Quality Assurance of Nutraceutical Health Claims: The Case for Antioxidants

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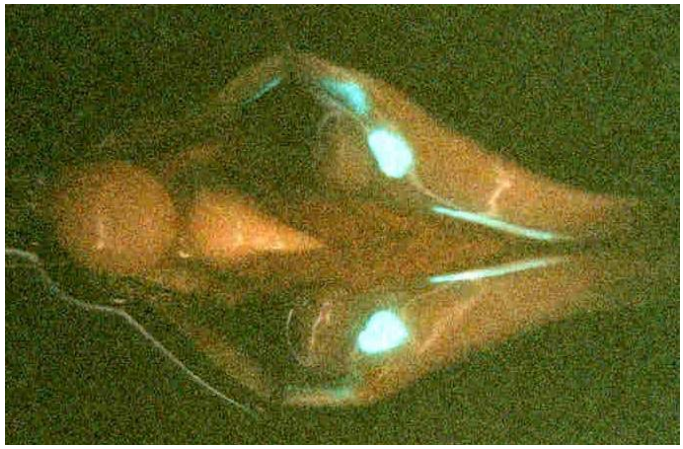
The consumer of nutraceuticals perceives natural products to be safer and more effective than synthetic analogues. For manufacturers and distributors credibility of claims is key. With reliable evidence derived from rigorous laboratory testing these natural products can hold their own against the synthetics.

Nutraceuticals are food-derived products containing ingredients with claimed health benefits. The term 'functional food' is used for any food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains. The potential of functional foods to mitigate disease, promote health and reduce health care costs is fueling the fastest growing sector in the food industry: nutraceuticals. Major food processing companies (Unilever, Nestlé and Kraft) are creating high price-margin food and nutraceutical products with claimed health benefits. These products are addressing markets estimated at over $50 billion in the US alone. In 2003 the world market was predicted to grow to $155 billion by 2005 (1).

Pholas dactylus, the Common Piddock

An active white blood cell producing free radicals



Pholas dactylus, opened to reveal glowing light organs

Free radicals and oxidative stress

Free radicals (molecules with unpaired electrons) and other reactive oxygen-containing species (ROS) are continually produced in the body and are continually destroyed by a range of substances known collectively as antioxidants (2). They have very important functions in the body, some good and some bad. However, when the production of these highly reactive molecules exceeds the supply of antioxidants to keep them under control oxidative stress occurs.

Oxidative stress occurs at sites of inflammation when billions of ROS-producing white blood cells accumulate and, instead of confining their activity to ridding the body of pathogens, excess free radicals and ROS injure and kill healthy tissue, damage DNA and attack enzymes. It is therefore not surprising that ROS are implicated in over 150 pathological conditions including cancer, heart disease, stroke, diabetes and premature ageing.

There is a current and growing interest in free radicals and oxidative stress in a wide range of research endeavours including: ageing, allergies and food intolerance, Alzheimer's disease, brain damage, cancer, critical care, diabetes, food testing, heart and vascular diseases, inflammation and inflammatory diseases, kidney disease and renal dialysis, materials testing, multiple sclerosis, neonates, obstetrics, pharmaceutical testing, reperfusion injury, respiratory medicine, sickle cell anaemia, sports medicine, surgery and toxicology – and others!

The food pharmacy

Much attention is focused on changing oxidative status by way of diet and supplements. Epidemiological studies over the past 20 years have led to an awareness that diets rich in fruits, vegetables and grain products can lead to significant reductions in chronic diseases, in particular certain cancers and heart disease (3). These protective effects have largely been attributed to their antioxidant activity. The principle antioxidants derived from food are vitamin E, beta-carotene and vitamin C. In addition, the trace element selenium is required for the proper functioning of the antioxidant enzyme glutathione peroxidase. There are a whole host of other antioxidant phytochemicals such as lycopene from tomatoes and allylic sulphides from garlic that are being credited with reducing the risk of cancer and heart disease. And the understanding that vitamin E is a generic term encompassing the family of tocopherols and tocotrienols is leading to much greater understanding of how best to use these phytochemicals therapeutically. What is fundamental to this whole story is that the body cannot manufacture these micronutrients so they must be provided in the diet.

