Chapter

Flavonoids and Pectins

Zhiping Zhang, Yanzhi He and Xinyue Zhang

Abstract

Pectins and flavonoids are two related groups of important secondary metabolites derived from plants. The interaction between pectins and flavonoids can affect their shelf-life stability, functionality, bioavailability, and bioaccessibility. In this chapter, we will concentrate on the current opinions on the flavonoids to understand how to classify this group of secondary metabolites, what biological and pharmacological activities they possess, and how to biosynthesize them in plants. We will then discuss the general strategies for the derivation of these small secondary compounds. The strategies comprise traditional plant extraction, chemical synthesis, and biosynthesis. We will also discuss the advantages and disadvantages of these three production strategies in the derivation of flavonoids and the future research directions in generating health-beneficial flavonoids using the biosynthetic strategy.

Keywords: flavonoids, pectins, secondary metabolites, interaction, pharmacological activity, biological activity, biosynthetic pathway, extraction, characterization, chemical synthesis, microbial cell factory, enzymatic synthesis, multienzyme synthetic system

1. Introduction

Peels represent a large percentage of the total weight of fruits, for example, 50–65% of *Citrus* fruits (lemon, lime, orange, and grapefruit) [1]. During processing of fruits for juice and oil extractions, the peels remain as the primary byproducts and become waste if not processed further, which can lead to serious environmental pollution [1]. Therefore, the fruit-processing industries are also interested in making use of these wastes.

The peels are also a good commercial source of pectins (polygalacturonic acid) and flavonoids [1]. The pectins are polysaccharide macromolecules contained in the primary cell wall of plants and involved in controlling cell wall ionic status, cell expansion, and separation [1]. Usually, the pectins are commercially extracted and isolated from *Citrus* peels and apple pomace. They are not only used as a gelling agent, dessert filling, or juice and milk stabilizer in food industry but also as a source of dietary fiber. Flavonoids are a large group of small secondary metabolites contained in the vacuoles and possess a wide range of biological activities, especially those with human health benefits [2, 3]. In the *Citrus* peels, flavonoids mainly include flavones (e.g., rhoifolin, isorhoifolin, diosmin, and neodiosmin), flavanones (e.g., eriocitrin, neoeriocitrin, narirutin, naringin, hesperidin, neohesperidin, poncirin, and neoponcirin), and flavonols (e.g., rutin) [4]. It has been reported that the highest concentrations of *Citrus* flavonoids occur in the peels [1]. Due to the importance of pectins and flavonoids in food, cosmetic, and medicinal industries, quite a number of studies have been focused on these two groups of compounds. Accordingly, a variety of approaches have been developed for efficient isolation of pectins and flavonoids from fruit peels and pomace. For example, to make a better use of yellow passion fruit rind, de Souza and colleagues have developed a strategy for sequential extraction of flavonoids and pectin [5].

As we know, pectins are abundant in the middle lamella of the plant cell walls with a gradual decrease in the content toward the plasma membrane, whereas flavonoids are naturally located within the cells [6]. Generally, flavonoids within the cells do not come into contact with the cell wall materials, such as pectins, celluloses, and hemicelluloses, prior to food processing. When fruits are processed and eaten, intracellular flavonoids can be released from the cells, leading to their interaction with substances like metal ions and plant cell wall materials [7, 8]. For example, procyanidins and anthocyanins can spontaneously bind to water-, chelator-, and sodium carbonate-soluble pectins. It is believed that the binding of flavonoids to cell wall materials results from noncovalent, hydrophobic, hydrogen bonding, and ionic interactions [9–11]. Recently, Chirug and colleagues have presented a novel possible mechanism that iron ions mediate the interaction between pectins and quercetin [6]. Such interaction might affect their shelf-life stability and functionality, as well as their bioavailability and bioaccessibility [6, 12]. Therefore, it could be of high importance to study their interaction. Since there are several reviews on the interaction [8, 13], we will not discuss it in this chapter. Instead, we will concentrate on understanding the current opinions on flavonoids, including the classification, biological activities, and biosynthetic pathway of these secondary compounds. We will then review the general strategies for derivation of these compounds, including the traditional plant extraction, chemical synthesis, and biosynthesis of these important small bioactive molecules in a microbial cell factory or an *in vitro* multienzyme synthetic platform. We will also discuss the advantages and disadvantages of these strategies and the future research directions in the field of flavonoid biosynthesis.

