Anthraquinones
Mireille Fouillaud, Yanis Caro, Mekala Venkatachalam, Isabelle Grondin,
Laurent Dufossé

To cite this version:

HAL Id: hal-01657104
https://hal.univ-reunion.fr/hal-01657104
Submitted on 6 Dec 2017

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
Anthraquinones

Mireille Fouillaud, Yanis Caro, Mekala Venkatachalam, Isabelle Grondin, and Laurent Dufossé

CONTENTS

9.1 Introduction

9.2 Anthraquinones’ Main Structures
  9.2.1 Emödin- and Alizarin-Type Pigments

9.3 Anthraquinones Naturally Occurring in Foods
  9.3.1 Anthraquinones in Edible Plants
    9.3.1.1 Rheum sp. (Polygonaceae)
    9.3.1.2 Aloe spp. (Liliaceae or Xanthorrhoeaceae)
    9.3.1.3 Morinda sp. (Rubiaceae)
    9.3.1.4 Cassia sp. (Fabaceae)
    9.3.1.5 Other Edible Vegetables
  9.3.2 Microbial Consortia Producing Anthraquinones, Empirically Used in Asian Productions
    9.3.2.1 Fuzhuan Brick Tea
    9.3.2.2 Katsuobushi (Karebushi)

9.4 Anthraquinones Used as Colorants
  9.4.1 Colors in Foods
  9.4.2 Anthraquinones and Derivatives
  9.4.3 Legislation and Use
    9.4.3.1 European Standards
    9.4.3.2 American Rules
    9.4.3.3 In Asia
  9.4.4 Colored Anthraquinones from Plants
    9.4.4.1 Madder Root (Rubia tinctorum Linn., Rubiaceae)
    9.4.4.2 Other Plants
  9.4.5 Colored Anthraquinone from Insects: Carminic Acid
  9.4.6 Colored Anthraquinones from Microbes: Arpink Red®, Natural Red™
    9.4.6.1 Arpink Red®, Natural Red™

9.5 Anthraquinones and Food Processing
  9.5.1 Stability in Model Systems
  9.5.2 Stability in Food Processing

9.6 Dr. Jekyll and Mr. Hyde? Biological Effects of Anthraquinones
  9.6.1 Benefits
    9.6.1.1 Anti-Tumor
    9.6.1.2 Antimicrobial, Antiviral, Antiparasitic
Phenolics are of outstanding importance in many foods consumed by humans. Among the most popular foods containing phenolics are wine, olive oil, coffee, and tea. Among the phenolics, anthraquinones are less known, as well as the foods containing this class of molecules, and their properties.

This chapter describes anthraquinones, their structure, and their occurrence in foods such as plants, fermented products, insects, and so on. Uses of anthraquinones are closely linked to their properties such as colorant, antioxidant, antimicrobial, and so on, sometimes only relying on an empirical knowledge.

As anthraquinone structure is based on an anthracenedione chemical backbone, they have often been associated with undesirable properties. This Dr. Jekyll and Mr. Hyde aspect is therefore presented in this chapter.

9.1 INTRODUCTION

Phenolics are of outstanding importance in many foods consumed by humans. Among the most popular foods containing phenolics are wine, olive oil, coffee, and tea. Among the phenolics, anthraquinones are less known, as well as the foods containing this class of molecules, and their properties.

This chapter describes anthraquinones, their structure, and their occurrence in foods such as plants, fermented products, insects, and so on. Uses of anthraquinones are closely linked to their properties such as colorant, antioxidant, antimicrobial, and so on, sometimes only relying on an empirical knowledge.

As anthraquinone structure is based on an anthracenedione chemical backbone, they have often been associated with undesirable properties. This Dr. Jekyll and Mr. Hyde aspect is therefore presented in this chapter.

9.2 ANTHRAQUINONES’ MAIN STRUCTURES

Anthraquinones, also called anthracenediones or dioxoanthracenes, are important members of the quinone family, and constitute a large structural variety of compounds among the polyketide group. Anthraquinones are structurally built from an anthracene ring with a keto group on position 9, 10 as basic core and different functional groups such as -OH, -CH₃, -OCH₃, -CH₂OH, -CHO, -COOH, and so on may substitute at various positions (Figure 9.1). Anthraquinones and their derivatives, produced as secondary metabolites in plants, lichens, insects, and higher filamentous fungi, occur either in a free form or as glycosides. These glycosides are formed when one or more sugar molecules, mostly glucose or rhamnose, are bound to the aglycone by an O-glycoside linkage to a hydroxyl group. At times, other complexes linked by C- or O- in the side chain can also be synthesized (Gessler, Egorova, and Belozerskaya 2013, Caro et al. 2012). The electronic absorption spectra is a characteristic feature of the parent compound 9, 10-anthraquinone and its dihydroxy- and diaminoderivatives, which permit understanding of the effects of the hydrogen bond, solvent polarity, and nature of substituents on the spectral shift (Shahid Khan 2012). These detailed studies are of great importance owing to the wide-ranging applications of anthraquinones in many fields, especially in the fields of dyeing. About
700 natural anthraquinoid pigments have already been identified from insects, plants, or microbes (Caro et al. 2012, Gessler, Egorova, and Belozerskaya 2013, Fouillaud et al. 2016). They are moreover considered “reactive dyes” as they can form covalent bonds, that is, with food components. Hydroxyanthraquinone derivatives can also easily form coordination complexes with several cations as metals. Opposite to direct dyes, their chemical structures are often much simpler, their absorption spectra show narrower absorption bands, and the dyeings are brighter (Hunger 2003).

9.2.1 Emodin- and Alizarin-Type Pigments

Based on the biosynthetic pathways, the pigment type is classified as an emodin or alizarin type. The emodin (6-methyl-1,3,8-trihydroxyanthraquinone)-type of anthraquinones shows a substitution on both aromatic rings and has a structure of 1,8 dihydroxyanthraquinone. Acetate-malonate pathway leads to the production of this type of anthraquinone pigments and contains basic aglycones such as emodin, aloe-emodin, physcion, and chrysophanol. Whereas the alizarin-type anthraquinones have one benzene ring unsubstituted with at least one hydroxyl group in position R1, and are typically synthesized by the shikimate-ο-succinylbenzoate pathway (Sajc et al. 1999, Caro et al. 2012).

9.3 ANTHRAQUINONES NATURALLY OCCURRING IN FOODS

9.3.1 Anthraquinones in Edible Plants

In higher plants, anthraquinone derivatives are found in a wide range of species (Caro et al. 2012). Even if the distribution of these compounds in edible plants and vegetables is less extensive than that of other phenolic molecules (e.g., flavonoids), it cannot be discounted.

A number of anthraquinones derivatives found in higher plants, especially in Rheum, Rumex, Rhamnus, Aloe, and Cassia species, are excellent examples of acetate-derived structures formed through the (acetate-malonate)-polyketide pathway. These plants’ anthraquinones show substitutions on both aromatic rings and have at least two hydroxyl groups in both the R1 and R8 positions. In contrast, in plants from the Rubiaceae family (e.g., Morinda, Rubia, and Galium species), the most common naturally occurring anthraquinones, such as alizarin, are synthesized via the chorismate/ο-succinylbenzoic acid pathway (Caro et al. 2012). In plants, anthraquinones are not only present under

For ages, the plants containing anthraquinones have been mainly exploited like purgative drugs and consumed as such. Emodin, physcion, chrysophanol, aloe-emodin, or rhein form the basis of a range of natural anthraquinones’ derivatives molecules found in purgative drugs of plant origin. The plant purgative drugs as senna (obtained from leaves and fruits of *Cassia angustifolia* and *C. senna*), cascara (obtained from bark extracts of *Rhamnus purshianus*), and frangula (obtained from bark extracts of *Rhamnus frangula*) are thus only suitable for medicinal and pharmaceutical uses. The free forms have little therapeutic activity in purgative drugs. They need to be under the form of water-soluble glycosides such as anthraquinone O- and C-glycosides, or dianthrone O-glycosides, in order to exert their action.

### 9.3.1.1 *Rheum* sp. (*Polygonaceae*)

The edible stem of the common rhubarb, that is, *Rheum rhaponticum* (also known as garden (English) rhubarb), is cultivated in various regions of Europe for culinary purposes (Figure 9.2).

In contrast, anthraquinone glycosides such as emodin-1-O-glycoside, chrysophanol-1-O-glycoside, emodin-8-O-glycoside, aloe-emodin-8-O-glycoside, rhein-8-O-glycoside, and chrysophanol-8-O-glycoside are a series of major active anthraquinoid compounds found in dried rhizome and root of some rhubarb species (*Rheum palmatum*, *R. officinale*, *R. tanguticum*, and *R. australe* or *R. emodi* wall [e.g., Meisson. also known as Himalayan rhubarb]). The aglycone structures, especially rhein, emodin, and chrysophanol, are also present in these “medicinal” rhubarb species.

The dianthrone derivatives, such as emodin dianthrone, physcion dianthrone, and sennosides A and B (i.e., dimers formed by oxidative coupling of two single anthraquinones), have also been characterized in these plants, especially in the roots (Teuscher and Lindequist 1994, Qhotsokoane-Lusunzi and Karuso 2001, Nunez Montoya, Agnese, and Cabrera 2006, Huang et al. 2007, Xiong et al. 2011).

Among these rhubarb species, *R. palmatum* extracts contained the highest amount of anthraquinones, for example, 34.0 milligrams/gram of dry material (Kosikowska, Smolarz, and Malm 2010).

