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The chemistry, distribution, and metabolic modifications of fruit flavonols

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Abstract

Fruits are considered as healthy foods because they provide a rich source of vitamins, antioxidants and other nutrients, including a range of essential bioactive flavonoid compounds. Flavonols, with diverse chemical properties and biological activities, are the most ubiquitous flavonoids that occur naturally in fruits and they are nutritionally important to animals and humans. Numerous investigations have emphasized that significant intake of dietary flavonols is associated with lower incidences of degenerative diseases. Here, we review current knowledge concerning the molecular structures, composition and distribution, regulation, and structural modification of fruit flavonols. In addition, we consider biotechnological approaches to enhance the levels of flavonols in plants or microorganism. An understanding of the factors determining production of flavonols in fruit crops will improve breeding programs and facilitate the production of fruits or bio-products with desirable contents of bioactive flavonols of benefit to humans.

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Introduction

With the improved awareness of nutrition and health worldwide, the demand for healthy dietary components has received increasing attention, and the consumption of nutrient supplements has been increasing. Numerous studies have emphasized that fruits confer a protective effect against human degenerative diseases such as diabetes, obesity, cardiovascular disease, and other chronic diseases, due to inherent richness in flavonoid compounds[1,2]. Fruits have been noted to play significant roles in nutrition and human health, especially as sources of vitamins, minerals, dietary fiber, and bioactive compounds[3–5].

Flavonols are by far the most widespread flavonoids, and naturally exist in plant vacuoles in the form of their glycoside derivatives. Currently, at least 15 flavonol aglycones have been identified in fruits, of which quercetin, kaempferol and myricetin are the most common ones. Glycosylation, hydroxylation, methylation and acylation enriches the types of flavonol derivatives present. It has been shown that flavonols play an important role in plant growth and development and resistance to stress, including regulating auxin transport, affecting pollen development, promoting lateral root formation, and responding to, and protecting against, ultraviolet (UV) light[6–10]. As bioactive compounds, flavonols are known to exhibit antioxidative, anti-inflammatory, anticancer and other pharmacological activities, and help prevention of cardiovascular disease and diabetes[11–13]. With the development of new chromatographic techniques and molecular biology methods, more flavonols have been identified in different fruits, and the metabolic mechanisms affecting flavonol accumulation have been analyzed. However, most reviews of flavonols in recent years have focused on health benefits and bioavailability[14,15] and information about metabolic mechanisms that determine the accumulation of specific flavonols in dietary fruits is often overlooked.

Here, we review current knowledge concerning molecular structures, distribution, biosynthetic mechanisms, transcriptional regulation, and metabolic engineering of fruit flavonols. Particular attention is paid to the roles of key structural enzymes, other proteins that add specific chemical modifications that affect structure and properties, and transcription factors important in regulating the biosynthesis pathway. Plant responses to environmental factors that influence accumulation of flavonols are also highlighted. Understanding the knowledge of molecular mechanisms controlling flavonol biosynthesis will facilitate future bioengineering programs to produce desirable levels of targeted bioactivities in our dietary fruits.

Chemistry and distribution

Structure of identified flavonols

Flavonols are constructed from 15-carbon skeletons and are composed of two aromatic rings (A and B ring) connected via a three-carbon chain (C ring) to form a basic diphenylpropane backbone (C6-C3-C6) with hydroxyl groups at the carbon 3 position (Fig. 1a). The A ring is normally formed from three malonyl-CoA molecules generated via the acetate