2. Classification and biological activities of flavonoids

Flavonoids belong to a class of secondary metabolites and comprise a large group of natural products that are widespread in higher plants but also found in mosses and liverworts [14, 15]. Chemically, flavonoid compounds have the basic structure of 15-carbon atoms with two phenolic rings connected by a 3-carbon chain [16], forming a C6-C3-C6 carbon framework (**Figure 1**). Generally, these small molecules can be divided into six major subclasses on the basis of the variations on the

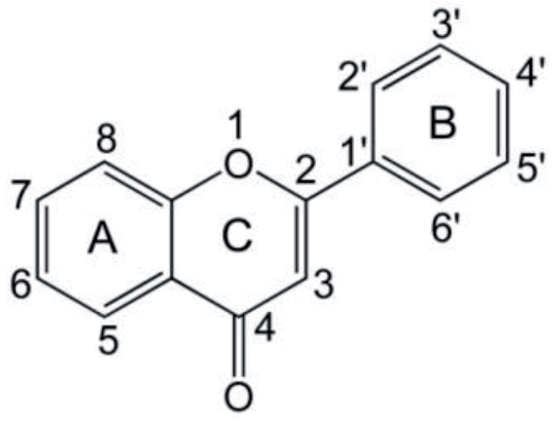

Figure 1.
Structure and atom numbering of flavonoid backbone.

heterocyclic C-ring and the degree of oxidation: the flavanols, flavones, flavonols, flavanones, anthocyanidins, and isoflavones [2, 16–18]. The flavonoids can exist in a free aglycone form but are often glycosylated (most commonly glucose), and the glycosylation in turn increases their water solubility [19].

The flavonoids are involved in the formation of plant pigments [20] and protect plants against pathogens, herbivores, and UV radiation [21]. However, the study of flavonoids, like that of most natural products, has emerged from the search of new compounds with promising pharmacological properties. After decades of endeavors, scientists have found that flavonoids possess a wide variety of biological and pharmacological properties, which leads to numerous studies on these secondary metabolites. These health-beneficial properties include antiangiogenic [22], antibacterial [23–27], anti-cancer [24, 28], anti-inflammatory [28–33], antiglycating [34], antimalarial [35], antimicrobial [36–42], anti-oxidant [26, 36, 38, 42–51], anti-platelet [48], anti-proliferation [52], agonistic/antagonistic [53], ammonia-lowering and regulation of urea cycle [54], anxiolytic [55], atheroprotective [56], cardioprotective and hypouricemic [57], cytotoxic [51, 58], endocrine disrupting [59], free radical-scavenging [31–33, 39, 40, 46, 51, 52, 58, 60–66], hepatoprotective [67], leishmanicidal [68], neuroprotective [69], photoprotective [43], and trypanocidal activities [68, 70]. In addition, the flavonoids can inhibit eukaryotic protein synthesis [71] and a variety of important enzymes such as aggrecanase [72], aldose reductase [30, 73], alpha-glucosidase [60], cholinesterase [26, 74], protein tyrosine phosphatase and acetylcholinesterase [75], and tyrosinase [44, 64].

