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GUEST EDITORIAL

Value of antioxidant capacity as relevant assessment tool for "health benefits" of fruit - understated or inflated?

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Consumption of fruit and vegetables is considered to be an inherent part of a healthy diet, but more so since plant antioxidants, and in particular polyphenols, have been linked through in vivo and epidemiological studies with positive health outcomes.[1–3] As a result, polyphenols have been elevated to "lifespan essentials", because scientific evidence indicated that they are needed by humans to achieve a full lifespan by reducing the risk of a range of chronic diseases.[4] No Dietary Reference Intake values exist for polyphenols, however, it has been suggested that their target intake value should be based on the total polyphenol content provided by the "5-a-day" portions of fruit and vegetables recommendation by the World Health Organisation.[5]

In spite of mounting evidence that fruit and vegetable intake should be within an adequate range to support health, the intake of the required recommended daily portions remains a challenge to many consumers due to various factors.[6,7] When considering the rural South African black population, Kucich and Wicht[8] in the current issue of the SAJCN identified availability and access (or food insecurity) as some of the reasons for the disparity between recommended and actual intake of fruit and vegetables. The authors suggest that an increased consumption of local indigenous fruits could provide a much-needed source of phenolics and antioxidants.[8] These wild fruits can play an important supplementary role in the diet of people during periods of food insecurity. A major methodological challenge, however, exists to evaluate the relative contribution of dietary polyphenols to the total antioxidant capacity (TAC) of a food and the validity of their maximal nutritional value as antioxidants in order to provide guidelines and advise consumers.

For comparative assessment of the relative "nutritional value" of ten indigenous fruits to that of blueberry and cranberry, well-known for providing high levels of antioxidants, Kucich and Wicht[8] employed an Antioxidant Potency Composite Index, by combining the equally weighted results of three antioxidant assays. These assays, i.e. Total Phenolic Content (TFC), Trolox Equivalent Antioxidant Capacity (TEAC) and Total Antioxidant Capacity (H-ORAC$_{FL}$ + L-ORAC$_{FL}$), reflect the ability of polyphenols to neutralise free radicals through hydrogen atom transfer and/or electron transfer.[9] ORAC values were used to indicate that consuming as little as 25 g of colpoon, Christmas berry or wild olive at least 8000 µmoles Trolox Equivalents (TEs) are added to the diet. The same amount of cranberries and blueberries would add 1600 and 3000 µmoles TEs, respectively. Louwrens et al.[10] recommended a daily TAC intake per person equalling 20 513 µmoles TE for the average South African consumer, based on ORAC values calculated for a diet compiled using the "5-a-day" concept.

To better understand the relationship between polyphenols, antioxidant capacity and health, and whether such a relationship has any value, the "Free Radical Theory of Ageing" needs to be examined. In 1954 Denham Harman, in his quest to explain ageing, had a "light bulb" moment, linking free radicals to ageing, although at that stage no literature existed to support this hypothesis. In a paper published in 2009 he describes the origin, evolution and eventual recognition of the "Free Radical Theory of Ageing".[11] Deleterious free radical reactions in biological systems, if unchecked, cause damage to cell structures such as lipids, proteins or DNA, inhibiting their normal function. The resultant impaired functionality may lead to "ageing" and degenerative diseases. The human antioxidant defence mechanism includes both enzymatic and non-enzymatic endogenous antioxidants, such as superoxide dismutase (SOD), catalase, glutathione peroxidase, coenzyme Q10 and glutathione, allowing cells to manage reactive oxygen species (ROS) by eliminating excess ROS to maintain redox homeostasis.[12,13] Exogenous antioxidants, and most notably polyphenols, provided by the diet, subsequently gained prominence over the past 25 years or more for their ability to scavenge free radicals, leading to the hypothesis that these phytochemicals could assist in maintaining redox homeostasis in the cell. In addition, excessive amounts of polyphenols ingested through dietary supplements have also been associated with adverse effects by acting as prooxidants resulting in the induction of oxidative stress[13], thereby suggesting the existence of critical thresholds for polyphenol intake to ensure their health beneficial effects. It becomes important to realise that the health benefits of polyphenol-enriched supplements, although making sense theoretically, have not been thoroughly evaluated and, that using a wrong dose and/or combinations of such supplements, could have detrimental health effects. Therefore, it is very important to accurately assess polyphenol intake instead of exclusively using the antioxidant activity of fruit and vegetables, which seems to provide unreliable and variable results. Various antioxidant assays[14] have been developed through the years to determine the TAC of plant foods with new assays or new versions of existing assays occasionally emerging[15–17], either to broaden the base of comparison or to focus on a specific mechanism of antioxidant activity. Whilst these efforts provide the nutritionists and food scientists with an array of assays to estimate the antioxidant activity of food, they have limited or lack relevance *in vivo*. For instance, many of the radical species utilised in these assays are unrelated to those effecting cellular oxidative stress. Furthermore, high antioxidant concentrations and the test environment employed (e.g. a low pH or non-buffered medium) are factors contributing to a lack of physiological and/or pathological relevance to oxidative stress.[18] The most widely used methods to provide a cumulative or TAC value for food items are the ORAC, DPPH and ABTS (or TEAC) assays.[19,20] The TFC or Folin-Ciocalteu assay, historically used to quantify the total polyphenol content of wine and later other foods, is in fact also an antioxidant method utilising a basic reaction mechanism of oxidation/ reduction.[21] It could be argued that the opposite may also be valid, as the so-called antioxidant assays only provide an estimate of redox-active compounds, and thus by implication polyphenols, especially for food products containing high levels of these phytochemicals.