---

**FIGURE 9.2** (a) *Rheum rhaponticum* leaves; (b) *Rheum rhaponticum* stems cooked in sugar syrup for pastry; (c) *Rheum tanguticum* rhizoma, common name Rhei Rhizoma in traditional Chinese medicine; (d) *Rheum palmatum*. 
Anyway, the variations in the anthraquinones’ glycoside content are also due to the different altitudes where the plants are grown (Li, Sun, and Feng 2010, Wang et al. 2013).

9.3.1.2 Aloe spp. (Liliaceae or Xanthorrhoeaceae)

Aloe, which consists of dried juice from the leaves of various Aloe species from the Liliaceae family (i.e., A. ferox [Cape aloes], A. barbadensis [Curacao aloes], and A. perryi [Socotrine aloes]) contains from 10 to 30 percent anthracene derivatives. Aloe dried juice obtained from the leaves of Aloe vera (= Aloe barbadensis Miller), is largely used in food and beverages for the aromatic and bitter taste. The leaf of the Aloe vera plant consists of two main parts: An inner central leaf pulp that produces and stores Aloe vera gel, and an outer leaf pulp that produces and transports Aloe vera latex. According to the International Aloe Scientific Council, the Aloe leaf can be processed into two types of juices for commercial use: Inner leaf gel juice and decolorized whole leaf juice. Inner leaf gel juice is only produced from the gelatinous fillet of the leaf. Decolorized whole-leaf juice is produced by grinding the leaves. The grinding is followed by the treatment of the extracted juice with activated charcoal to remove aloe “latex.” Approximately 80 phenolic anthraquinone’s derivatives are produced by the pericyclic cells, located just below the epidermis. They can be found in some Aloe vera preparations. The main anthraquinone derivative of Aloe’s latex is aloin. This is a mixture of two diastereomers, termed aloin A and aloin B (Sehgal, Winters, Scott, David, et al. 2013; see Figure 9.3).

Aloin A, also called barbaloin, is the major C-glycoside anthraquinone in Aloe’s latex. When oxidized, it yields the free aglycone aloe-emodin. Aloinosides A and B, and the aglycone anthraquinone chrysophanol are also present in some Aloe varieties (Caro et al. 2012). Because of some adverse pharmacological effects of aloe constituents on consumers, the European Economic Community (EEC) listed aloin as a marker of aloe occurrence in food, and limited the amount of aloin to levels of 0.1 ppm in foods and beverages, and 50 ppm in alcoholic beverages (European Community Directive 88/388; EEC 1988). In contrast, the fresh mucilaginous gel in the leaves obtained from Aloe vera does not contain high level of anthraquinone derivatives. The fresh mucilaginous gel from A. barbadensis has been widely used for ages as a raw material or additive for health drinks, health foods, and health supplements. Recent studies indicated that commercial stabilized Aloe gel consumed as a beverage was not genotoxic or toxic in vivo (Sehgal, Winters, Scott, David, et al. 2013; see Figure 9.3).

![Aloe vera leaves](image1)

**FIGURE 9.3** (a) Aloe vera leaves; (b) main anthraquinones found in Aloe vera: Aloin A, aloin B, and the free aglycone form aloe-emodin.
Concerning *Aloe vera* preparations, the United States Food and Drug Administration (USFDA) then specified that anthraquinones’ levels should be kept below 50 ppm, and Cosmetic Ingredient Review (CIR) concluded that a concentration of anthraquinones below 50 ppm in a product is adequately safe (CIR 2007). In 0.5% *Aloe vera* solution for oral consumption, aloin should not exceed 10 ppm. EEC foodstuff regulation allows a maximum of 0.1 ppm aloin to be used for flavoring purposes in food and drinks (Müller et al. 1996).

### 9.3.1.3 *Morinda* sp. (Rubiaceae)

Root of Indian mulberry (*Morinda citrifolia* L., Rubiaceae) commonly known as noni, also potentially contains natural anthraquinone derivatives such as damnacanthal, morindone, rubiadin, and rubiadin-1-methyl ether (Deng et al. 2009, Bussmann et al. 2013) (Figure 9.4). Noni products (fermented or unfermented juices or powders) have been used in traditional medicine and also as nutritional supplement in foodstuffs. Noni’s fruit puree (from which seeds had been removed), as well as food products derived from the puree, did not contain any detectable amount of anthraquinone derivatives (Bussmann et al. 2013). However, noni products that did contain seeds or leaf material did contain significant amounts of anthraquinone derivatives. To alleviate safety concerns for food uses, noni products should be derived only from fully ripe noni fruits. Therefore, any seed material needs to be removed during the production process (Bussmann et al. 2013).

### 9.3.1.4 *Cassia* sp. (Fabaceae)

Cassia gum, which comes from the purified flour from the endosperm of the seeds of *Cassia tora* and *Cassia obtusifolia*, is an authorized food additive (CAS Registry Number 11078-30-1) (Figure 9.5). It is mainly used as a thickener, emulsifier, foam stabilizer, moisture retention agent, or texturizing agent in diverse processed foods (cheese, frozen dairy desserts and mixes, meat products, and poultry products).

It has recently been evaluated according to its anthraquinone content: Rhein, emodin, aloe-emodin, and physcion where identified (Kim et al. 2004). Maximum use levels

---

**FIGURE 9.4** Indian mulberry (*Morinda citrifolia* L. plant and root’s powder) and the main anthraquinones present in its roots.
for cassia gum ranged from 2.5 grams/kilogram food in frozen desserts and 3 grams/kilogram food in cheeses to 3.5 grams/kilogram food in meat and poultry products. The concentration of anthraquinones in Cassia gum obtained from an isopropanol extraction purification step was below the 0.5 milligrams/kilogram detection limit. Moreover, traditional Cassia gum, containing approximately 70 milligrams/kilogram of total anthraquinones, was not mutagenic or clastogenic in mammalian cells.

Nevertheless, in a submission to the European Commission, use levels for Cassia gum only up to 2.5 grams/kilogram food were considered, with a maximum of 1.5 grams/kilogram food for processed meat and poultry products (EFSA 2006).

Cassia fistula Linn. ripe pods and leaves have been used for a long time in Thai traditional medicine (locally called “Khun”). They have been used as a laxative drug by boiling with water. Ripe pods and leaves of Cassia species contain several anthraquinones both in aglycone and glycoside forms such as rhein, aloe-emodin, chrysophanic acid, and sennosides (Dave and Ledwani 2012). The content of total anthraquinone glycosides in the dried leaves of C. fistula was evaluated at 0.36 percent w/w (average value). The laxative activity depends on the amount of total anthraquinone glycosides for which the Standard of ASEAN Herbal Medicine recommended should not be less than 0.5 percent of dried leaf raw materials. In the European Pharmacopoeia, the percentage of hydroxyanthracene glycosides in C. angustifolia dried leaves recommended should not be less than 2.5 percent. The recommended dose of hydroxyanthracene glycosides in the Senna leaf extract is 15–30 milligrams (Heilpflanzen-Welt 1993a,b, EMEA 2006). Thus, the dose of C. fistula decoction leaf extract equivalents to the dose of senna leaf extract should be 1–2 grams while the dose of the dried leaves should be 4–9 grams (Sakulpanich and Gritsanapan 2009).

9.3.1.5 Other Edible Vegetables
Anthraquinone derivatives can also be found at lower amounts in other types of vegetables and herbs. For example, a study has screened a variety of vegetables (cabbage lettuce, beans, and peas), herbs and herbal-flavored liquors for their content in the “free” (aglycone) anthraquinones emodin, chrysophanol, and physcion. The vegetables showed a large batch-to-batch variability, from 0.04 to 3.6, 5.9 and 36 milligrams total anthraquinones per kilogram.
fresh weight in peas, cabbage lettuce, and beans, respectively (Mueller et al. 1999). Physcion predominated in all vegetables. In herbs, grape vine leaves, couch grass root, and plantain herb, anthraquinones’ contents ranged below 1 milligrams/kilogram (dry weight).

9.3.2 Microbial Consortia Producing Anthraquinones, Empirically Used in Asian Productions

Asia is often a precursor in using natural substances from microbes, based on empirical evidences. Well-known examples are alcoholic beverages made from rice (Japanese sake or Chinese huangjiu), red soya bean cheese, anka (red rice), processed meat (sausage, ham), fish paste, and so on (Dufossé 2006). Because of the renewed interest in natural food components in relation with health, anthraquinones have recently been carefully studied in some ancient processed foods.

9.3.2.1 Fuzhuan Brick Tea

Fuzhuan brick tea (*Camelia sinensis* var. *sinensis*), a traditional fermented Chinese drink, has been demonstrated to contain a mixed microscopic fungal population producing anthraquinones (mainly emodin and physcion; Figure 9.6). The strains are involved in the red color, the flavor, and certainly also in the health benefits, showing antidysenteric effects, anti-food born spoilage, and anti-pathogenic microorganisms (Anke et al. 1980, Anke, Kolthoum, and Laatsch 1980, Mo, Zhu, and Chen 2008, Mo et al. 2008, Ling et al. 2010, Singh et al. 2005). The manufacturing process implies several steps of tea leaves’ treatments (mixing, grinding, steaming, cooling, etc.) followed by a solid-state fermentation step (15–17 days) (Mo, Zhu, and Chen 2008, Xu et al. 2011). The main

![Image](image_url)

**FIGURE 9.6** (a) Fuzhuan brick tea (fermented leaves of *Camelia sinensis* var. *sinensis*), (b) *Eurotium cristatum* on agar nutritive medium: The main fungus involved in the fermentation, (c) emodin and physcion structures: Two main anthraquinones produced by the fungi during the fermentation of the tea leaves.
microorganisms involved are *Debaromyces*, *Aspergillus*, *Verticillium* and *Eurotium* spp. Several species of *Eurotium* were identified from the fermented material (10^5 CFU/g dry weight of readymade Fuzhuan brick tea), but *Eurotium* sp. FZ (*Eurotium cristatum*) was the predominant strain characterized during the fermentation (Ge et al. 2016, Mo, Zhu, and Chen 2008, Xu et al. 2011, Qi and Sun 1990).

