram depending on the product (Prior et al., 2003).

The assay has been applied to a wide range of samples including food, beverages and plasma, as was detailed by Prior et al. (2003) and has been successfully applied to samples of a complex nature (Zulueta et al., 2009). Increasingly, ORAC is being applied in the areas of cosmetics and neutraceuticals (Cornelli, 2009), with a twofold aim. Firstly, in product development, to establish the antioxidant affect of specific ingredients and the impact of formulation preparations on these antioxidant properties. The second area is in product marketing, where consumer interest in antioxidants has increased considerably (Mertz et al., 2009).

Any visit to local supermarkets, health food stores or larger grocery chains reveals an increasing number of products being marketed for their antioxidant abilities. In Australia, the main method of reporting antioxidant capacity of a product is by ORAC values. These ORAC values are quoted on product packaging, and sometimes near the base of nutrition information panels (NIP).

A key area of concern is units used for reporting the antioxidant capacity values. As mentioned previously, the units used are micromoles of antioxidant standard equivalent per litre or per kilogram (μM T.E./L or kg). These units are understood in the scientific community however understanding is more limited in the wider community, in particular the consumer groups. In Australia, most nutritional information for different food components has been reported in mass units, thus, conversion of ORAC

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values into the mass units is desirable for more effectively communicating antioxidant capacity. Mass units have several advantages in that they increase the communication of antioxidant capacity of foods to the consumer, law makers, manufactures of processed products, and for labelling purposes.

The work presented in this paper involved investigating and measuring the ORAC values of a range of food products. The ORAC values are presented in mass units, in contrast to the conventional presentation of the molar units. In this study, the FLUOstar OPTIMA microplate reader (BMG LabTechnologies) was used in the assay as a semi-automated method to measure the ORAC value of products. The experimental approach used is presented and the results are reported in mass and molar units for comparison purposes.

2. Materials and methods

Chemicals: Fluorescein disodium salt (FL), 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), monosodium phosphate monohydrate, disodium phosphate heptahydrate, hydrochloric acid, acetone, acetic acid, hexane and randomly methylated β-cyclodextrin (RMCD). All chemicals were obtained from Sigma–Aldrich (Australia).

Reagent and standard preparation: 75 mM aqueous phosphate buffer was prepared to 2 L volume with monosodium phosphate monohydrate and disodium phosphate heptahydrate, and the pH adjusted to 7.4 using 1 M hydrochloric acid (HCl) prepared from 10 M HCl stock. All subsequent working solutions were prepared in phosphate buffer. A stock solution of fluorescein (FL) was prepared monthly at 0.7 mM concentration, and the final working solution of FL was achieved by serial dilution of the stock with buffer to a final concentration of 70 nM. The working FL solution was prepared from stock daily. AAPH peroxyl radical donor was prepared to 35 mM concentration by dissolution in phosphate buffer. Due to the thermal sensitivity of AAPH, the working solution was prepared just prior to analysis. The Trolox antioxidant standard stock solution was prepared weekly at a concentration of 10 mM and serial dilutions with buffer from the stock were performed daily to achieve a range of 6.25–100 μM for preparation of calibration plots. A 7% RMCD solvent solution was prepared in methanol as required to act as a solubility enhancer for extracted lipophilic antioxidants from a given sample (Huang et al., 2002b). An acetone/water/acetic acid (AWA) 70:29.5:0.5 (v/v/v) solvent was prepared for the extraction of hydrophilic antioxidants. Both lipophilic and hydrophilic extracts were diluted in phosphate buffer prior to analysis.

Sample preparation and extraction: Samples were obtained locally from Coles supermarkets in Port Melbourne, Victoria, Australia, and stored according to manufacturer's instructions. In the case of processed products, once opened the products were homogenised and refrigerated at ≤4 °C until use. All samples were homogenised and analysed on the same day, to minimise product degradation. All samples were also extracted and analysed in triplicate.