In spite of these caveats, dietary TAC has also been used to assess the relationship between the cumulative antioxidant capacity of a food and health outcomes in humans. Several studies indicated an inverse relationship between antioxidant intake and disease risk[22–29] fuelling the interest in these dietary constituents as health promotors. In some instances, threshold values of antioxidants that will reduce certain disease risks, were suggested.[23,26] While interpretation of the outcomes associated with daily intake of a threshold level of antioxidants may seem straight forward, many factors confound perceived outcomes and interpretation. Visioli et al.[3] noted that the level of evidence from epidemiological studies on antioxidant-related wine consumption and health, varies greatly, probably due to inherent difficulties to accurately estimate dietary habits and lifestyle. Furthermore, they pointed out that the methods currently used in epidemiological studies for evaluating antioxidant intake via foods and beverages are inadequate and challenging.

Interpretation of threshold intake levels of TAC for a perceived positive health outcome is further confounded by the lack of a standardised assay. The ORAC assay has gained popularity, not least because of the effort Prior and co-workers have put into the development of a method that takes into account both hydrophilic and lipophilic antioxidants, but also to create a large ORAC database of selected foods.[16,30] The data generated by various TAC assays are also employed by industry and even by consumers as yardsticks to compare food products, in order to identify the "best" product or the one with the "highest" TAC value. This gave rise to terms such as "superfoods" and "superfruits", yet with little *in vivo* evidence and sometimes only an *in vitro* TAC value as basis. In a 2012 press release, the United States Department of Agriculture announced the removal of their USDA ORAC Database for Selected Foods from their Nutrient Data Laboratory website.[31] The main reason given was the "mounting evidence that the values indicating antioxidant capacity have no relevance to the effects of specific bioactive compounds, including polyphenols in human health". Furthermore, it stated that "ORAC values are routinely misused by food and dietary supplement manufacturing companies to promote their products and by consumers to guide their food and dietary supplement choices". This begs the question - have we all been misguided or overstating the positive health impact of antioxidants and the value of antioxidant measurements in food? Prior[9] concluded that antioxidant capacity assays do have relevance to *in vivo* health outcomes, providing that the advantages, disadvantages and shortcomings of the particular *in vitro* assay are defined and understood.

In the final analysis of the value of the TAC of fruit as a relevant assessment tool for "health benefits" as employed by Kucich and Wicht[8], we can argue that such a value is adequate to provide the analyst with a global content value for phytochemicals, including polyphenols that undergo and modulate redox type reactions. However, these assays may not reflect the structural complexity of these compounds that governs their physicochemical properties related to their absorption, metabolism and excretion. Characterisation and accurate quantification of individual polyphenols in foods are critical to assess their relevance and contribution to a healthy diet. The development of the Phenol-Explorer, another web-based database, was a step in the right direction. This comprehensive database on individual polyphenol content in foods also allows retrieval of data on the biotransformation and pharmacokinetics of dietary polyphenols, in addition to data on the effects of food processing on polyphenol content of food.[32-34] Mounting scientific evidence points to important roles for polyphenols and their metabolites in cellular responses that are independent of their antioxidant activity *per se*.[35–39] Virgili and Marino[36] concluded that "… the "antioxidant hypothesis" is to be considered in some cases an intellectual "shortcut" possibly biasing the real understanding of the molecular mechanisms underlying the beneficial effects of various classes of food items".

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