Further studies were conducted, aiming at standardizing Fuzhuan tea industrial production, through identifying and optimizing the synthesis of antioxidants and antimicrobial substances during the fermentation (Xu et al. 2011, Huang et al. 2010, Liu et al. 2010, Abe et al. 2008, Cao, Zhao, and Liu 1998). These research works stated that emodin was present in all dark teas samples but that physcion was only detectable in the teas fermented by *E. cristatum*. As they found that *Beauveria* sp. (an entomogenous fungus occurring in the stored brick tea) was a part of the fungal community, probably acting as a protectant against insects, they assessed that a microbial consortia should be used as starter cultures to improve the quality of Fuzhuan tea fermentations.

**9.3.2.2 Katsuobushi (Karebushi)**

The Asian empirical knowledge about the involvement of non-mycotoxigenic strains of *Eurotium* (*E. rubrum*, *E. repens*, and *E. herbariorum*) in the production of natural anthraquinoid compounds in foods is illustrated through the fermentation of fishes. These fungi are already extensively used as starter cultures in Japanese manufactures of katsuobushi (or karebushi), that is, fermented slices from bonito (*Katsuwonus pelamis*) (Dimici and Wada 1994; Figure 9.7). These fungi were demonstrated to produce

![figure](image_url)

**FIGURE 9.7** Anthraquinones in fermented *Katsuwonus pelamis*: (a) slices of katsuobushi (karebushi), (b) microscopic structures of *Eurotium* sp. (conidiophores colored with lactophenol blue) involved in anthraquinones production during the fermentation, (c) two of the main anthraquinones produced by *Eurotium herbariorum* in katsuobushi: Asperflavin and physcion-10,10'-bianthrone.
effective antioxidant extrolites, which participate in the suppression of lipid oxidation in fermented fish and therefore in the long shelf life. Their extrolites also participate in giving a deep red color to the final product (Manabe 2001, Pitt and Hocking 2009). Among them physcion, physcion-10,10’-bianthrone, catenarin, questin, asperflavin, and questinol were clearly identified (Miyake et al. 2014).

Another fungal species \textit{A. glaucus}, already known to produce various anthraquinones such as 10,10’-dimer of emodin and physcion, catenarin, cynodontin, emodin, erythroaglaucin, helminthosporin, physcion, questin, rubrocristin, tritisporin, variecolorquinone A (Fouillaud et al. 2016), is nominatively involved in katsuobushi fermentation (Nout and Aidoo 2010).

Obviously, a panel of secondary metabolites may occur in an uncontrolled manner, in a large majority of traditional indigenous fermented foods, as they naturally contain anthraquinone-producing microbial strains. Therefore, this is still a challenge to provide a warranty for the stability of the composition, the quality, and food safety.

### 9.4 ANTHRAQUINONES USED AS COLORANTS

#### 9.4.1 Colors in Foods

We have known for a long time that colors are key factors for human and animal behaviors. Their attractive or repulsive effects are carefully studied as they highly influence the consumer’s attitude. The “buying act,” especially in foods, is undoubtedly based on the perception the consumer obtains from the color, as color and flavor are closely connected (Garber, Hyatt, and Starr 2000). In industrial fields, colorants are commonly used to enhance the product’s natural color when its components are unable to provide a sufficient or attractive hue; they can also standardize the color and the appearance of products. Sometimes they are applied to restore the color that has been lost during processing. Until the middle of the nineteenth century, dyeing molecules used in cosmetics, drugs, and foods were of natural origin (mainly plants, animals, minerals). The situation rapidly changed when the first synthetic dyestuff was discovered (1856). The easier mass production conditions and the cheaper costs of chemical processes have made them the main sources of dyes in most industries (Hunger 2003). Nowadays about 7000 synthetic dyestuffs are known but consequently due to health risks, especially towards children (allergies, hyperactivity, risks on sexual development; Blendford 1995, Hunger and Sewekow 2003, Kobylewski 2010, FSA 2011), many regulations appeared in industrialized countries to regulate and control the introduction and the use (dose) of dyes in daily-use products. A quite recent awareness therefore led consumers to prefer foodstuffs, or more generally products, containing natural colorants and additives. Even if “natural” does not mean harmless, this undoubtedly applies a strong pressure on the market.

#### 9.4.2 Anthraquinones and Derivatives

Anthraquinoid molecules decline a wide range of nuances in shades of brown, purple, red, orange, and yellow. Their structures are relatively stable and they demonstrate a superior brightness compared to azopigments (Caro et al. 2012). In food industries, only few anthraquinones are already manufactured and marketed, either coming from insects
(carminic acid), plants (alizarin from European madder roots), or microbes (Arpink red™ or Natural red™). These molecules present a great interest in the field of dyeing, even if they are sometimes described as “Dr. Jekyll or Mr. Hyde” (Dufossé 2014b).

9.4.3 Legislation and Use

Today, each country has its own regulations and European, American, and Asian standards highly diverge about authorizations and conditions of use for dyes.

9.4.3.1 European Standards

In the European Union, the directive referred to as the EU Directive 94/36/EC (Color Directive), then 89/107/EEC, has been implemented throughout national legislations that locally rule the specific uses of dyes (Mapari et al. 2009). About 100 authorized colorants used in foodstuffs are given E-numbers and tested for biosafety before commercialization. About 40 percent of them are from natural origins (Caro et al. 2012). A quite recent CEE regulation (CE n°1223/2009) (EEC 2009) intends to unify the rules concerning colorants used in cosmetics, but the work is presently under progress.

The European Food Safety Authority (EFSA) first fixed in the UE a detection limit of anthraquinones below 0.5 milligrams/kilogram (EFSA 2006). Specific maximum residue limits for food were then defined with a new regulation (EC), No. 1146/2014, effective from May 18, 2015 (EEC 2014). A European maximum residue limit of 0.01 milligrams/kilogram was applicable for the presence of anthraquinones in food, irrespective of the origin of the foodstuff. Taking into account state-of-the-art research, the specific standard values were adapted to the limit of quantification. According to matrix, these maximum residue limits may deviate from the common 0.01 milligrams/kilogram and are listed for anthraquinones in annex V of regulation (EC) 396/2005 (EEC 2005).

9.4.3.2 American Rules

In the United States, the specific use of colors is outlined by the United States Food and Drug Administration (USFDA) in the Code of Federal Regulation, Title 21 (21 CFR, titles 73-82) (USFDA 2001). Twenty-nine “exempt colorants” (synthetic or natural), are available in the United States for foods without being submitted to the rigorous requirements applied to all the certified ones. Indeed, a list of nine molecules was termed “the permitted list” including dyestuffs, which achieved certification procedures and were proved to be more or less harmless. However, very few of the dyeing molecules had therefore been extensively tested for safety and the harmlessness of synthetic colorants has been replete with controversies and contradictions (Francis 2002). This is particularly true for azoïc dyes in red hues, presently authorized over the world, but exhibiting the above-mentioned negative effects on human health (Greenhawt and Baldwin 2009, USFDA 2011, Weiss 2012, Yilmaz, Ergun, and Yilmaz 2014). The conclusions of the Southampton study (McCann et al. 2007) thus led to the obligation for food companies to apply a label mentioning that some “azo-dyes (i.e., synthetic dyes) may have an advert effect on activity and attention in children” (EFSA 2008).

Today it is noteworthy that, under the pressure of regulatory agencies, now more than ever, extensive, lengthy and costly toxicity studies are required for the commercialization of new dyes.
9.4.3.3 In Asia

The concern in Asian countries about the impact of food additives on health is relatively recent. As an example, European madder root extract (i.e., madder color) has been long accepted for use as a food additive in Japan and South Korea. It was present among food additives that were already marketed or used on the date of the amendment of the Japanese Food Sanitation Law in 1995. Thus, it appeared in the List of Existing Food Additives. As a coloring agent, its food use was limited to wakame, kelp, meat, fresh fishes, shellfish, whale meat, tea, beans, and vegetables (Dufossé 2014b). Nevertheless, due to its extensive use as a food colorant, its safety has been recently studied in Japan. The analysis of the biological effects of the coloring compounds extracted from madder roots clearly indicated that this dyestuff exerts a carcinogenic potential in animals organs and cells (Inoue,Yoshida, Takahashi, Fujimoto et al. 2009, Inoue, Yoshida, Takahashi, Shibutani, et al. 2009, Ishii et al. 2014). The Japanese Ministry of Health, Labor, and Welfare of Japan then concluded that no acceptable daily intake (ADI) could be established for this substance. Madder color was then delisted and prohibited for use in foods (JFAEC 2004).

9.4.4 Colored Anthraquinones from Plants

Historically, plants have been used for the extraction of a majority of natural colorants (pepper, red beet, grapes, and saffron) before being replaced by synthetic dyes. A renewed interest in natural colorants has increased their commercial availability. The most common plant pigments from edible plants, fruit, and vegetables are carotenoids, chlorophylls, anthocyanins, and betalains. Nevertheless, anthraquinones’ derivatives are common aromatic compounds in plant pigments. They are the largest group of plant quinones, better known for their use as mordant dyes as well as bird repellants. The anthraquinone derivatives occur in many different higher plants and are generally present as anthraquinones glycosides in young plants. The anthraquinone-based pigmented compounds present in plants are often under 5 percent (dry weight) (Caro et al. 2012).

