Food Research International 74 (2015) 10–36

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Review

Antioxidants: Characterization, natural sources, extraction and analysis

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ARTICLE INFO

Article history:
Received 11 June 2014
Received in revised form 3 April 2015
Accepted 12 April 2015
Available online 20 April 2015

Keywords:
Antioxidant
Chemical structures
Health benefits
Extraction
Identification
Quantification

ABSTRACT

Recently many review papers regarding antioxidants from different sources and different extraction and quantification procedures have been published. However none of them has all the information regarding antioxidants (chemistry, sources, extraction and quantification). This article tries to take a different perspective on antioxidants for the new researcher involved in this field. Antioxidants from fruit, vegetables and beverages play an important role in human health, for example preventing cancer and cardiovascular diseases, and lowering the incidence of different diseases. In this paper the main classes of antioxidants are presented: vitamins, carotenoids and polyphenols. Recently, many analytical methodologies involving diverse instrumental techniques have been developed for the extraction, separation, identification and quantification of these compounds. Antioxidants have been quantified by different researchers using one or more of these methods: in vivo, in vitro, electrochemical, chemiluminescent, electron spin resonance, chromatography, capillary electrophoresis, nuclear magnetic resonance, near infrared spectroscopy and mass spectrometry methods.

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http://dx.doi.org/10.1016/j.foodres.2015.04.018
0963-9969/

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1. Introduction

Antioxidants found in food are a heterogeneous category of molecules (Tuberoso, Boban, Bifulco, Budimir, & Pirisi, 2013). Antioxidants are compounds or systems that can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. They can use several mechanisms: (i) scavenging species that initiate peroxidation, (ii) chelating metal ions so that they are unable to generate reactive species or decompose peroxides, (iii) quenching $^{*}O_2^-$ preventing formation of peroxides, (iv) breaking the auto-oxidative chain reaction, and/or (v) reducing localized O_2 concentrations (Asimi, Sahu, & Pal, 2013).

The anti-oxidative effectiveness of these compounds depends on their chemical characteristics and physical location within a food (proximity to membrane phospholipids, emulsion interfaces, or in the aqueous phase) (Watanabe et al., 2000). Antioxidants (e.g., flavonoids, phenolic acids, tannins, vitamin C, vitamin E) have diverse biological properties, such as anti-inflammatory, anti-carcinogenic and anti-atherosclerotic effects, reduce the incidence of coronary diseases and contribute to the maintenance of gut health by the modulation of the gut microbial balance (Bartoszek & Polak, 2012; Boffetta et al., 2010; Cardona, Andrés-Lacueva, Tulipani, Tinahones, & Queipo-Ortuño, 2013; Díaz et al., 2012; Fernández-Marín et al., 2012; Fu et al., 2011; Hamrouni-Sellami et al., 2013; Li & Beta, 2011; Machmudah et al., 2012; Ratnasooriya & Rupasinghe, 2012; Strati & Oreopoulou, 2011; Toro-Funes et al., 2012).

The aim of this study is to describe: the main classes of antioxidants existing in fruit, beverages, vegetables and herbs; the different procedures to determine total antioxidant activity; and the different extraction, separation and quantification procedures used to evaluate their presence in food. The article tries to take a different perspective on antioxidants for the new researcher involved in this field, collecting important knowledge related to this topic.

2. Classification of antioxidants

Antioxidants cover different classes of compounds which can interfere with oxidative cycles to inhibit or retard the oxidative damage of biomolecules. The major classes of compounds with antioxidant activity are: vitamins (vitamin C and vitamin E), carotenoids (carotenes and xanthophylls) and polyphenols (flavonoids, phenolic acids, lignans and stilbenes).

2.1. Vitamins

2.1.1. Vitamin C

Vitamin C (L-ascorbic acid; here after, 'ascorbic acid' and 'ascorbate' refer to 'L-(+)-ascorbic acid' and 'L-ascorbate' Fig. 1a) is unique among vitamins for several reasons. It is believed to be the most important hydrophilic antioxidant (Lykkesfeldt, 2000) being effective in scavenging superoxide radical anions, hydroxyl radicals, hydrogen peroxide, reactive nitrogen species and singlet oxygen. Vitamin C can acts as a reactive oxygen species (ROS) scavenger (>1000 mg/kg) and inhibits oxidation, however, at low levels, vitamin C (<100 mg/kg) can catalyze oxidation (in muscle tissue) (Ahn, Grün, & Mustapha, 2007).

Vitamin C has, at a structural level, 4 –OH groups which can donate hydrogen to an oxidizing system (Brewer, 2011). Resonance forms can also be written for the form of vitamin C that has lost one electron, making the radical semidehydroascorbate (SDA) much more stable, and thus much less reactive, than most other free radicals (Buettner, 1993).

The antioxidant mechanism of vitamin C was proposed by Bendich, Machlin, Scandurra, Burton, and Wayner (1986). At pH 7, the ascorbate anion (AH^-) is the predominant form present, due to the acidic nature of ascorbic acid (AH_2). This compound can undergo a reversible oxidation process and form dehydroascorbic acid (A), with ascorbyl radical formation ($A^{-\bullet}$). The ascorbyl radical is relatively unreactive and may react with other free radicals and the propagation of free radical reactions may be stopped (Eq. (1)):

$$2A^{-\cdot} + H^+ \rightarrow AH^- + A^{\cdot} \quad (1)$$

The reaction between the peroxyl radicals and ascorbate has been proposed as (Bendich et al., 1986):

$$ROO^{\cdot} + AH^- \rightarrow ROOH + A^{-\cdot} \quad (2)$$

$$A^{-\cdot} + O_2 \rightarrow A + O_2^{-\cdot} \quad (3)$$

$$O_2^{-\cdot} + ROO^{\cdot} + H^+ \rightarrow O_2 + ROOH \quad (4)$$

$$O_2^{-\cdot} + AH^- + H^+ \rightarrow H_2O_2 + A^{+\cdot} \quad (5)$$

$$A^{-\cdot} + ROO^{\cdot} + H^+ \rightarrow A + ROOH \quad (6)$$

In the case of in vivo cellular assays, ascorbate efficiency, is higher at low concentrations (one molecule of ascorbate can trap 2 molecules of peroxyl radical), while at high concentrations the efficiency decreases exponentially to 0 (Bendich et al., 1986). The superoxide anion (reaction 4) or the ascorbyl radical (reaction 6) reacts with a peroxyl radical at very low concentrations of ascorbate. At higher concentrations of ascorbate, reaction 4 propagation competes with the chain termination

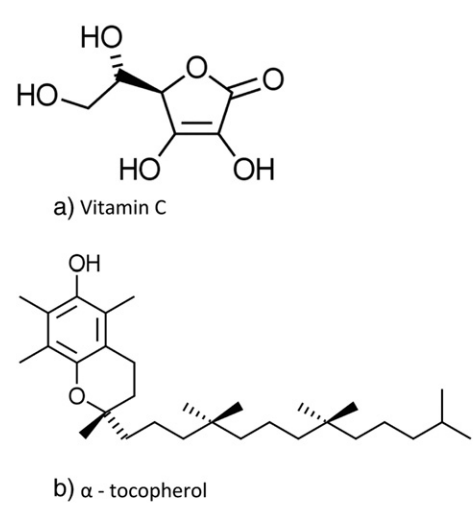

Fig. 1. a. Vitamin C. b. α-Tocopherol.