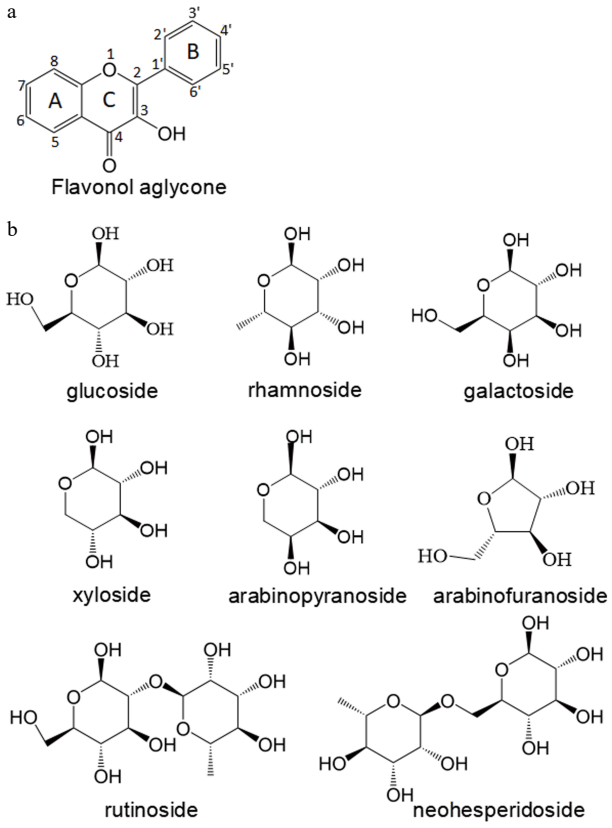

Fig. 1 General structure of (a) flavonol aglycones and (b) main glycosides.

pathway and exhibits a characteristic hydroxylation pattern at the carbon 5 and 7 sites. The B ring carbon originates from *p*-coumaroyl-CoA produced from phenylalanine via the shikimate pathway, and is often hydroxylated at carbon 4′, 3′4′, or 3′4′5′ positions (Table 1). Among the flavonol aglycones identified in fruits, kaempferol is the predominant structure and most other types including quercetin, myricetin, isorhamnetin, morin, laricitrin, gossypetin, kaempferide, natsudaidain, quercetagetin, syringetin, sexangularetin, rhamnetin are considered to be kaempferol derivatives carrying substituted hydroxyl groups or methyl groups at the different positions of the flavonol skeletons (Table 1). For example, quercetin, a 3′-hydroxykaempferol, is widespread in fruits[16]. Morin is hydroxylated at the 2′ carbon of kaempferol and accumulates mainly in mulberry[17]. Kaempferide, a 4′-*O*-methylkaempferol, occurs in grape (*Vitis vinifera*)[18]. Galangin and fisetin are not regarded as kaempferol derivatives, however. Galangin has no OH group on the B ring and has been reported in grape[19] and blueberry (*Vaccinium* L.)[20]. Fisetin is not hydroxylated at the 5-carbon position of the A ring and occurs in mulberry[17]. Hydroxylated flavonol aglycones are highly unstable *in vivo*, and methylation modifications help to enhance stability. Isorhamnetin, with methylation at the 3′ site, is the most common methylated flavonol aglycone and occurs mainly in pear (*Pyrus communis* L.)[21] and peach (*Prunus persica* L.)[22].

Flavonols are most frequently found in nature in the form of glycosides due to the unstable physicochemical properties of their aglycones. Most of the sugar ligands attached to flavonol aglycones are glucoside, galactoside, rhamnoside, xyloside, and arabinoside, and these sugar moieties usually accumulate in the form of mono-, or diglycosides in fruits (Fig. 1b). The glycosidic linkage can be divided into *O*-glycosidic bonds and C-glycosidic bonds. Sugar ligands are generally attached to an oxygen atom at carbon 3, 5, 7, 8, 3′, 4′, or 5′ positions to form flavonol *O*-glycosides, of which 3-oxyglycosides are the most common ones, while flavonol *C*-glycosides are attached to the carbon atom at position 6 or 8. In addition, hydroxyl or acyl groups can also be attached to the parent ring of flavonols, which contribute to the structural diversity of flavonols and play an important role in their diverse biological functions.

Flavonol compounds in fruits

Different types of flavonol metabolites are found in specific fruit, and sugar moieties are most commonly attached to an oxygen atom at carbon 3 (Table 2). With the development and application of high-resolution mass spectrometry, more and more flavonol compounds have been identified, among which kaempferol and quercetin glycosides are the most common dietary flavonols and can be detected in most of fruits, while the distribution of other flavonol glycosides is relatively limited (Table 2). Apple (*Malus domestica*) is well-

Table 1. Summary of flavonol aglycones identified in fruits.