Various plant parts, including roots, leaves, twigs, stems, heartwood, bark, wood shavings, flowers, fruits, rinds, hulls, and husks, can serve as natural colorant sources. However, the dried roots of higher plants are usually used to extract the dyestuff containing anthraquinone derivatives.

9.4.4.1 Madder Root (Rubia tinctorum Linn., Rubiaceae)

The mixture of color compounds extracted from dried roots of European madder (Rubia tinctorum Linn., Rubiaceae) is one of the oldest red dyes used throughout the history in Europe, Asia, and Northern and Southern America. European madder roots contain from 2 to 3.5 percent (dry weight) of anthraquinones glycosides (Caro et al. 2012, Dufossé et al. 2014, Dufossé 2014b). The roots’ bark contains a higher amount of dyestuff than the wooden parts. During storage, hydrolysis of some glycosides occurs, which is completed under acidic conditions. The color shades of madder vary from scarlet, carmine red, pink (high content of pseudopurpurin or purpurin, called pink madder or rose madder), to red with a bluish tint (alizarin lakes) (Figure 9.8). European madder roots contain an impressive number of anthraquinone derivatives; a total of more than 36 have been identified in Rubia tinctorum roots, even if a part of these compounds is believed to be artifacts formed during extraction or drying of the dyestuff. Fifteen anthraquinones’ derivatives from Rubia tinctorum roots play an important role in dyeing and are grouped together.
FIGURE 9.8  The main structures of glycones (a, b) and aglycones (c, d) present in madder root: (a) Lucidin primeveroside, (b) ruber-ythric acid, (c) alizarin, and (d) purpurin
in the Color Index as C. I. Natural Red 8. The yield of *Rubia tinctorum* roots from the three-year-old plant is between 3–5 tons per hectare producing about 150–200 kilograms of dye (Saxena and Raja 2014).

The composition of the extracted anthraquinone derivatives differs between the varieties of *Rubia*. Other madder plants yielding anthraquinone red dyes include Indian madder (*Rubia cordifolia Linn*) and Naga madder (*R. sikkimensis*).

The main anthraquinone derivatives isolated from plants in *Rubia* spp., and also in *Galium* spp. (another Rubiaceae) are usually alizarin (orange-red), purpurin (dark red), pseudopurpurin (orange), lucidin-primeveroside (red), nordammacanthal (orange), rubiadin (yellow), and munjistin (orange-red) (Kawasaki, Goda, and Yoshihira 1992, Westendorf, Pfau, and Schulte 1998, Caro et al. 2012). In some *Rubia* species, the anthraquinone alizarin is bound to the disaccharide primeverose (6-O-β-D-xylopyranosyl-β-D-glucose) to build up the anthraquinone glycoside ruberythric acid (golden-yellow) (Derksen et al. 2003). Surprisingly, these phenol anthraquinone derivatives are not formed via the polyketide pathway, but through a more elaborate sequence, involving shikimate and an isoprene unit through the chorismate/O-succinylbenzoic acid pathway. Such structures, like the potent mutagenic-alizarin, rubiadin, and lucidin pigments, do not contain the characteristic meta-oxygenation pattern of phenol anthraquinone, and often have oxygenation in only one aromatic ring (Caro et al. 2012).

The roots of the plant are rich in the highly colored, naturally occurring glycosidic anthraquinoid compounds ruberythric acid and lucidin-primeveroside. Alongside, the corresponding aglycones can be readily formed by deglycosylation, particularly during extraction of the pigments, and free aglycones or glycosides can also be extracted, depending on the polarity of the solvent used for the process. For the production of a commercially useful dye extracted from European madder, the glycoside ruberythric acid has to be hydrolyzed to the water-soluble aglycone alizarin. Alizarin (1,2-dihydroxyanthraquinone) is the main red dye found in commercial madder color. It is also known as Pigment Red 83 or C. I. Mordant Red 11. An intrinsic problem is the simultaneous hydrolysis of the glycoside lucidin-primeveroside to the unwanted lucidin and rubiadin aglycones proved to be mutagenic (Kawasaki, Goda, and Yoshihira 1992, Westendorf, Pfau, and Schulte 1998). Purpurin (1,2,4-trihydroxyanthraquinone; C. I. Natural Red 16) is a minor component in the European madder roots, but is the main dye (bright red crystals) in addition with munjistin (orange-red crystals) extracted from Indian madder (*Rubia cordifolia*). Both alizarin and purpurin contained in European madder and Indian madder, respectively, are only sparingly soluble in water, but are freely soluble in alcohol, ether, acetone, and alkaline solutions. It has been demonstrated that alizarin can be extracted from the roots of *R. tinctorum* with methanol at 25°C with an extraction yield of 2.9 grams/kilogram of dried material (De Santis and Moresi 2007). This yield can be increased to 4.0 grams/kilogram by means of microwave assisted extraction, with purpurin being extracted from *R. tinctorum* at a yield of 2.1 grams/kilogram by microwave-assisted extraction (Dabiri et al. 2005). It has also been demonstrated that extraction of *R. tinctorum* in methanol/water mixtures can be conducted at lower temperatures and in shorter times to obtain similar yields by application of ultrasound assisted extraction (Cuoco et al. 2009).

9.4.4.2 Other Plants

Several other species, although producing colored anthraquinones, are not considered viable contributors to the natural dye market. This includes *Anchusa tinctoria*, *Lithospermum* spp. (Boraginaceae), *Carthamus tinctoria* (Asteraceae), and *Galium*
species (Rubiaceae). *Galium tinctorium*, *Galium mullugo* (great lady’s bedstraw or wild madder), *Galium verum* (yellow lady’s bedstraw), and *Galium aperine* (goosegrass or cleavers) are, however, considered to produce inferior dyes compared with the red pigments obtained from European madder (Rymbai, Sharma, and Srivastav 2011).

9.4.5 Colored Anthraquinone from Insects: Carminic Acid

Carminic acid, carmine, cochineal extract are produced in Peru, Bolivia, Mexico, Chile, and Spain (Canary Islands), from the dried bodies of female cochineal insects (*Dactylopius coccus*), primarily grown on *Opuntia cacti*. The pigments can create red, orange, purple, and pink shades, depending on formulation.

These dyes are allowed by most of the food laws in different countries, such as the Food and Drug Administration (FDA) of the United States, and the European Union, where food additive identification code is E120 (Müller-Maatsch and Gras 2016).

The chemical structure of carminic acid, the main pigment of cochineal, consists of a glucose unit, which is attached to an anthraquinone (Figure 9.9).

Carminic acid is soluble in water, alcohol, acid, and alkaline solutions. It presents good light stability and its color varies depending on pH. Because of its carbonyl and hydroxyl groups, carminic acid is ideally suited to coordination bonding with metals, creating carmine. Some cationic metal complexes can form lakes, giving precipitates of different colors (Borges et al. 2012).

The coloring is currently used in a variety of products such as ice creams, yogurts, fruit drinks, candies, alcoholic drinks, and meat products.

While carmine is considered as a safe and effective natural alternative to synthetic red color FD&C Red #40, manufacturers have faced pressure to replace it for vegans, vegetarians, shoppers seeking kosher and halal products, plus those suffering from the “ick” factor (Watson 2013). This “ick” factor is the main consumer issue for carminic acid, carmine, and cochineal extract and it started when these colorings were implicated in adverse reactions, that is, anaphylactic shock reaction in a small number of people due to impurities in the preparation, not due to the pigment itself. In 1998, it was reported that IgE-mediated allergy might be caused by the consumption of carmine due to the presence of protein or protein-derived residues. In another case an anaphylactic reaction

![Figure 9.9 Carminic acid.](image_url)
occurred in a 35-year-old woman after she ingested mixed-fruit yogurt colored with carmine. Acute allergic reactions after the ingestion of orange beverages, strawberry milk, and red-colored cocktail, all containing carmine, were also mentioned (Beaudouin et al. 1995, Baldwin, Chou, and Solomon 1997, Acero et al. 1998). A major 38-kd cochineal allergen was cloned, expressed, and characterized by Ohgiya et al. in cochineal extract, protein allergen that could be a phospholipase or related enzyme, which are both known to be allergens in other insects (Ohgiya et al. 2009).

Companies producing carminic acid, carmine, and cochineal extract should better communicate to researchers and consumers these observations (e.g., improvements made in extraction processes, in order to minimize or to suppress the “protein” or “peptidic” content). Consumer lobbying groups are now requesting/looking for replacement products and companies producing food colorants are selling other pigments as alternatives to what they both now call “crushed bug juice.”

Another drawback of carmine products is not scientific nor technical but linked to the market as prices are highly volatiles (from a stable level of 15 USD per kg it surged in 2010–2011 up to 120 USD per kilogram and moderated down again to 15 USD per kilogram—a previous price peak occurred during the 1995–1996–1997 years). This fact also prompted manufacturers to seek alternatives, such as natural red color from tomatoes, beetroots, grape skins, and purple carrots. The world’s largest food color company, Chr. Hansen, which sources one-third of global carmine production, decided in 2011 to explore whether it would be commercially viable to produce carmine with a controlled fermentation process (proof of concept test). The genome of the cochineal insect has been sequenced and candidate genes identified (Watson 2013). The host organism for industrial application is unknown up to now (genetically modified organism [GMO] could be a yeast or a filamentous fungi), as are the future regulatory status of that carmine and the consumer perception of such a colorant (GMO-derived; insect genes inserted in an eukaryotic microorganism). Another issue not addressed up till now by scientists from universities, research centers, and private companies is also important: Who is truly producing the anthraquinones present in Dactylopius coccus: The insect itself, as hypothesized above, or the symbiotic microflora living in the insect? (Ramirez-Puebla et al. 2010). Isolation, screening, and cultivation of these microorganisms in conditions that mimic symbiosis should be investigated.