3. Biosynthetic pathway of flavonoids

After several decades of efforts, the pathway for flavonoid biosynthesis has been largely deciphered even though quite a number of details remain unknown (**Figure 2**). The flavonoids and their derivatives are biosynthesized by a variety of enzymes. These enzymes belong to different families [76], mainly including 2-oxoglutarate-dependent dioxygenase (2-ODD), cytochrome P450 hydroxylase, short-chain dehydrogenase/reductase (SDR), *O*-methyltransferase (OMT), and *O*-glycosyltransferase (GT). The 2-ODD, cytochrome P450, and SDR enzymes constitute the major pathway for flavonoid biosynthesis [76], and the OMT and GT enzymes are involved in modification of flavonoids. The involved 2-ODD enzymes mainly comprise flavanone 3-hydroxylase (F3H), flavonol synthase (FLS), flavone synthase I (FSI), anthocyanidin synthase (ANS), and flavonol 6-hydroxylase (F6H) [17, 76–81]. The related cytochrome P450 enzymes contain cinnamate 4-hydroxylase (C4H), isoflavone synthase (IFS), flavanone 2-hydroxylase (F2H), flavone synthase II (FSII), flavonol 6-hydroxylase (F6H), flavonoid 3′-hydroxylase (F3’H), flavonoid 3′,5′-hydroxylase (F3′5′H), isoflavone 2′-hydroxylase (I2′H), and isoflavone 3′-hydroxylase (I3′H) [17, 18, 76, 80, 82, 83]. The SDR enzymes participating in flavonoid biosynthesis include dihydroflavonol 4-reductase (DFR) and anthocyanidin synthase (ANR) [76]. Interestingly, the flavone synthase (FS) activity is specified either by a 2-ODD (FSI) or a P450 (FSII) enzyme in a plant species-dependent manner [84, 85]. Similarly, the flavonol 6-hydroxylase (F6H) activity is also endowed either by a 2-ODD [81, 86] or P450 [87, 88] enzyme in different plant species. These findings further increase the complexity of flavonoid biosynthesis.

Basically, biosynthesis of flavonoids can be arbitrarily divided into three major stages. The first stage (a.k.a the phenylpropanoid pathway) includes three successive chemical reactions catalyzed by phenylalanine ammonia-lyase (PAL), cinnamate 4-hydroxylase (C4H), and 4-coumaroyl:CoA ligase (4CL), respectively, to convert L-phenylalanine to 4-coumroyl-CoA. In addition, L-tyrosine can also

