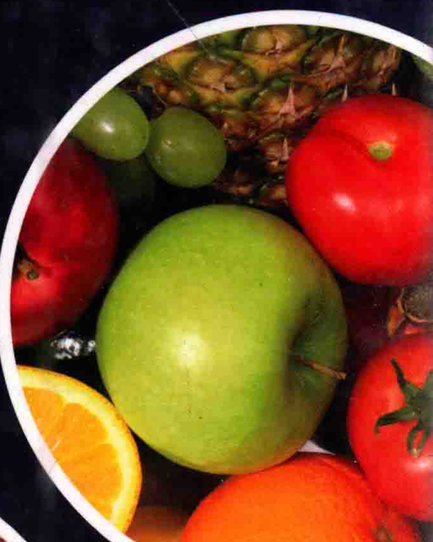
Analysis of Antioxidant-Rich Phytochemicals

EDITED BY • ZHIMIN XU • LUKE R. HOWARD



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A John Wiley & Sons, Ltd., Publication

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Registered office: John Wiley & Sons, Ltd, The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, UK

Editorial offices: 9600 Garsington Road, Oxford, OX4 2DQ, UK
The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, UK
2121 State Avenue, Ames, Iowa 50014-8300, USA

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Library of Congress Cataloging-in-Publication Data

Analysis of antioxidant-rich phytochemicals / edited by Zhimin Xu and Luke R. Howard.
p. cm.
Includes bibliographical references and index.
ISBN 978-0-8138-2391-1 (hard cover : alk. paper)
1. Antioxidants. 2. Phytochemicals–Analysis. I. Xu, Zhimin, 1964- II. Howard, Luke R.
QK898.A57A53 2012
613.2'86–dc23

2011035811

A catalogue record for this book is available from the British Library.

Wiley also publishes its books in a variety of electronic formats. Some content that appears in print may not be available in electronic books.

Set in 11.5/13.5pt Times New Roman by Thomson Digital, Noida, India
Printed and bound in Singapore by Markono Print Media Pte Ltd

1 2012

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Preface

Antioxidant-rich phytochemicals in plants and agricultural food products have recently become an attractive subject for food, biomedical and nutrition scientists, and food producers. Thousands of articles in this area are published each year and numerous related research projects are being carried out in institutes and companies. Compared to synthetic food antioxidants, the antioxidants in natural sources are generally recognized as safe for food applications. Also, most of the phytochemicals have been confirmed to have health-promoting functions in preventing various human epidemiological diseases, such as cardiovascular diseases, cancers, obesity, and diabetes. In addition to antioxidant function, many phytochemicals have been found to alter cell signalling pathways and gene expression, and thus have the ability to regulate numerous physiological functions involved in the pathogenesis of various chronic diseases.

Antioxidant-rich phytochemicals are micro-constituents in plants and agricultural food products. They differ from proteins, carbohydrates, and lipids, which are macro-nutrients that are abundant in plants and food products. The type and quantity of antioxidant-rich phytochemicals vary significantly from source to source. Different types of the antioxidant-rich phytochemicals may have different antioxidant and other biological activities and bioavailability. Although most phytochemicals have UV absorption, using traditional spectrophotometeric methods to quantify the antioxidants is not practical as they could be significantly masked or interfered with by many other compounds in the sources. Thus, the analysis methods for antioxidant-rich phytochemicals are more complicated and sophisticated than the methods used for macro-nutrient compounds.

Chromatography techniques with different detectors followed by skillful sample preparation are usually applied to quantify these antioxidants in natural sources. These techniques offer sensitive and specific analysis methods for most of the antioxidants. This is the first book that particularly covers and summarizes the details of sample preparation procedures and methods developed to identify and quantify various types of natural antioxidants in plants and food products. In the book, the principle of quantification methods for natural antioxidant-rich phytochemicals is introduced and current methods used in the determination of antioxidants in different sources are reviewed and summarized by experts in the field. As a handbook of analysis of natural antioxidant-rich phytochemicals, the book provides useful information for many researchers in this area to learn ideal analysis methods for the antioxidants they are examining. The book may also serve as a lecture resource for courses in food analysis, functional foods, and nutrition.