In order to account for fat and water soluble antioxidants, the same sample is extracted using 2 different solvent systems. Fat soluble, or lipophilic, antioxidant compounds are extracted initially, generally with hexane or a similar solvent (Prior et al., 2003) and then the extracts are treated with a solubility enhancer such as methyl-β-cyclodextrin (Mercader-Ros et al., 2010). Water soluble, or hydrophilic, compounds are then extracted using any one of a range of solvents cited in literature. The solvents used vary as researchers tailor their studies, and consequently their solvent systems, to a selected range of products or samples. Commonly used solvents include, but are not limited to, methanol, acetone, water and phosphate buffer (Amorati et al., 2006).

In most techniques, phosphate buffer is used to dilute all extracts, as the FL probe is most stable at pH 7.4 (Gomes et al., 2005). The strength and pH of this buffering solution are important factors, as is solubility, especially of the lipophilic extracts.

The extraction process was conducted in 2 stages. Firstly, samples to be analysed for both hydrophilic and lipophilic antioxidant capacity were isolated. This involved samples being weighed and extracted for lipophilic antioxidants using hexane as the solvent. This extraction process was repeated twice. The hexane fractions were separated from the remaining sample solids, and combined and blown to dryness at room temperature with nitrogen. This process yielded oily or fatty residue on the inner surface of the collection tube. The residue was redissolved with acetone, followed by dilution with RMCD solution to enhance solubility. The final solution was dissolved in phosphate buffer prior to analysis. The remaining sample solids were then re-extracted using the AWA solvent system, diluted in phosphate buffer and analysed separately. This allows both lipophilic and hydrophilic fractions to be extracted, buffered and analysed independently.

Snack bar and chocolate samples were homogenised prior to subjecting them to the extraction processes, as described above. Care was taken when solubilising chocolate samples to ensure that even dissolution of the solids was achieved. The lipophilic extraction was conducted first followed by the hydrophilic extraction process. The hydrophilic extraction process was modified to include a pre-dissolution step where the chocolate sample was dissolved in acetone first, followed by AWA solvent system. The final extract was then diluted in phosphate buffer.

The edible portion of fresh fruit samples (mango, orange, blueberries, and pear) was homogenised and then weighed. This was then subjected to both the lipophilic and the hydrophilic extraction processes. Inedible portions such as seed, stalk or skin were discarded, except in the case of oranges as not all the pith could be completely removed. Dried apple was homogenised and extracted with both hydrophilic and lipophilic solvent systems directly, without rehydration, as the product can be consumed in either the dried or rehydrated form.

Processed or canned fruits were homogenised together with their juice or syrup. The homogenised sample was weighed and subjected to both the hydrophilic and lipophilic extraction processes. Fruit juice samples were centrifuged and an aliquot taken followed by dilution in 75 mM phosphate buffer (pH 7.4). This sample was only subjected to the hydrophilic extraction process as the fat content of the sample was known to be negligible.

Tea bags were cut open and the contents from each bag weighed and steeped in 200 mL of near boiling water (≥92 °C) for 5 min for infusion. The leaves were strained and the infusion cooled, centrifuged, and then diluted in 75 mM phosphate buffer. Upon visual inspection of the bag contents, all teas containing citrus were all subjected to both lipophilic and hydrophilic extraction processes. These teas appeared to have small amounts of peel or rind which were expected to have a measurable lipophilic antioxidant capacity.

Instrumentation: The FLUOstar OPTIMA microplate reader system as used by Volden et al. (2009), was equipped with 2 injector pumps and fitted with FLUOstar OPTIMA software system (version 2.0). Fluorescence intensity measurements were taken at regular intervals using an excitation wavelength of 485 nm and an emission wavelength of 520 nm. The instrument sensitivity (gain) was set to 1600 as this was found to be optimal for analysing a broad range of samples in the same experimental run. Both pumps were primed with 4 mL of reagent (FL for pump 1 and AAPH for pump 2) from beaker reservoirs prior to the assay.