As a conclusion, the Dr. Jekyll aspect of carminic acid and derivatives is the excellent stability in food formulations (between 2004 and 2009 a 76 percent increase in new European food product launches listing carmine as an ingredient was observed; the increase was also linked to the consequences of the “Southampton six” study, warning for hyperactivity in children related to the occurrence in food of six artificial colorants, including three sulfonated mono-azo red dyes—E122 carmoisine/azorubine, E124 Ponceau 4R, E129 Allura Red AC), whereas the Mr. Hyde aspects include (1) allergenicity in some cases, (2) not vegan-vegetarian-kosher-halal, (3) price versatility.

9.4.6 Colored Anthraquinones from Microbes: Arpink Red®, Natural Red™

Anthraquinone compounds have been considered among the most abundant microbial natural pigments. If few bacteria species have been proven to produce anthraquinones (Streptomyces spp. [Balachandran et al. 2016, Duraipandiyan, Al-Dhabi, and Ignacimuthu 2016]; Photorhabdus temperata [Ahn et al. 2013]), anthraquinoid molecules are widespread among fungi and lichens, giving color to spores, sclerotia, sexual
bodies, and other developmental structures (Yu and Keller 2005, Fouillaud et al. 2016). However, only rare experiences have been up to date, successful in industrial production of microbial anthraquinones.

9.4.6.1 Arpink Red®, Natural Red™

Arpink Red™ is the first anthraquinoid-type pigment that has been produced from microbes (fungus *Penicillium oxalicum var armeniaca*) and used in an industrial setting. This natural colorant, initially manufactured and commercialized by the Czech company Ascolor Biotech s.r.o., received in 2004 a two-year temporary approval by the EU for distribution as a food additive, exclusively in the Czech Republic (Dufossé 2006) (Figure 9.10).

In 2006 the file for homologation was still under progress at the European Food Safety Authority (EFSA). The situation is still not clear as Ascolor Biotech s.r.o. or the new Biomedical s.r.o. did not send the required data to authorities till later on (WHO 2006).

9.4.6.1.1 Arpink Red®

This colorant was the first one produced by fermentation and bioprocess engineering using the strain *Penicillium oxalicum var armeniaca* CCM 8242, obtained from soil (the variety was morphologically described). The fungus produces a pigment (C25H26O14, MW= 550 Da) up to 2 grams/liter of culture medium, providing a raspberry-red color in an aqueous solution. It is stable at pH over 3,5. Neutral solutions are even stable after 30 minutes of boiling and the color shade does not change in relation with pH (WO 9950434; CZ 285721; EP 1070136; US 6340586, cited in Sardayan [2004]). The liquid culture conditions allow the crystallization of a red powder including carminic acid (Sardaryan 1999). Spectral analysis of the red-colored mixture obtained through the extraction process showed to contain no more than 52 percent (dry weight) of colored substances. Toxicological data about the pigment, produced by Ascolor Biotech s.r.o, examined oral toxicity in mice, 90-day subchronical toxicology, dermal irritation/corrosion, eye irritation/corrosion, anti-tumor effectiveness, micronucleus tests in mice, AMES tests, antibiotic activity, and presence of mycotoxins. The results allowed its acceptance by the Codex Alimentarius Commission (Rotterdam meeting, March 11–15, 2002) and its safety assessment during the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 63rd meeting in Geneva, June 8–17, 2004). A specific formulation of the colorant was moreover patented as a food supplement, supposed to develop prophylactic and therapeutic anticancer activities (patents n° WO 2002011563 A1 [Sardaryan 2002, 2006]).

![General structure of Arpink Red®](image-url)
9.4.6.1.2 Natural Red™ In 2009, a new patent (CZ patent n° 302696) was filled in by the Czech company Biomedical s.r.o., dealing with the fungus *Penicillium oxalicum* var *armeniaca* CCM 8374. The strain produced an exogenous red pigment of anthraquinone type (Sardaryan 2009). The organism was obtained from genetic modifications (site-directed mutagenesis) applied to the former strain *P. oxalicum* var *armeniaca* CCM8242, coupled with culture medium optimization and guidance of the biosynthetic pathways. A molecular weight of 378.32 Da was determined for the major chromophore and the production of the anthraquinone type pigment was improved up to 5–10 grams/liter.

The recommended use of Arpink red® was 100 milligrams/kilogram in meat products and in non-alcoholic drinks, 200 milligrams/kilogram in alcoholic drinks, 150 milligrams/kilogram in milk products including ice creams, and 300 milligrams/kilogram in confectionery products (Codex Alimentarius 2002).

According to the patent filled in 2009 (Natural Red™), ethanol–water solutions of the dye (2 to 50 percent) can be prepared from aqueous solutions. The product can be used at concentrations between 50 and 400 milligrams/kilogram. It seems that antibiotics are also produced along with the pigment. However, no significant effect was found when testing bacteria (G+ or G-). All toxins detected in the mix were in low concentrations. Therefore, in the range of recommended quantities, it was asserted that the use of this product did not present any inconvenience, according to the presented tests.

The formulation of the final mixture commercialized for dyeing foodstuffs, may include maltodextrine (around 65 percent) along with the chromophore (around 35 percent) (Dufossé 2014a).

9.5 ANTHRAQUINONES AND FOOD PROCESSING

Investigation on the stability of anthraquinones is important for the evaluation of health foods, cosmetics, and pharmaceuticals containing these secondary phenolic metabolites. Several factors such as pH, temperature, light, water, or oxidants have been recognized to affect their stability. However, the available information concerning the effect of food processing on the degradation of anthraquinones is more limited. On the other hand, many studies have been carried out to evaluate their stability in model systems.

As little is known about the decrease in the content of anthraquinones during food processing, forced degradation studies provide an insight and information about the storage and intrinsic stability conditions of food products containing anthraquinones.

9.5.1 Stability in Model Systems

Temperature shows a clear effect on anthraquinone stability. It has been established that molecular structure of quinones is a determining factor for their thermal stability and strongly influences their thermal decomposition (Sousa et al. 2012). These authors found that 9,10- anthraquinone starts to decompose above 215°C. A clear effect of the different substituted groups attached to the skeleton of anthraquinones has been mentioned. Zhang et al. investigated the thermal behavior of five free anthraquinones having the same skeleton (1,8- dihydroxy anthraquinone) from rhubarb using thermogravimetry (TG), differential thermogravimetry (DTG), and different thermal analysis technique (Zhang et al. 2009). Similar TG and DTG curve shapes were observed for chrysophanol, emodin, physcion, aloe-emodin, and rhein. But due to the different substituted groups, they exhibit different
mass loss features. For example, chrysophanol, emodin, and physcion displaying the same substituted group $R_1$ (CH$_3$), but different groups $R_2$, showed different thermal behavior. It was found that the decomposition point of chrysophanol is near to physcion and their mass loss occur between 189–293°C and 200–317°C, but the emodin is stable before 281°C and its temperature range is 245–332°C due to the substituted hydroxyl group.

Concerning the stability of aloin A, Ding et al. evaluated the effects of varying pH, temperature, and light conditions, usually encountered in processing, and characterized its major degradation products (Ding et al. 2014). Aloin (also named barbaloin) is an anthraquinone-C glycoside which occurs naturally as a mixture of two diastereoisomers: Aloin A (configuration at C10, C1:S, S) and aloin B (C10, C1:R, S). The effect of pH on aloin A stability was evaluated at pH 2.0, 3.0, 5.0, 7.0, and 8.0. The thermal stability of aloin A was studied at four different temperatures (4, 30, 50, and 70°C) at the same pH values (pH 7.0), all involved in industrial treatments. The photostability test occurred in protection from light, under natural light, and strong light (4000 lx) conditions (at pH 7.0 and room temperature). It was reported that aloin A decomposed quickly at high temperature or under neutral-basic conditions, but light did not promote the degradation of aloin A. The main degradation products of aloin A were identified as aloe-emodin, elgonica-dimers A and B at pH 5.0 or below, and elgonica-dimers were mainly formed at 4°C as well. In addition, it was shown that 10-hydroxyaloins A and B were found under any condition except at pH 2.0 and 3.0, and they were mainly formed under high temperature, neutral-basic and any light conditions. Several studies have been previously carried out with aloin. For example, Zonta et al. (1995) have mentioned a remarkable decrease of aloin A content with the increase in pH value and temperature. Compositional variations of aloin have been studied by Pellizzoni et al. (2011) have evaluated the stability of the main Aloe fractions and aloe-based commercial products under different storage conditions. It was mentioned that aloin stability was not increased by ascorbate nor by the antimicrobial agents used.

Narayanan et al. (2015) carried out forced degradation studies on aloe emodin and emodin by HPTLC. Various degradation parameters were evaluated such as oxidation (6 percent v/v hydrogen peroxide for 3 hours), acid (0.1 N HCl for 2 hours) and alkaline (0.1 N NaOH for 2 hours) hydrolysis, photolysis (sunlight or UV-254 nm for 8 hours), hydrolytic and thermal degradation (dry heat at 105°C for 8 hours). A significant degradation of aloe-emodin and emodin under acid hydrolysis was reported, whereas these anthraquinones were found less susceptible to base degradation. A moderate thermal degradation was also mentioned. Both anthraquinones underwent moderate oxidative and photolytic degradation. In constrast, aloe emodin was found more susceptible to hydrolytic degradation than emodin.