Aglycones	5	6	7	8	2′	3′	4′	5′	6′	Reference
Kaempferol	OH	H	OH	H	H	H	OH	H	H	[16]
Quercetin	OH	H	OH	H	H	OH	OH	H	H	[16]
Myricetin	OH	H	OH	H	H	OH	OH	OH	H	[16]
Galangin	OH	H	OH	H	H	H	H	H	H	[19,20]
Gossypetin	OH	H	OH	OH	H	OH	OH	H	H	[30]
Kaempferide	OH	H	OH	H	H	H	OCH_3	H	H	[25]
quercetagetin	OH	OH	OH	H	H	OH	OH	H	H	[31]
Laricitrin	OH	H	OH	H	H	OCH_3	OH	OH	H	[25]
Morin	OH	H	OH	H	OH	H	OH	H	H	[17]
Isorhamnetin	OH	H	OH	H	H	OCH_3	OH	H	H	[16]
Natsudaidain	OCH_3	OCH_3	OCH_3	OCH_3	H	H	OCH_3	OCH_3	H	[32]
Syringetin	OH	H	OH	H	H	OCH_3	OH	OCH_3	H	[33]
Sexangularetin	OH	H	OH	OCH_3	H	H	OH	H	H	[34]
Rhamnetin	OH	H	OCH_3	H	H	OH	OH	H	H	[17]
Fisetin	H	H	OH	H	H	H	OH	OH	H	[17]

Table 2. Distribution of divergent flavonols in fruits. The first three listed compounds are the major flavonols in each fruit.