Zhimin Xu and Luke R. Howard

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Chapter 1

Important Antioxidant Phytochemicals in Agricultural Food Products

Zhimin Xu

Abstract

Antioxidant phytochemicals are secondary plant metabolites widely present in the plant kingdom. Most of the phytochemicals are phenolic derivatives with monohydric or polyhydric phenols. Numerous clinical studies have confirmed that antioxidant phytochemicals can prevent some cholesterol-related and oxidation-induced chronic diseases. The antioxidant properties and health benefits of phytochemicals in different agricultural food plants have been intensively studied in recent years. This chapter will discuss the phytochemicals in common fruits, vegetables, and grains and their potential to reduce the risk of epidemiological disease. As more and more consumers are becoming concerned about health functions of foods, the information of this chapter will be useful for scientists in the food, plant breeding and physiology, medicine, and epidemiology areas to understand and utilize natural antioxidant phytochemicals in health-promoting food and other products.

Keywords: antioxidant; phytochemical; phenolic; polyphenolic; antioxidation; plants

1.1 Introduction

Antioxidant phytochemicals generally possess one or more hydroxylated aromatic or phenolic rings, which contribute to their antioxidant

Analysis of Antioxidant-Rich Phytochemicals, First Edition.
Edited by Zhimin Xu and Luke R. Howard.

activity. They are widely distributed in plants and synthesized by shikimate and chorismate biosynthesis from the essential precursor L-phenylalanine (Haslam, 1993; Herrmann, 1995). The synthesis reaction involves a series of deamination, enzymatic conversion, and enzymatic hydroxylation reactions, and generates a variety of the phytochemicals with one or more phenolic rings (Herrmann, 1989). Based on the number and pattern of phenolic rings in the structure, antioxidant phytochemicals may be simple structures such as phenolic acids, which have only one phenolic ring, or complicated polyphenolics and tannins, which have two or more than two phenolic rings, respectively. The phenolic phytochemicals can also link with different moieties, such as sugars, long carbon chains, and phytosterols to form complex phenolic derivatives. Thousands of phenolic derivative phytochemicals have been found in the plant kingdom. The major physiological function of antioxidant phytochemicals in plants is to defend against oxidative and environmental stress, such as UV radiation, microbes, pathogens, parasites, etc. (Croteau et al., 2000). Most phytochemicals are also responsible for the colors of plants. For example, anthocyanins, one class of polyphenolics are purple, black, or red pigments. In edible plants such as fruits and vegetables, phytochemicals also contribute to attributes like astringency, bitterness, or a spicy taste (Lule and Xia, 2005).

Phenolic acids, derivatives of hydroxybenzoic and hydroxycinnamic acids, are divided into subgroups and may be found in many different types of agricultural products. Protocatechuic, caffeic, coumaric and chlorogenic acids are phenolic acids found in abundance in fruits and vegetables. Ferulic acid is a phenolic acid commonly found in grains, especially in grain bran. Polyphenolics are a group of flavonoids, which are divided into anthocyanins, isoflavones, flavones, flavonols, flavanols, and flavanones. Anthocyanins are present in high levels in berries, and isoflavones are abundant in beans. The flavonol quercetin is largely present in apples, while catechin, a flavanol, is found in teas and coffees. Grapefruits are rich in flavanones, such as naringenin. Tannins are a group of polymerized polyphenolics present in berries and red wines. Some tannins existing in fruit juice and wine have a molecular weight over 2000 daltons and are not water-soluble (Khanbabaee and van Ree, 2001). Also, some antioxidant phytochemicals in grain germ and bran, such as tocols and oryzanols, are lipid-soluble.

Antioxidant phytochemicals have recently become an attractive subject for scientists in many different research areas. The scope of research in phytochemicals is no longer limited to the functionality associated with plants and organoleptic properties, but has been expanded to include their functionality in human health, which is linked to various epidemiological diseases and micro-nutrition. Most phytochemicals in natural agricultural sources have been generally recognized as bioactive or health-promoting compounds, which play an important role in preventing cardiovascular diseases, cancers, obesity and diabetes, lowering blood cholesterol level, and reducing inflammatory action (Halliwell, 1996).