Ali et al. (2014) have evaluated a simultaneous determination of diacerein, rhein, and emodin using an accurate, sensitive and selective thin-layer chromatography–densitometry method. Different stress conditions, including hydrolysis, oxidation, and photolytic degradation of diacerein, were analyzed in bulk powder and different pharmaceutical formulations. These authors observed that diacerein was degraded under hydrolytic and oxidative degradation conditions to give one degradation product, rhein, whereas the drug was stable upon exposure to photolytic degradation conditions. The thermal stability of anthaquinones has also been studied in course of extraction processing. Several studies have been carried out with pressurized hot water extraction (PHWE). Barrera Vasquez et al. (2015) evaluated the effect of temperature, pressure,
and water flow rate on the extraction yield of four anthraquinones (soranjidiol, rubiadin, rubiadin 1-methyl ether and 2-hydroxy-3-methyl anthraquinone) from aerial parts of *Heterophyllaea pustulata* Hook f. It was mentioned that extractions at higher temperature (220°C) gave lower yields of anthraquinones, apparently due to the thermal decomposition of these compounds. Investigating PHWE water extraction of anthraquinones of *Morinda citrifolia* roots, using alizarin or 1,2-dihydroxy anthraquinone as a standard compound, Shotipruk et al. (2004) have determined the effects of extraction temperature (110, 170, and 220°C) and water flow rate on extraction yield and rate of extraction, and reported that alizarin was stable up to 220°C. Studying PHWE water extraction of the anti-cancer damnacanthal (3-hydroxy-1-methoxy anthraquinone-2-aldehyde) from roots of *Morinda citrifolia*, Anekpankul et al. (2007) mentioned its decomposition at higher temperature than 170°C. It has therefore been established that anthraquinones usually decompose at high temperatures. It is also appropriate to pay particular attention to matters of hydrolytical degradation of glycoside anthraquinones.

Wianowska demonstrated hydrolytical unstability of glycoside forms of hydroxy-anthraquinones during their extraction from *Rumex crispus* roots in different pressurized liquid extraction conditions using a methanol/water mixture as an extractant (Wianowska 2014). Different solvent compositions, extraction temperatures, pressures, and static extraction times were investigated, examining concentration changes of some monoglycosides (emodin-8-O-β-D-glucopyranoside, chrysophanol-8-O-β-D-glucopyranoside, and physcion-8-O-β-D-glucopyranoside) and their aglycones (emodin, chrysophanol, and physcion). A gradual concentration increase of all the examined aglycones was observed with temperature increase (50–150°C), followed by a decrease of aglycones concentration above 150°C, attributed to their thermal decomposition. Several studies were previously dedicated to the barbaloin stability. Yasuda et al. studied its concentration in fifteen products containing aloe. A decrease was observed in liquefied products up to 50 percent after storage for 1 month in a cold, dark place. These authors also explored the stability and degradation pathways of barbaloin suspension in an aqueous solution over the pH range of 1.1–8.4 (Yasuda et al. 1997, Yasuda et al. 2000). It was found a 1.15 days half-life of barbaloin at pH 7.2 (20°C), and 5 days at pH 3.4 (20°C). It was mentioned that barbaloin is converted to dimers and then to trimers during storage.

Duterman and Chauser-Volfson (2006) compared the decrease in the content of three secondary phenolic metabolites (two C-glycosides: barbaloin, aloeresin, and one O-glycoside: aloenin). They used (1) a suspension of *Aloe arborescens* powder in water after storage for up to 45 days, and (2) harvested leaves stored for up to 3.5 months at 4°C in darkness. A rapid degradation of aloenin was observed during storage in water, while a gradual and slow degradation was measured for the two C-glycosides, compounds known for their resistance towards hydrolysis. On the other hand, it was shown that the variation in the relative amounts of these three glycosides was quite similar in stored leaves. Several years’ stability was mentioned for these three secondary phenolic metabolites when stored as dry powder.

9.5.2 Stability in Food Processing

Concerning the anthraquinones stability in food processing, McDougall et al. (2010) have evaluated the effects of several cooking methods on the polyphenolic composition...
of garden rhubarb *Rheum rhabontigen*. Total polyphenolic content, anthocyanin content, and total antioxidant capacity were studied. Products were analyzed by liquid chromatography–mass spectrometry, leading to a putative identification of 40 polyphenol compounds, including anthraquinone, stilbene, anthocyanin, and flavonol derivatives. Four cooking regimes were developed: Blanching (boiling water), slow cooking (70–80°C), fast cooking (100°C), and baking (180°C), from 2 to 30 minutes. Most cooking regimes, except blanching, increased total polyphenol content and overall antioxidant capacity, compared to the raw material. The authors found a yield increase of all components but the initial increase in the content of some anthraquinone aglycones was followed by their destruction with increasing cooking time. For example, a dramatic reduction in the relative amounts of the anthraquinone aglycones between 5 and 10 minutes of baking, accompanied by a contents decrease of the anthraquinone glycoside derivatives were observed. It was suggested a breakdown of the anthraquinone dimer derivatives to form anthraquinone monomer glycosides.

Yen and Chung (1999) investigated the effects of heating on water extracts from *Cassia tora* L. seeds, prepared under different degrees of roasting. It was found that the total content of anthraquinones in water extracts was in the order of unroasted > 150°C roasted > 200 °C roasted, indicating that anthraquinones were degraded by thermal treatments. Wu and Yen (2004) have analyzed the contents of chrysophanol, emodin, and rhein in *C. tora* seeds, showing also that the unroasted samples contained the highest anthraquinones content. The authors have mentioned that anthraquinones were degraded to a free form (aglycon) by roasting treatment.

### 9.6 DR. JEKYLL AND MR. HYDE? BIOLOGICAL EFFECTS OF ANTHRAQUINONES

As anthraquinones are not yet widely applied as dietary supplements or food colorants, research work needs to extend to the knowledge concerning their potential roles on human and animal health. In recent years, anthraquinones are increasingly attracting attention of the pharmaceutical community as they include a wide diversity of pharmacologically active compounds (Xie et al. 2010, Zhang et al. 2010, Matsuda et al. 2001, Riecken et al. 1990, Firuzi et al. 2011, Zhou and Chen 1988, Zhou et al. 2006, Izhaki 2002). The review of Fouillaud et al. (2016) highlights some selected bioactive effects of a large panel of anthranoid molecules (Fouillaud et al. 2016). Their positive or negative effect(s) due to the 9,10- anthracenedione structure and its substituents are still not clearly understood and their potential roles or effects on human health are today strongly discussed among scientists. Extending the knowledge about these widespread molecules may help to open doors toward innovative and useful natural substances, potentially usable in daily diets.

#### 9.6.1 Benefits

**9.6.1.1 Anti-Tumor**

Cancer development largely results from an uncontrolled growth of malignant cells in which cell proliferation surpasses cell death. Deregulation of apoptosis, occurring in a
majority of cancer types, has since become a non-negligible target for anticancer strategies and pro-apoptotic compounds are thus under active investigations (Xie et al. 2010).

**Emodin.** Huang et al. (2007) and other teams clearly demonstrated that anthraquinones, such as emodin, aloe-emodin, and rhein, inhibit the growth and proliferation of various cancer cells, such as lung adenocarcinoma, myelogenous leukemia, neuroblastoma, hepatocellular carcinoma, bladder cancer, and others through cell death and survival’s modulation (Olsen, Bjorling-Poulsen, and Guerra 2007, Chen et al. 2014, Meggio et al. 2004). Emodin also demonstrated its capacity to reduce toxicity and to enhance efficacy in combination chemotherapy with standard drugs (arsenic trioxide and docosahexaenoic acid, or gemcitabine) against tumor cells (Srinivas et al. 2007, Brown, Bellon, and Nicot 2007, Guo et al. 2012). Emodin might also suppress the growth of cancers by reducing tumor neovascularization and decreasing macrophages’ migration inhibitory factor expression. It also attenuates tumor cell-induced metastasis (Zhang, Hu, and Chen 2015, Ma et al. 2015). Following the pharmaceutical hits with emodin, studies about anthraquinones were expanded with the addition of bromo, nitro, amino or bromoacetamido groups, and other compounds such as citreorosein (ω-hydroxyemodin) were also proved to be active (Lu et al. 2012, Lim et al. 2014).

**Chrysophanol,** found in rhubarb, is chemically closely related to emodin. It stimulates reactive oxygen species (ROS) production, mitochondrial dysfunction, loss of ATP, and DNA damage in J5 human liver cancer cells, which leads to necrotic cell death (Lu et al. 2010, Pandith et al. 2014).

**Physcion,** the orange pigment found in the roots of curled dock (*Rumex crispus*; yellow dock in the United States) and also in rhubarb, has antitumor and antifungal properties. Recent research suggests that physcion effectively inhibited a part of the pentose–phosphate pathway responsible for constructing the cellular building blocks necessary for rapid growth of cancer cells. The inhibition of cancer cell proliferation and tumor growth in nude mice xenografts takes place without obvious toxicity (Lin et al. 2015).

**Rhein** (4,5-dihydroxyanthraquinone-2-carboxylic acid), used in clinical studies on animal disease models or functional cells, exerted multiple functions including anticarcinogenesis, antioxidant, anti-inflammation, and immunosuppression (Hu et al. 2015, Tsang and Bian 2015, Huang, Chu, and Chao 1991, Chang et al. 1996, Zhang et al. 2005).

**Damnacanthal,** present in noni plants, targets several tyrosine kinases and also proved its antitumor effects (Garcia-Vilas, Quesada, and Medina 2015).

**Purpurin.** The antigenotoxic effect of purpurin against a range of environmental carcinogens has previously been observed in *Drosophila melanogaster*. The compound also clearly inhibited the formation of hepatic DNA adducts in mice exposed to carcinogens (Marczylo et al. 1999, Marczylo, Sugiyama, and Hayatsu 2003, Takahashi et al. 2007).