Products that claim to enhance health, but are not officially drugs, are more appealing to consumers than prescription drugs. And when these products are made with ingredients derived from natural materials consumers perceive these products to be safer and more effective than a synthetic analogue. In order to meet the aspirations of discerning consumers the industry must be able to produce products with quantifiable and reproducible activity. It is now essential that industry is able to demonstrate adequate quality control of materials before, during and after processing, and quality assurance of finished products. Furthermore, if the industry is to thrive and gain support from traditional medicine it must present facts not hyperbole.

Antioxidant concentration versus antioxidant capacity

Measuring the concentration of specific antioxidants in a sample is one approach to quality assurance. However, this approach can be misleading because the sum of the individual antioxidants present in a sample may not match the total antioxidant activity of the sample. This can be explained in two ways: first the actual ability of the individual antioxidants in the sample to quench free radicals and other reactive species, that is their antioxidant capacity, may vary weight for weight, and secondly the complex mixture of molecules in natural materials may act in synergy leading to enhanced, or perhaps diminished, antioxidant capacity, compared to the sum of the individual antioxidants. It is therefore more revealing to use the total relative antioxidant capacity (RAC) of a unit weight of the sample. This is analogous to the specific activity of enzymes in which enzyme activity is expressed per weight of protein.

Factors affecting differences in antioxidant capacity of materials from different sources may be related to conditions of cultivation, storage and transportation prior to and during processing. In addition, the treatment of nutraceutical ingredients and finished products before, during and after manufacture of products may effect their total antioxidant capacity. For example, irradiation of ingredients as well as of the finished product will lead to loss of antioxidant activity because free radicals produced during irradiation may attack some or even all of the antioxidants present in the sample. And if all the antioxidants are attacked but free radical production continues then pro-oxidants, which are molecules when attacked by free radicals produce even more free radicals, may be generated, sometimes from antioxidants that have lost their own activity (4).

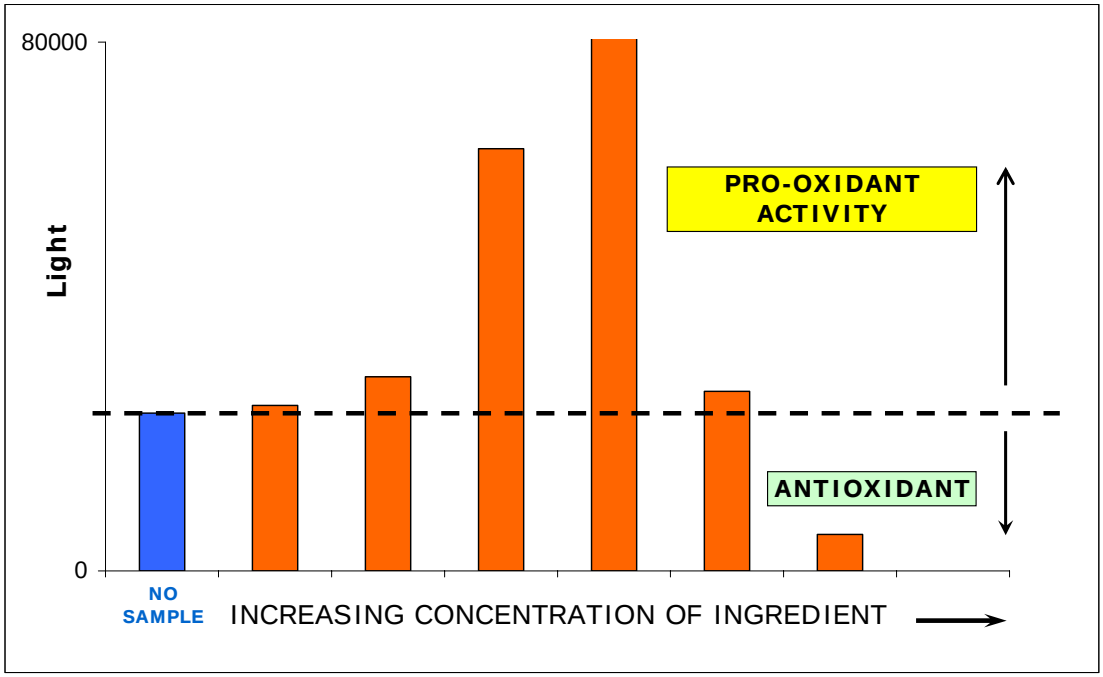
During food processing and manufacture of nutraceuticals free radicals can be produced from: grinding, compacting, exposure to UV light, drying, heating and especially solubilising materials by sonication. It is, therefore desirable to monitor the RAC of the ingredients and of the finished product throughout the various stages of manufacture and if necessary to change procedures to avoid losses. To obtain valid use-by dates the manufacturer should also test the finished product during storage. The cost benefit of doing this testing is obvious in relation to manufacturing efficiency but it also will give added confidence to the discerning customer and thus afford a competitive advantage to the product.