1.2 Lipid Oxidation and Antioxidant Property of Phenolic Derivative Phytochemicals

Antioxidants are substances that inhibit the generation and reduce the number of oxidation-initiating free radicals, which eventually helps to prevent or delay oxidation reactions, such as lipid auto-oxidation. Lipids, essential components in animal and human cell membranes, are very vulnerable substances that are readily oxidized when exposed to free radicals, light, oxygen, pro-oxidants, and high temperatures (Frankel, 1999). In food systems, lipid oxidation causes serious deterioration in food quality during the storage of lipid-containing foods (Decker and Xu, 1998). The oxidation of lipids produces undesirable rancid odors and oxidation products, and decreases the sensory and nutritional quality of food. The primary products of lipid oxidation are hydroperoxides, which are unstable and further decompose into various secondary compounds such as alkanes, alkenes, aldehydes, ketones, alcohols, esters, acids, and hydrocarbons. Also, some lipid oxidation products are harmful to a variety of mammalian cells. They can affect cell division and proliferation, resulting in cell inflammation, and increase the risk of developing chronic diseases (Berliner et al., 1995). Lipid auto-oxidation is a major type of lipid oxidation, in which lipids react with oxygen through a free radical mechanism (Asghar et al., 1988). Below are the steps of free radical lipid auto-oxidation (RH indicates a fatty acid; ROOH indicates a hydroperoxy fatty acid; $^{\bullet}$ denotes a free radical):

Radical initiation:

$$RH + O_2 \rightarrow R^{\bullet} + {}^{\bullet}OH$$

Radical propagation:

$$R^{\bullet} + O_2 \rightarrow ROO^{\bullet}$$
$$ROO^{\bullet} + RH \rightarrow R^{\bullet} + ROOH$$
$$ROOH \rightarrow RO^{\bullet} + HO^{\bullet}$$
$$RO^{\bullet} + RH \rightarrow R^{\bullet} + ROH \rightarrow \text{Oxidation products}$$

Radical termination:

$$R^{\bullet} + R^{\bullet} \rightarrow RR$$
$$R^{\bullet} + ROO^{\bullet} \rightarrow ROOR$$
$$ROO^{\bullet} + ROO \rightarrow ROOR + O_2$$

Lipid oxidation is not restricted to the fatty acids and triglycerides of foods. Another important food lipid, cholesterol, can also be oxidized by free radicals (Maerker, 1987; Xu et al., 2001). Cholesterol is present at significant levels in food from animal sources, such as egg yolk, meat, and milk products. It is an essential molecule for humans as a component of cell membranes and as the precursor of steroid hormones and bile acids. Most cholesterol in the human bloodstream is carried by low-density lipoprotein (LDL) particles. High levels of LDL cholesterol, known as "bad cholesterol," is directly associated with various cardiovascular diseases. Cholesterol oxidation products from foods, or those produced by human metabolism, are toxic and harmful to blood vessel tissue cells (Kumar and Singhal, 1991; Lyons and Brown, 1999). They can trigger the development of a progressive thickening of the artery wall due to the accumulation of the oxidation products in LDL particles. Eventually, this can lead to the formation of plaque, which results in cardiovascular diseases and the formation of certain types of cancers (Morel and Lin, 1996; Wilson et al., 1997). Lipid oxidation reactions that occur in the cell membrane may also lead to various types of cancer, as these reactions result in damage to the membrane due to mutations that arise during the cell-duplication process (Jadhav et al., 1996).

Phenolic antioxidants can quench and terminate the free radicals without being transformed to new free radicals in the system.