More recently, the bioactivity of the major constituents of *R. cordifolia* roots has been explored by Biswas et al. (2015). The study indicated that the most bioactive fraction of the plant extract and purpurin showed primarily monophenolase inhibition and to a lesser extent diphenolase inhibitory activity. In addition, results of enzyme kinetic analysis showed they reversibly inhibited tyrosinase in a competitive manner. Molecular
docking results implied that the possible inhibitory mechanisms might be attributed to purpurin interaction with copper ion, coordinating three histidine residues of tyrosinase. Authors concluded that this finding could be of importance in prevention of the undesirable enzymatic browning reaction of food products, as well as hyper-pigmentation of human skin (Biswas et al. 2015).

9.6.1.2 Antimicrobial, Antiviral, Antiparasitic
To date, most of the anthraquinones studied, isolated from various sources (plants, microbes), exhibited more antibacterial than antifungal activities. One aspect of the mechanism was elucidated by Daly et al. (2015). The polyhydroxyanthraquinone ω-hydroxyemodin (OHM) was identified as a suppressor of quorum sensing (QS) which is controlling the production of a virulence factor, essential for causing tissue infections by *Staphylococcus aureus* (through agr inhibition f. i.). Decreased dermonecrosis with OHM treatment was associated with enhanced bacterial clearance and reductions in inflammatory cytokine transcription and expression at the site of infection. Furthermore, OHM treatment enhanced the immune cell killing of *S. aureus in vitro* in an agr-dependent manner. These data suggest that bacterial disarmament through the suppression of *S. aureus* QS may bolster the host innate immune response and limit inflammation.

Several anthraquinones are able to inhibit the replication of viruses, or even directly kill enveloped or un-enveloped strains. Alizarin, quinalizarin, rhein, hypericin, and protohypericin, but also other anthraquinones derivatives as emodin, aloe-emodin, emodin anthrone, emodin bianthrone chrysophanic acid, and hypericin showed activity against several strains of human or animal viruses, clearly distinguishable from cytotoxic effects on cells (Barnard et al. 1992, Li et al. 2014, Sydiskis et al. 1991, Lin et al. 2008, Semple et al. 2001, Kubin et al. 2005, Shuangsoo et al. 2006). Aloe-emodin, moreover, showed dose-dependent inhibition of virus-induced cytopathic effects. A significant antileishmanial activity has also been demonstrated for 1,8-dihydroxy-3-methoxy-6-methylanthraquinone against *Leishmania major* (Awaad et al. 2014).

9.6.1.3 Antioxidant and Chelation Properties
Oxidative stress contributes to free radical–mediated diseases such as aging, atherosclerosis, cancer, ischemic heart disease, diabetes, hyperlipidaemia, hepatotoxicity, and neurodegenerative diseases. Natural and synthetic anthraquinones and their derivatives (Emodin, aloe-emodin, alizarin, physcion, etc.) clearly demonstrated their antioxidant potential (Li, Li, and Wang 2009, Kosalec et al. 2013, Firuzi et al. 2011, Brash and Havre 2002, Fiorentino et al. 2007, Heo et al. 2008, Zargar et al. 2011). From their quinoid structures, they are bound to participate in redox reactions, exhibiting antioxidant or pro-oxidant properties. According to Yen et al. (2000), the basic anthrone chemical structure exhibited the role of electron acceptor, and the hydroxy substituent accompanied with methylations are multifunctional antioxidants, combining both chain-breaking and metal-chelating properties.

9.6.1.4 Excretion Functions: Laxative, Diuretic Activities
Anthranoïd laxatives of natural origin, mainly extracted from plants (emodin, aloe-emodin, and chrysophanol) are widely used, even since ancient times (Evans and Evans 2002, Bruneton 2009, Van Gorkom et al. 1999). Senna, cascara, frangula, rhubarb, and aloe are commonly used for their laxative effects (IARC 2002). It is believed that the presence of hydroxyl groups, in position 1 and 8 or 9 of the aromatic ring system, are essential for the purgative action (Paneitz and Westendorf 1999). Because
of their chemical structure, emodin glycosides (and other anthraquinones) are carried unabsorbed to the large intestine in mammals, where metabolism to the active aglycones takes place by intestinal bacterial flora. The aglycone exerts its laxative effect by damaging epithelial cells, which leads directly and indirectly to changes in absorption, secretion and motility (Van Gorkom et al. 1999, Mueller et al. 1999). One main target is the inhibition of the ion transport (Cl⁻-channels) across colon cells, contributing to the laxative effect (Izhaki 2002, Rauwald 1998). Moreover Na⁺/K⁺-ATPase (pump) was inhibited by those 1,8-dihydroxyanthrones/anthraquinones that bear an additional phenolic hydroxyl group. Interference with oxidative ATP production, as an additional effect, may explain the known synergistic action described for the combination of different anthrones/anthraquinones or anthranoid drugs, respectively (Rauwald 1998).

The diuretic action of emodin and aloe-emodin is probably due to this Na⁺-K⁺-ATPase inhibition (Zhou and Chen 1988). 1,3,6,8-trihydroxymethylanthraquinone has been used in a patented laxative preparation for intravascular injection, active by stimulating the neuromuscular junction of the bowel wall (Mobley 1991).

Nevertheless, studies in humans have also suggested tumor-promoting activities for these laxatives. Although the short-term use of these substances is generally safe, long-term utilization cannot be recommended.

9.6.1.5 Other Identified Biological Activities

Effects on lipid and glucose metabolism. Recent findings about the therapeutic potential against diabetes mellitus of several naturally occurring anthraquinones and their derivatives (including emodin, physcion, cascacin, catenarin, chrysophanol, and rhein) were highlighted in Chien et al. (2015) and Mishra et al. (2014). Emodin, for example, demonstrated a dose-dependent antidiabetic effect (reductions in blood glucose) and lipid-modulating effects (serum total cholesterol, triglycerides, free fatty acids, and malonaldehyde) that involve, in part, upregulation of L-type calcium channel expression in the pancreas and heart of dyslipidaemic-diabetic rats (Zhao et al. 2009). Emodin also caused dose-dependent increases in the plasma superoxide dismutase activity.

Estrogenic activity. Insufficiency of endogenous estrogen secretion is known to cause several physical disorders in postmenopausal women, such as osteoporosis, hypercholesteremia, and symptoms of menopause. Synthetic estrogen-replacement therapy has been reported to be effective for these diseases. Emodin was mentioned for its high estrogenic activity. Conversely to aloe-emodin and chrysophanol, the compound is able to bond with human ERα and Erβ, competing with 17β-estradiol. Concerning the structure–activity relationships of anthraquinones regarding the estrogenic activity, it is quite clear that the unchelated hydroxyl group is essential for a strong competency (Matsuda et al. 2001, Fain, Zaitsev, and Ryabov 2004).

Vasorelaxant or contractile effects. Emodin dose-dependently relaxed isolated vascular rings of several vessels in animal and humans (Huang, Chu, and Chao 1991, Huang et al. 1991). Emodin can also induce muscle contracture, simultaneously depressing twitch amplitude. It seemed to be caused myogenically and it suggests that muscle contraction induced by emodin was dose-dependent (Cheng and Kang 1998).
9.6.2 Risks: Cytotoxicity, Carcinogenic Effects

9.6.2.1 Aloe Constituents

Several studies have attempted to determine whether or not Aloe vera is toxic to animals or humans. Many of the adverse effects of Aloe vera preparations should be related to the anthraquinones content and more particularly to the aloin level. Recently, the U.S. Department of Health and Human Services, in the National Toxicology Program (NTP/NCTR), has demonstrated a dose-dependent increase in large intestinal tumors in rats, chronically exposed to Aloe vera non-decolorized whole-leaf extract (in daily drinking water containing 60 ppm of aloin for nearly their entire lifetime; NTP Technical Report, 2013). In the study, the increased incidence of colon adenomas and carcinomas was related to intake of non-decolorized Aloe vera leaf extracts (unpurified, high anthraquinone level), supporting the notion that preparations containing aloe latex phenolic compounds, such as anthraquinones, are responsible mediators of the adverse effects on the colon. A more recent study has confirmed that Aloe vera whole-leaf extract is an intestinal irritant in rats and mice and a carcinogen of the large intestine in rats (Boudreau et al. 2013). Concerning the compound aloe-emodin in Aloe vera, it was reported that it induced micronucleus frequencies in in vitro micronucleus test in mouse lymphoma L5178Y cells (Müller et al. 1996). There are thus concerns for various adverse side effects usually related to oral intake of Aloe latex rather than Aloe gel (Dell’Agli et al. 2007).

9.6.2.2 Madder Root Compounds

A number of long-term genotoxicity studies on rats demonstrated positive results for madder color, suggesting that the carcinogenicity is based on genotoxicity. Madder root causes DNA adducts in the kidneys, livers, and colons of rats and provide clear evidence that madder color exerts unequivocal carcinogenicity (Westendorf, Pfau, and Schulte 1998, Inoue, Yoshida, Takahashi, Fujimoto, et al. 2009, Yokohira et al. 2008, Inoue, Yoshida, Takahashi, Shibutani, et al. 2009). Rubiadin, the major contributor to madder color, plays the role of an initiator as well as a promoter of carcinogenic effects.