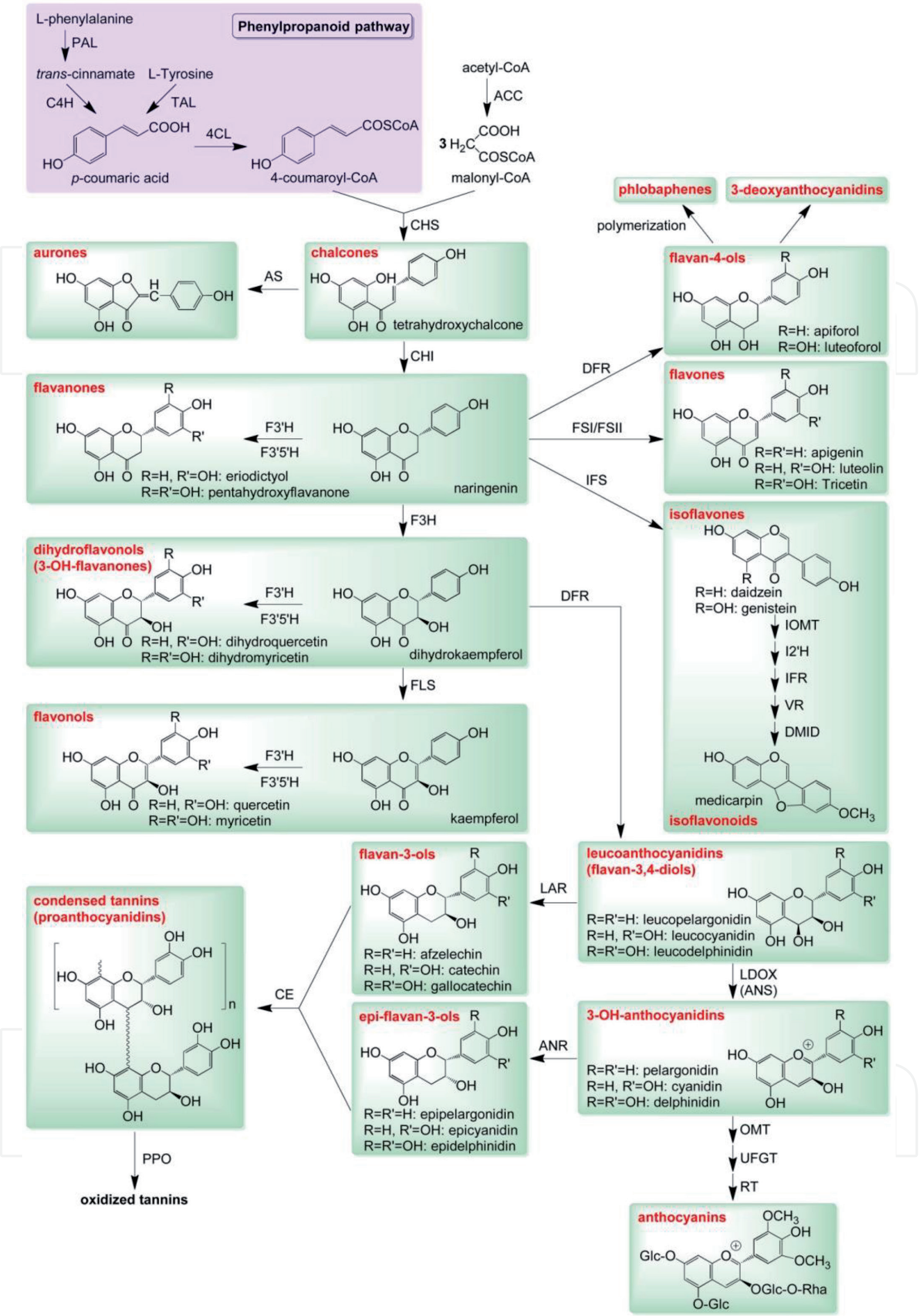

Figure 2.

Schematic of the biosynthetic pathway leading to the major subclasses of flavonoids. Adapted from [10, 12, 68]. 4CL, 4-coumaroyl:CoA ligase; ACC, acetyl CoA carboxylase; ANR, anthocyanidin reductase; ANS, anthocyanidin synthase; AS, aureusidin synthase; C4H, cinnamate 4-hydroxylase; CE: condensing enzyme; CHI, chalcone isomerase; CHS, chalcone synthase; DFR, dihydroflavonol 4-reductase; DMID, 7,2'-dihydroxy-4'-methoxyisoflavanol dehydratase; F3H, flavanone 3-hydroxylase; F3'H, flavonoid 3'-hydroxylase; F3'5'H, flavonoid 3',5'-hydroxylase; FLS, flavonol synthase; FSI/FSII, flavone synthase I/II; I2'H, isoflavone 2'-hydroxylase; IFR, isoflavone reductase; IFS, isoflavone synthase; IOMT, isoflavone O-methyltransferase; LAR, leucoanthocyanidin reductase; LDOX, leucoanthocyanidin dioxygenase; OMT, O-methyltransferase; PAL, phenylalanine ammonia-lyase; PPO, polyphenol oxidase; RT, rhamnosyltransferase; TAL, tyrosine ammonia-lyase; UFGT, UDP flavonoid glucosyltransferase; VR, vestitone reductase.