Methods of measurement

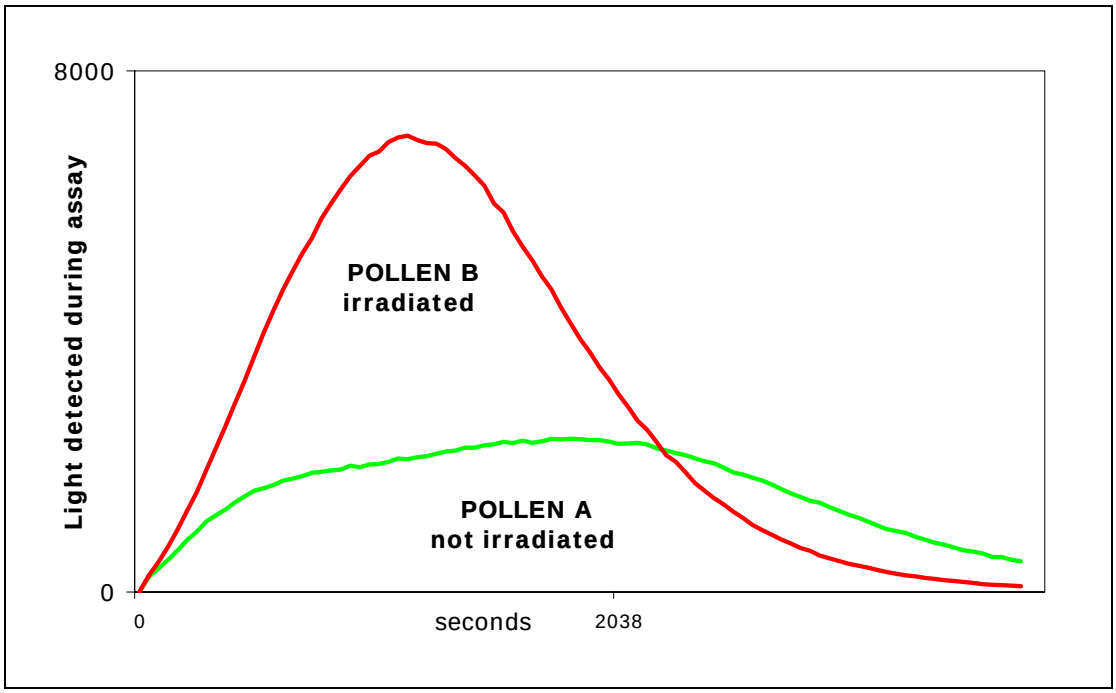
Many analytical methods exist for the measurement of concentration and purity of individual ingredients. Other methods aim to measure the functional capacity – that is the activity – of a material to neutralize free radicals. Functional methods are more closely related to the final biochemical function of the material in the body. In most of these methods the sample is exposed to free radicals and/or oxidants and the degree of quenching of the free radicals/oxidants is measured and sometimes quantified against a standard of known concentration. Such tests include: TEAC (Trolox-Equivalent Antioxidant Capacity) involving production of an coloured intermediate which is decolourised on exposure to antioxidants; ORAC (Oxygen Radical Absorbance Capacity) or TRAP (Total Peroxyl Radical Trapping) in which peroxyl radicals are generated and used in the presence of a fluorescent molecule which decays when attacked by the radicals; bleaching of Crocin. There are others. ORAC (5) is the best known and has been used extensively to score foods depending upon their antioxidant capacity. All these tests have their particular deficiencies: lack of reproducibility, especially between laboratories; time taken to complete; sensitivity. Moreover, none of these identifies pro-oxidants nor the formation of pro-oxidants as a function of concentration. Knowledge of the latter is fundamental to a consideration of formulations and dosages. However, recent tests presented below, are able to quantify both antioxidant and pro-oxidant capacity and thus provide a much needed tool for assessing possible changes in materials and products during manufacture as well as the effect of concentration in formulas.