The hydroxyl groups on the phenolic ring contribute to the antioxidant function by donating electrons to eliminate free radicals in a system. As the phenolic radical intermediates are relatively stable due to resonance occurring on the phenolic ring, they do not initiate a new free radical chain reaction. Furthermore, the phenolic radical intermediates can react with other free radicals to terminate the chain reaction. Phenolics may also suppress reactive oxygen and nitrogen species formation by deactivating related enzymes and chelating free radical-producing metal ions. The number of free hydroxyl groups on phenolic rings is correlated to the antioxidant activity of a phenolic compound (Cotelle, 2001). Hydroxycinnamic acid derivatives were found to have higher antioxidant activity than their corresponding hydroxybenzoic acid derivatives because the –CH=CH-COOH linked to the phenyl ring may enhance the stability of the resonance (Rice-Evans et al., 1996). The number and position of hydroxyl groups in phenolic rings are also important to the antioxidant activity of flavonoids (Bors and Michel, 2002).

Unlike natural antioxidant phytochemicals, which are accumulated and excreted in biological systems, there is a group of antioxidants that are artificially synthesized. The most common synthetic antioxidants used in foods are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butylhydroquinone (TBHQ), and propyl gallate (PG). They are all phenolic derivatives and have either a mono- or polyhydroxyl group on the phenolic ring. The use of synthetic antioxidants is restricted because of food safety concerns, which are increasing around the world. Even though only a small amount of these artificial antioxidants are used, they are still a concern owing to of potential harmful health problems from long-term consumption (Kotsonis et al., 2001). They could be the promoting agents that target liver, lung, and stomach tissues to alter their gene expression (Pitot and Dragon, 2001). However, the antioxidant phytochemicals from agricultural products are usually considered to be GRAS (generally recognized as safe). With this advantage, more and more consumers and food developers prefer using phytochemicals as natural antioxidants to replace synthetic antioxidants in food products. Foods labeled "all natural" or "no artificial" ingredients are becoming common in the markets. Furthermore, the benefits of reducing the risk of chronic diseases by a higher daily consumption of fruits, vegetables and grains have been confirmed in a number of studies. Many studies have suggested that antioxidant phytochemicals in these agricultural food plants play an important role in preventing chronic diseases (Block et al., 1992; Boyer and Liu, 2004; Delgado-Vargas and Paredes-Lopez, 2003; Harborne and Williams, 2000; Ness and Powles, 1997; Steinmetz and Potter, 1996).

1.3 Antioxidant Phytochemicals in Fruits

1.3.1 Berries

The most important antioxidant phytochemicals in berries are anthocyanins, a group of polyphenolics responsible for the black, purple, or red colors of these fruits. The profiles of anthocyanins and health benefits of different berry varieties – such as blackberries, raspberries, blueberries, cranberries, bilberries, and strawberries – have been studied extensively (Seeram, 2009). Compared to other berries, bilberries have more than 15 different types of anthocyanins (Lätti et al., 2008; Yue and Xu, 2008), and the content and composition of anthocyanins in berries is largely dependent on the growth environment. There is large variation of total anthocyanin content in bilberries harvested in different geographic areas, ranging from 19.3 to 38.7 mg/g dry weight (Lätti et al., 2008). Delphinidin and cyanidin sugar-conjugated derivatives dominated in those bilberry samples. Also, the total anthocyanin content in the concentrated bilberry extracts could be up to 24% (Zhang et al., 2004). Besides anthocyanins, berries also have significant quantities of flavan-3-ols, hydroxybenzoic and hydroxycinnamic acid derivatives, condensed and hydrolyzable tannins, and other antioxidant phytochemicals (Howard and Hager, 2007). These compounds and anthocyanins directly contribute to the antioxidant capacity of berries. The order of antioxidant activity from high to low of different berries using a LDL oxidation model was blackberries, raspberries, blueberries, and strawberries (Heinonen et al., 1998). This study also suggested that the bioavailability and bioactivity of different anthocyanins was variable. The health function of anthocyanins in preventing obesity and diabetes was also reported. Mice that were fed cyanidin-3-O-glucoside in their diet had significantly lower body weight gain typically induced by the high-fat diet, and white and brown adipose tissue weights were also significantly lower after 12 weeks (Tsuda et al., 2003). In another study, mice fed a high-fat (35%) diet plus purified anthocyanins from blueberries also had lower body weight gains and less body fat than the