participate in the flavonoid biosynthesis via two successive enzymatic reactions catalyzed by tyrosine ammonia lyase (TAL) and 4CL, respectively. The second stage is crucial for the biosynthesis of flavonoids, in which the backbones of major subclasses of flavonoids are formed. This stage begins from the formation of chalcone by conversion of the 4-coumroyl-CoA from the first stage and the malonyl-CoA from carboxylation of acetyl-CoA. Chalcone synthase (CHS), an entry point enzyme into the pathway, catalyzes this chemical reaction by conversion of one molecule of 4-coumroyl-CoA and three molecules of malonyl-CoA to one molecule of chalcone (e.g., tetrahydroxychalcone). Then, the chalcone molecule is cyclized to form a flavanone (e.g., naringenin) by chalcone isomerase (CHI) and an aurone (e.g., aureusidin) by aureusidin synthase (AS). The flavanone can be further converted to dihydroflavonol by F3H and then flavonol by FLS. Alternatively, the flavanone molecule can also be converted to a flavone by FS, a flavanol by DFR, an isoflavone by IFS, and an anthocyanidin by a series of successive enzymatic reactions catalyzed by F3H, DFR, and leucoanthocyanidin dioxygenase (LDOX), respectively. The resulting anthocyanidin molecule can be further modified to form anthocyanins by a series of chemical modifications by OMT, UDP flavonoid glucosyltransferase (UFGT), and rhamnosyltransferase (RT). The third stage is mainly involved in various chemical decorations of flavonoids. Generally, natural flavonoids are often extensively modified by chemical reactions, including glycosylation and methylation [76], acylation [89], sulfonation [90, 91], prenylation [92, 93], and galloylation [94], which further contribute to the structural and functional diversity of flavonoids.

4. Derivation of flavonoids

Due to the intrinsic health benefits possessed by flavonoids, numerous approaches have been developed during the past decades for the derivation of a wide range of flavonoids. Basically, these approaches can be divided into three major categories: traditional plant extraction, chemical synthesis, and biosynthesis.

4.1 Traditional plant extraction via organic solvents

Traditionally, flavonoids are extracted from various plant species, which currently remains the most commonly used methods. During the past decades, researchers have developed plenty of methods to improve the yield and purity of flavonoids derived from plants. Generally, the plant tissues are air-dried and ground into powder for extraction via organic solvents (most commonly methanol and ethanol), and the extracts are then subjected to successive fractionation with other organic solvents (most commonly petroleum ether, chloroform, ethyl acetate, and n-butyl alcohol), followed by repeated silica gel and Sephadex LH-20 column chromatographies [44, 95]. The yield of plant-derived flavonoids can be improved by ultrasonic wave- [96], microwave- [97], and enzyme-assisted extraction [98]; aqueous two-phase extraction [99]; and a combination of these modifications [100]. The isolated flavonoids are then subjected to polyamide thin plate chromatography (TLC), high performance liquid chromatography (HPLC), electrospray ionization mass spectrometry (ESI-MS), and nuclear magnetic resonance (NMR) analyses to determine their identity and purity [2, 3]. Due to the high solubility of most flavonoids in organic solvents, this strategy often demonstrates a high efficiency in the derivation of flavonoids from plant tissues. However, the disadvantage of the plant extraction is obvious. Due to the very low content of most flavonoids in plant tissues, the extraction and isolation of flavonoids often requires multiple steps and plenty of time, labor, and organic solvents, which greatly increase the production cost. Moreover, different plant tissues often need to develop different approaches for processing, which makes the extraction more complicated and further increase the cost for the production of flavonoids. Therefore, this approach is not cost-effective, and it is crucial to develop alternative strategies to reduce the cost for producing flavonoids.

4.2 Chemical synthesis of flavonoids

Another approach for producing flavonoids is chemical synthesis. Basically, there are two strategies for chemical synthesis of flavones, that is, the chalcone route and the Baker-Venkataraman method [101]. Even though there are a few successful examples, chemical synthesis of flavonoids is often very complicated and involved in many steps [2]. It requires toxic reagents and extreme reaction conditions [3, 102]. Chiral synthesis and subsequent modifications further increase the difficulty of this approach in the production of flavonoids [3]. Moreover, the multistep chemical reactions often produce quite a number of intermediate products with a high similarity in structure, which further increases the difficulty in purification of the desired products. Therefore, chemical synthesis is not economically feasible for the mass production of flavonoids [3].

