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Measurement of antioxidant activity

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A B S T R A C T

Antioxidants play an important role in food preservation by inhibiting oxidation processes and contributing to health promotion rendered by many dietary supplements, nutraceuticals and functional food ingredients. Antioxidant activity can be monitored by a variety of assays with different mechanisms, including hydrogen atom transfer (HAT), single electron transfer (ET), reducing power, and metal chelation, among others. Understanding the principle mechanisms, advantages and limitations of the measurement assays is important for proper selection of method(s) for valid evaluation of antioxidant potential in desired applications. This contribution provides a general and up-to-date overview of methods available for measuring antioxidant activity and the chemistry behind them.

1. Introduction: lipid oxidation and antioxidants

Lipid oxidation is a major cause of food quality deterioration, and has been a challenge for manufacturers and food scientists alike. Lipids are susceptible to oxidative processes in the presence of catalysts such as heat, light, enzymes, metals, metalloproteins and microorganisms, giving rise to the development of off-flavours in foods and loss of essential amino acids, fat-soluble vitamins and other bioactive molecules (Shahidi & Zhong, 2005). When occurring in the human body, it is a cause for oxidative stress and thought to exert destructive cellular effects associated with pathophysiology of a number of diseases and health conditions, including inflammation, atherosclerosis and ageing, among others (Dalton, Shertzer, & Puga, 1999; Davies, 2000; Floyd & Hensley, 2002; Kruidenier & Verspaget, 2012). Lipids in living organisms undergo oxidation reactions during normal aerobic metabolism (Beckman & Ames, 1998). Unsaturated fatty acids in membrane phospholipids and cholesterol, especially LDL-cholesterol, are generally the major reactants affected by such reactions, causing irreversible cellular and tissue damage.

Oxidation of lipids may be via autoxidation, photooxidation, thermal oxidation and enzymatic oxidation, most of which involve free radicals and/or other reactive species as the intermediate (Shahidi, 2000; Vercellotti, St. Angelo, & Spanier, 1992). Autoxidation, defined as the spontaneous reaction of atmospheric oxygen with lipids, is the most common process leading to oxidative deterioration/damage in food and biological systems (Shahidi & Zhong, 2005). The process can be accelerated at higher temperatures as experienced during deep fat frying, which is referred to as thermal oxidation, with increased formation of free fatty acids and polar matters, foaming, colour and viscosity (Perkins, 1992). Photooxidation involves excitation of photosensitizer and energy transfer to lipid molecules or oxygen. Enzymes such as lipoxygenases also catalyse oxidation of fatty acids and are usually inactivated

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by thermal processing of food. It has been widely accepted that lipid oxidation occurs via a free radical (e.g. lipid radicals, $R^•$; alkoxyl, $RO^•$; peroxyl, $ROO^•$; and hydroxyl, $^•OH$) chain reaction mechanism. The chain reaction proceeds through three stages of initiation, propagation, and termination that lead to a series of complex chemical changes (Shahidi & Zhong, 2005). More detailed pathways of lipid oxidation have been elucidated and explained elsewhere by Schaich (2005) and Shahidi and Zhong (2010). In general, lipid oxidation produces lipid hydroperoxides and conjugated dienes (or trienes) as primary oxidation products, which are unstable and further break down to a wide range of secondary oxidation products, including alcohols, aldehydes, ketones, hydrocarbons, volatile organic acids and epoxy compounds, among others, some of which give rise to a bad odour at very low threshold values and are responsible for the "off-flavour" notes of oxidatively deteriorated foods. A variety of polymeric products is also generated during the termination stage of the oxidation reaction as a result of radical–radical coupling or radical–radical disproportionation. The formation of polymers accounts for increased viscosity of highly oxidized vegetable oils after deep frying. Polymeric products produced from membrane phospholipid oxidation lead to reduced fluidity of the membranes, affecting membrane transport and cell signalling, as observed in Alzheimer's disease (Lyras, Cairns, Jenner, & Jenner, 1997).