Indeed, rubiadin aglycones and lucidin are found to be positive to bacterial mutagenicity tests, as well a number of other anthraquinone compounds like 1-hydroxy-2-methylantraquinone, lucidin-ω-methyl ether, lucidin-ω-ethylether, xanthoprupurin, 7-hydroxy-2-methyl-anthraquinone, and lucidin-primeveroside (Kawasaki, Goda, and Yoshihira 1992, Westendorf, Pfau, and Schulte 1998, Yasui and Takeda 1983, Ishii et al. 2014). Alizarin from madder color also exerts promotor potential in the kidney, but the effects are much weaker than with rubiadin. From structure mutagenicity studies, it was concluded that 1,3-dihydroxyanthraquinones that bear a methyl (CH3) or hydroxymethyl (CH2OH) group in position R2, for example, rubiadin and lucidin aglycones from madder color, respectively, are mutagenic. For direct mutagenicity an oxygenated state of the benzylic carbon-2 is required. Mutagenic studies about lucidin, more particularly, showed that a reactive compound is formed from the metabolism of the pigment, which then reacts with DNA and possibly other macromolecules to form covalent adducts with adenine and guanidine under physiological conditions. Other 1,3-dihydroxyanthraquinones that do not possess a methyl or hydroxymethyl group in position R2, such as the orange pigment nordamnacanthal and the orange-red munjistin pigment, are not found to be mutagenic, since the dehydration to the exomethylene compound is not possible under physiological conditions (Kawasaki, Goda, and Yoshihira 1992, Westendorf, Pfau, and Schulte 1998).
In conclusion, rubiadin, and more generally madder color, can induce carcinogenicity and should be dealt carefully as a significant carcinogen against humans (Inoue, Yoshida, Takahashi, Fujimoto et al. 2009).

9.6.2.3 Common Vegetables
The genotoxicity of several anthraquinones found in a variety of vegetables (cabbage lettuce, beans, and peas) was investigated in the comet assay, the micronucleus test, and the mutation assay in mouse lymphoma cells (Mueller and Stopper 1999). Emodin was genotoxic, whereas chrysophanol and physcion showed no effects. Indeed, pure emodin has toxic and direct gene mutagenic properties to *Salmonella typhimurium* TA1537 (Fullbeck et al. 2005). Another study mentioned that emodin was clearly genotoxic in mouse lymphoma cells, but also inhibits cell invasiveness in human cancer cells (Huang, Shen, and Ong 2004). This is probably due to the dose-dependent action. However, complete vegetable extract on its own did not show any effect in the micronucleus test. Taking into consideration the measured concentrations of anthraquinones, estimated daily intakes, the genotoxic potency, as well as protective effects of the food matrix, authors concluded that the analyzed constituents do not represent a high priority genotoxic risk in a balanced human diet (Mueller et al. 1999).

9.6.2.4 Senna Ingredients
1,8-Dihydroxyanthraquinone, the aglycone moiety of the laxative ingredient of senna, was formerly marketed as a laxative under the trade name Dantron®, but human drug products containing Dantron® (IARC, 1990) were withdrawn from commerce in the United States in 1987 after it was shown to cause intestinal tumors in experimental animals (IARC 1990, 2002).

9.7 IMPROVING INDUSTRIAL SCALE PRODUCTION OF ANTHRAQUINONES FOR FUTURE APPLICATIONS
Since the dawn human history, plants have been a well-known, renewable, and almost fully controllable source of food additives. However, to match the high increase of the world’s demand and to compete with the efficacy of chemical synthesis, the raw material productions, thus the agricultural yields, should be incredibly enhanced and improved. Thus, the need for extended agricultural surfaces, huge water volumes used for the plants’ growth, and today’s financial strategies (short-term profits) cause plants to be an expensive way of producing useful chemicals for food or daily products. This high cost is bearable when the product has a high added-value (as pharmaceutical drugs or luxury cosmetics), but it cannot be easily supported if the final compound is of low price on the global market. In our societies, where we search for what’s cheap right now, the plant-based productions are facing increasing difficulties.

9.7.1 Plants
New solutions appeared with the development of plant cells cultures, allowing the in vitro production of biomolecules and ensuring uniform quality and continuous delivery (Rymbai, Sharma, and Srivastav 2011). These processes have already been applied at a laboratory scale with the culture of callus tissues and cells of *Frangula alnus*, *Frangula*
rupestris, or *Rhamnus purshiana* (Rhamnaceae) in order to produce anthraquinoid drugs (emodin, aloe-emodin, chrysophanol, and physcion) (Van den Berg and Labadie 1984, Van den Berg and Labadie 1988, Van den Berg, Radema, and Labadie 1988b, a, Suzuki and Matsumoto 1988, Van den Berg and Labadie 1989, Van den Berg 1991, Sajc et al. 1999). Unfortunately, the productivity was not high enough at the moment, whatever the improvement factors they tried (maximum 0.5 percent of anthraquinoid glycosides w/w dry weight). However, the cultured tissues accumulated higher amounts of free anthraquinone aglycones compared to corresponding plants. In vitro shoot multiplication of *Frangula alnus* was then obtained on woody plant medium with indole-3-acetic acid and 6-benzylaminopurine. The highest anthraquinone production was in the shoots grown on the Murashige and Skoog medium (MS medium) with addition of 1-naphthaleneacetic, thidiazuron and chitin (Dörnenburg and Knorr 1994, Namdeo 2007). Good results of elicitation came from a study from Komaraiah et al. (2005) on *Morinda citrifolia* cells suspension cultures (noni fruit). Enhancement of accumulation of anthraquinones in plant cell cultures was accomplished by treatment with elicitors such as polyunsaturated fatty acids, methyl jasmonate, salicylate, and nitric oxide, coupled with ultrasonication and a controlled feeding of the carbon source in the growth medium. The anthraquinone production was increased up to 16.74 milligrams/gram of dry weight, which was more than a four-fold increase above the control cultures (Komaraiah et al. 2005). However, in vitro cultures of plants for industrial production are undoubtedly still in its infancy. Strategies need to develop informations based on cellular and molecular levels. Cell cultures, should then provide new continuous and reliable means for the commercial processing of even rare plants, and the chemicals they provide. This is the basis for the production of commercially acceptable levels of compounds for health benefits.

### 9.7.2 Microbes

Since the food company DSM has gained EU approval for food use of fungal originated β-carotene, produced from the fermentation of *Blakeslea trispora* in 2000 (EEC 2000), industrial interest on microbial metabolites has been revived, and new investigations have been ongoing to develop stable, continuous, and cost-effective microbial products ever since. Indeed, the past decade was a period of great improvement for microbial metabolites synthesis and knowledge about different ways to increase yields have been greatly extended. Anthraquinones in microbes belong to secondary metabolites, the pathways of which generate a great diversity of compounds, arising from a few key intermediates. Therefore, the biotechnological approach is the royal road to improve anthraquinonoid metabolites production.

Four major fronts are currently ongoing:

- Overall analysis of gene expression, that is, genomics, proteomics, metabolomics, fluxomics, and transcriptomics to better understand the production pathways and general metabolisms as well as the genes and the molecules involved.
- Molecular techniques to carry out metabolic engineering, to modify and improve particular biosynthetic pathways. Further metabolic engineering to optimize already existing or exogenous biosynthetic pathways coupled with the use of powerful computational algorithms, and databases based on the above-mentioned «omic» sciences (genomics, proteomics, and metabolomics) (Chen and Nielsen 2013).
• Production of interesting new metabolites in alternative hosts that have already been given GRAS status by the U.S. Food and Drug Administration (Generally Recognized as Safe), to be used in the food industry (P. roquefortii, Aspergillus oryzae, A. sojae, A. japonicus, Mortierella vinacea, M. alpina, Fusarium monoliforme, F. veneratum, Saccharomyces cerevisiae, etc.; Duran, De Conti, and Teixeira 2009, USFDA 2015a,b). Some long-ago studied bacteria or fungi (Escherichia coli, Corynebacterium glutamicum, Bacillus subtilis, Aspergillus niger, Aspergillus oryzae, Penicillium chrysogenum, Saccharomyces cerevisiae) are already operated in industry for enzymes, nutraceuticals, or pharmaceuticals. Indeed, the numerous years of research done on the selected strains led to high robustness and remarkable tolerances against various stresses under industrial conditions. This is the guarantee of stable and efficient production levels.

• Extensive use of Design of Experiment (DOE) is also of great interest to improve the conditions of metabolites production, combining the main optimal physicochemical parameters: Temperature, oxygen, carbon, nitrogen and other nutrient sources, pH regulation, light exposure, and physiological stage of the fungi. A side goal is to decrease the total production costs using by-products of agroindustrial origin as low-cost alternative substrates for microbial metabolites production (Sánchez 2009).

9.8 CONCLUSION

As it was presented throughout the chapter, anthraquinones constitute a large group of natural compounds that occur in many foods, from plants such as Aloe to more elaborated products such as Asian fermented tea or tuna. They are able to bring color to food, and the biological properties described for some anthraquinones are broad, for example, antimicrobial, antiviral, antiparasitic, antitumor, antioxidant, chelatant, diuretic, laxative, and so on.

Legislation is also an important point to address when using anthraquinones for food use. Many countries already set values of maximum limits, some (Europe) being less permissive than others (Asia).

As a concluding remark, the global biological effect of anthraquinones and derivatives formed during food processing or in the human body still need to be studied in order to have a clear picture of these compounds.

REFERENCES


Dufossé, L. 2014a. Personal communication about Natural Red™.


EFSA. 2006. Opinion of the scientific panel on food additives, flavourings, processing aids and materials in contact with food on a request from the commission related to an application on the use of Cassia gum as a food additive (Question N° EFSA-Q-2003-134). The EFSA Journal 389:1–16.

EFSA. 2008. Scientific opinion of the panel on food additives, flavourings, processing aids and food contact materials (AFC) on a request from the commission on the results of the study by McCann et al. on the effect of some colours and sodium benzoate on children’s behaviour. The EFSA Journal 660:1–54.


Mobley, J. R. 1991. 1,3,6,8-trihydroxymethylanthraquinone: Patent US 5039707 A.