4.3 Biosynthesis of flavonoids

Since the biosynthetic pathway of flavonoids is largely elucidated in plants [20], other promising alternative strategies have been developed to produce these secondary compounds [2, 103–106]. One of these alternative strategies is to produce flavonoids in a microbial cell factory. It has been well known that *Escherichia coli* and *Saccharomyces cerevisiae* are the two most commonly used model organisms for the construction of a microbial cell factory. There are quite a few paradigms for the production of flavonoids using this strategy. For example, eriodictyol has been produced using ʟ-tyrosine as a substrate in *E. coli* BL21(DE3) genetically modified by *TAL, 4CL, CHS, CHI, F3H*, and *F3'H* genes and the production can reach up to 107 mg/L by further introducing three other genes *acs*, *accBC*, and *dtsR1* to enhance the availability of malonyl-CoA [103]. Kaempferol has been produced in a microbial cell factory by introducing a *de novo* biosynthetic pathway into *S. cerevisiae*, and the biosynthesis has been further improved by introducing two more pathways to enhance the generation of acetyl-CoA and malonyl-CoA [107]. Obviously, this strategy circumvents some inherent disadvantages of traditional plant extraction and chemical synthesis. However, not all genetically modified microbes can produce desired products due to the well-known complexity of a microbial cell system, the incompatibility of artificially synthesized genetic elements in host cells, the growth inhibition of host cells by desired and intermediate products, and the instability of an engineered biosystem itself [2, 108].

Recently, we have developed an *in vitro* platform to produce flavonoids by constructing a multienzyme synthetic system to convert naringenin into kaempferol in one pot [2]. After optimizing a series of reaction parameters, including the components and pH value of the buffer system, reaction temperature and time, and total amount and ratio of the enzymes, the production yield can reach up to 37.55 ± 1.62 mg/L within 40–50 min with a conversion rate of 55.89% ± 2.74% [2]. The advantages of this strategy are obvious. It is time- and labor-saving. The reaction conditions are easy to control accurately. Due to the clearness in the buffer components and the lack of complex physiological regulation as occurred in the microbial cell factory, it is possible to easily make further optimization in the future.

It is also much easier to purify desired products from this *in vitro* synthetic system than from the cell factory because of the simplicity of the components in the system. In addition, the strategy is highly cost-effective because of the cheap chemicals and recombinant proteins used in this system. More importantly, the system is easy to scale up and therefore possesses a huge industrialization potential. It also provides a guide for other secondary metabolites to produce economically. However, problems still exist in this production strategy. For example, due to the lack of P450-reductase function, prokaryotically expressed cytochrome P450 enzymes lose their enzymatic activities [109]. To achieve a functional expression, Leonard and colleagues fused a plant P450 enzyme gene *F3′5′H* with its redox partner cytochrome P450 reductase gene *cpr* from *Catharanthus roseus* and successfully produced a hydroxylated flavonol quercetin from *p*-coumaric acid in *E. coli* by simultaneous coexpression of the fusion protein with 4CL, CHS, CHI, F3H, and FLS [110], which provides a guide to solve this kind of problem. To further improve the efficiency of the biosynthetic system, future research should be focused on screening key enzymes with high activities from various plants, mutation of genes encoding key enzymes to enhance their activities, and immobilization of the highly active enzymes to inert carriers.

5. Conclusions

Pectins and flavonoids are two distinctive classes of bioactive secondary metabolites presented in the fruit peels and used in food industry. The flavonoids can be divided into six major subclasses, including the flavanols, flavones, flavonols, flavanones, anthocyanidins, and isoflavones, and their flavonoid biosynthetic pathway has been largely elucidated. These natural small compounds possess a wide range of health-beneficial properties and can be derived by traditional plant extraction via organic solvents, chemical synthesis, and biosynthesis by constructing a microbial cell factory or an *in vitro* multienzyme synthetic system.

Conflict of interest

The authors declare that they have no competing financial interests.

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