Lipid oxidation has long been classified as the major deterioration process affecting both the nutritional and sensory quality of foods, especially lipid-based products (Yanishlieva & Marinova, 2001). It occurs during harvesting, processing and storage of foods, and causes chemical spoilage, resulting in rancidity and/or deterioration of the nutritional value, colour, flavour, texture and safety of the food products. In edible fats and oils such as butter, margarine, and salad/cooking oils, oxidative rancidity observed as the obvious manifestation of "off-flavour" development is the major cause of food wastage (Scott, 1997). Lipid oxidation is also responsible for quality deterioration of meat (Min & Ahn, 2005) and dairy products (Collomb & Spahni, 1996), fruits and vegetable crops. While lipid oxidation in food poses challenge to food quality preservation, oxidative stress *in vivo* renders destructive and lethal effects by oxidizing membrane lipids, cellular proteins, enzymes and DNA, thus disrupting the cells (Winrow, Winyard, Morris, & Blake, 1993). Owing to its detrimental effects on food quality and human health, concerns about lipid oxidation have been addressed in the food and feed as well as the health care industries, leading to intensive efforts to improve oxidative stability of oils and fats by different means.

Oxidation of fats/oils and lipid-containing products may be prevented by excluding the initiator and promoter elements during processing and storage. This explains why packaging of highly oxidation-susceptible products usually avoids heat and uses materials that are not metal or transparent, while under vacuum or inert gas. When used as nutraceuticals or pharmaceuticals, the highly unstable polyunsaturated lipid components are protected by encapsulation. The membrane of the capsule protects the core material from undesirable effects of light, moisture and oxygen, thus increasing the shelf-life of encapsulated materials (Shahidi & Han, 1993). Nanoencapsulation technique has attracted much attention as an effective and efficient delivery method for bioactive ingredients and drugs such as fish oils and omega-3 polyunsaturated fatty acid (PUFA) concentrates. In spite of the many advantages of nanoencapsulation, toxicity issues related to possible overdosing remains to be a major concern.

Among the many methods employed for controlling lipid oxidation, use of antioxidants is the most effective, convenient and economical means. Food manufacturers use antioxidants to stabilize food lipids and thus prevent quality deterioration of the products. Antioxidants are also used in health related areas for disease risk reduction and health promotion due to their ability to protect the body against oxidative damage. Antioxidants are substances that when present in food or in the body at very low concentrations, delay, control or prevent oxidative processes leading to food quality deterioration or initiation and propagation of degenerative diseases in the body. Antioxidants that fit in this definition include free radical scavengers, singlet oxygen quenchers, inactivators of peroxides and other reactive oxygen species (ROS), metal ion chelators, quenchers of secondary oxidation products and inhibitors of pro-oxidative enzymes, among others (Shahidi & Zhong, 2007). These substances exert their inhibitory effect against oxidation processes through different mechanisms and with varied activities. They are broadly classified by their mechanism of action as primary and secondary antioxidants. Primary antioxidants such as tocopherols and some phenolic compounds inhibit the chain reaction of oxidation by acting as hydrogen donors or free radical acceptors and generation of more stable radicals. The inhibition reaction is considered to be in competition with the propagation step of lipid oxidation and yields stable products that will not initiate new free radicals or bring about a rapid oxidation via a chain reaction (Nawar, 1996). Secondary antioxidants prevent or retard oxidation by suppressing the oxidation promoters, including metal ions, singlet oxygen, pro-oxidative enzymes and other oxidants. Reducing agents can reduce lipid peroxides and related oxidants through redox reactions and are also referred to as oxygen scavengers. Some secondary antioxidants such as ascorbic acid can regenerate primary antioxidants by replenishing hydrogen atom, thus inhibiting depletion of important primary antioxidants. Other secondary antioxidants promote decomposition of hydroperoxides into non-radical species, or absorb UV radiation thus protecting lipids from UV-induced photo-oxidation. An in-depth discussion of antioxidant mechanisms has already been provided elsewhere (Shahidi & Zhong, 2010).

Antioxidants may occur naturally in plants, animals and microorganisms or may be synthesized by chemical means. Higher plants and their constituents provide a rich source of natural antioxidants, such as tocopherols and polyphenols which are found abundantly in spices, herbs, fruits, vegetables, cereals, grains, seeds, teas and oils. Antioxidants from marine origin such as algae, fish/shellfish and marine bacteria, have also been considered (Amarowicz, Karamac, & Shahidi, 1999; Athukorala et al., 2003; Shahidi & Amarowicz, 1996). In addition, by-products from the food and agricultural industries have been explored for their potential use as antioxidants. For example, hulls, shells and skins of nuts and cereals, citrus peels and seeds, canola meal and fish viscera extracts have been found to possess antioxidant activity (Cumby, Zhong, Naczk, & Shahidi, 2008; Liyana-Pathirana, Dexter, & Shahidi, 2006; Shahidi, Alasalvar, & Liyana-Pathirana, 2007). Naturally occurring antioxidants can