ABEL: Analysis By Emitted Light Tests for Antioxidant Capacity and Free Radical Detection

There are five different assays, developed by Knight Scientific Limited (KSL) for quantifying antioxidant and pro-oxidant capacity. These assays are based on the use of a substance that emits light in the presence of free radicals and oxidants (6). In these tests samples containing unknown antioxidants are challenged with defined oxidants: superoxide (high concentration), superoxide (enzymatically produced), hydroxyl radical, peroxynitrite and hypochlorous acid, in the presence of the luminescent material. The resulting light is related to the antioxidant activity of the sample. Information derived from these tests can be converted into relative antioxidant capacity scores that enable comparisons to be readily made between different materials and batches. In addition, the assays can be used to assess antioxidant capacity at different concentrations and to identify those ingredients that do not follow typical dose responses but are pro-oxidant at some concentrations and antioxidant at others. Such unusual behaviour is known as hormesis in toxicology and is extremely important when deciding on recommended dosages.



An ingredient in a nutraceutical product under development exhibiting concentration dependent pro- and antioxidant activity (hormesis): Bars are peak light detected in an antioxidant assay with peroxynitrite and Pholasin.. NOTE: Pro-oxidant activity increases with increased light compared to the no sample control; antioxidant activity correlates to reduction in the amount of light detected.



Two identical samples of pollen challenged with peroxynitrite in the presence of Pholasin Sample B was irradiated. Note the greater the amount of light emitted the LOWER the antioxidant capacity

In a sixth assay, the test material is exposed to white blood cells, usually in a sample of diluted blood. In this cell activation assay the cells are stimulated to produce free radicals in the presence of the test material, This assay can reveal if the material is acting directly on the cell, effecting the free radical production by the cell, or simply quenching (or enhancing) the free radicals produced by the cell.

The luminescent material, which is unique to these tests is Pholasin, a protein extracted from cultivated specimens of the marine bioluminescent mollusc, Pholas dactylus, the common piddock .

These assays can be run on either microplate or tube luminometers. The results are highly reproducible with a cv of less than 3% within and between assays. Testing of samples can be carried out in house using commercial kits or sent to contract testing laboratories.

These tests have been used for QC of materials before, during and after processing and for QA of finished products. They have also been used to determine the total antioxidant capacity (TAC) of blood from people, horses, dogs, rodents, birds and badgers with the advantage that only a small amount (5µL of serum or plasma) is needed for a test. Such assays have been used to monitor changes in TAC following intervention studies with horses (7)

Manufactures and suppliers of products based on natural materials need to be able to satisfy themselves and their customers that the products they are selling can be matched precisely, batch to batch, for their efficiacy. ABEL assays can provide scientific data : • to demonstrate batch to batch uniformity on natural ingredients • to exclude materials that may have lost antioxidant capacity during irradiation and other treatments, • to assess synergistic effects when ingredients are mixed together in products, • to understand how activity changes with increasing or decreasing concentration and • to monitor changes that might occur during manufacture. With reliable and reproducible evidence derived from the robust testing of nutraceuticals, these products will be able to stand up to spurious arguments favouring the use of synthetic alternatives to naturally derived ingredients. Quality assurance of nutraceutical products on which to base statements regarding their potential benefits to the consumers is the way forward for the fastest growing sector in the food industry. .

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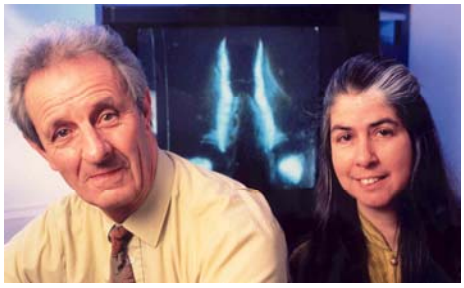

Drs. Robert and Jan Knight

Dr. Jan Knight gained a first class honours degree from the Open University in 1978 in Science. After a stint in industry she took her PhD at London University in the field of marine biochemistry, spending much of her time at the Marine Biological Association in Plymouth UK. Since 1984 she has been involved in inflammatory disease research, free radical research and measurement of the antioxidant capacity of people, animals and ingredients. In 1990 she and her husband Robert founded Knight Scientific Ltd. of which she is managing director.

Dr. Robert Knight has a first degree in chemistry and a PhD in biochemistry from St Thomas' Hospital Medical School, London (1957). He spent five years in Uganda at the East African Trypanosomiasis Research Organisation. His wide ranging research has included, biochemistry, chemistry, haematology, mircrocopy, palaeontology, parasitology, taxonomy. and toxicology