Fruit species	Divergent flavonols	Reference
Apple 	Quercetin 3-*O*-rhamnoside; Quercetin 3-*O*-galactoside; Quercetin 3-*O*-arabinoside; Quercetin 3-*O*-glucoside; Quercetin 3-*O*-xyloside; Quercetin 3-*O*-robinobioside; Quercetin 3-*O*-rutinoside; Quercetin 3-*O*-neohesperidoside; Kaempferol 3-*O*-galactoside; Kaempferol 3-*O*-arabinoside; Isorhamnetin 3-*O*-galactoside; Isorhamnetin 3-*O*-glucoside; Rhamnetin 3-*O*-rutinoside	[10,35]
Pear 	Quercetin 3-*O*-glucoside; Isorhamnetin 3-*O*-galactoside; Isorhamnetin 3-*O*-rutinoside; Isorhamnetin 3-*O*-malonylglucoside; Isorhamnetin hexoside; Isorhamnetin 3-*O*-malonylgalactoside; Isorhamnetin 3-*O*-glucoside; Isorhamnetin; Quercetin 3-*O*-galactoside; Quercetin 3-*O*-galactosyl-glucoside; Quercetin 3-*O*-rutinoside; Quercetin 3-*O*-arabinoside; Quercetin *O*-acetylhexoside; Quercetin 5-*O*-malonylhexosyl-hexoside; Quercetin 7-*O*-malonylhexosyl-hexoside; Quercetin 4′-*O*-glucoside; Kaempferol 3-*O*-galactoside; Kaempferol 3-*O*-rutinoside; Kaempferol 3-*O*-acetylglucoside; Kaempferol 3-*O*-rhamnoside; Rhamnetin hexoside;	[21,36,37,38]
Peach 	Quercetin 3-*O*-glucoside; Isorhamnetin 3-*O*-rutinoside; Isorhamnetin 3-*O*-glucoside; Quercetin 3-*O*-rhamnoside; Quercetin 3-*O*-galactoside; Quercetin 3-*O*-rutinoside; Kaempferol 3-*O*-rutinoside; Kaempferol 3-*O*-glucoside	[22,28]
Loquat 	Quercetin 3-*O*-glucoside; Quercetin 3-*O*-galactoside; Kaempferol 3-*O*-sophoroside; Quercetin 3-*O*-rhamnoside; Quercetin 3-*O*-rutinoside; Quercetin 3-*O*-neohesperidoside; Quercetin 3-*O*-sophoroside; Quercetin-3-O-galactosyl-glucoside; Quercetin 3-*O*-sambubioside; Kaempferol 3-*O*-neohesperidoside; Kaempferol 3-*O*-sambubioside; Kaempferol 3-*O*-rhamnoside; Kaempferol 3-*O*-glucoside; Kaempferol 3-*O*-rutinoside	[39,40]
Hawthorn 	Quercetin 3-*O*-galactoside; Quercetin 3-*O*-glucoside; Quercetin 3-*O*-rutinoside; Kaempferol 3-*O*-glucoside; Kaempferol 3-*O*-neohesperidoside; Sexangularetin; Sexangularetin 3-*O*-neohesperidoside; Sexangularetin 3-*O*-glucoside	[27,34]
Grape 	Quercetin 3-*O*-glucoside; Quercetin 3-*O*-glucuronide; Myricetin 3-*O*-glucoside; Quercetin 3-*O*-galactoside; Quercetin 3-*O*-rutinoside; Quercetin; Kaempferol 3-*O*-galactoside; Kaempferide coumaroylhexoside; Kaempferol 3-*O*-glucoside; Myricetin 3-*O*-glucuronide; Myricetin dihexoside; Myricetin glucoside-glucuronide; Isorhamnetin 3-*O*-glucoside; Isorhamnetin 3-*O*-glucoside; Isorhamnetin glucuronide; Isorhamnetin coumaroylglucoside; Isorhamnetin; Laricitrin 3-*O*-glucoside; Syringetin-dihexoside; Syringetin 3-*O*-glucoside; Syringetin 3-*O*-galactoside	[18,23,41]
Blueberry 	Quercetin 3-*O*-galactoside; Quercetin 3-*O*-rhamnoside; Quercetin 3-*O*-rutinoside; Quercetin 3-*O*-glucoside; Quercetin 3-*O*-pentoside; Quercetin 3-*O*-glucoside acetate; Quercetin 3-*O*-arabinoside; Myricetin 3-*O*-galactoside; Myricetin 3-*O*-glucoside; Myricetin 3-*O*-pentoside; Myricetin 3-*O*-rhamnoside; Kaempferol 3-*O*-rutinoside; Kaempferol 3-*O*-glucoside; Laricitrin 3-*O*-galactoside; Laricitrin 3-*O*-glucoside; Laricitrin 3-*O*-rhamnoside; Laricitrin 3-*O*-pentoside; Isorhamnetin 3-*O*-galactoside; Isorhamnetin 3-*O*-rhamnoside; Isorhamnetin 3-*O*-glucoside; Syringetin 3-*O*-glucoside; Syringetin 3-*O*-rhamnoside; Syringetin 3-*O*-pentoside; Syringetin 3-*O*-galactoside	[20,25,42]
Bayberry 	Myricetin 3-*O*-rhamnoside; Quercetin 3-*O*-galactoside; Quercetin 3-*O*-rhamnoside; Myricetin 3-*O*-glucoside; Myricetin deoxyhexoside-gallate; Quercetin 3-*O*-glucuronide; Quercetin 3-*O*-arabinoside; Kaempferol 3-*O*-rhamnoside; Kaempferol 3-*O*-galactoside; Kaempferol 3-*O*-glucoside; Isorhamnetin 3-*O*-rhamnoside; Isorhamnetin 3-*O*-glucoside	[11,43]
Mulberry 	Quercetin 3-*O*-rutinoside; Kaempferol 3-*O*-glucoside; Quercetin 3-*O*-glucoside; Quercetin 3-*O*-rhamnoside; Quercetin 3-*O*-galactoside; Quercetin 3-*O*-glucuronide; Quercetin; Myricetin 3-*O*-rhamnoside; Isorhamnetin 3-*O*-glucoside; Myricetin; Kaempferol; Isorhamnetin; Fisetin; Morin; Rhamnetin; Galangin; Kaempferide	[17]
Strawberry 	Quercetin glucuronide; Quercetin pentoside; Kaempferol coumaroylhexoside; Quercetin 3-*O*-glucoside; Quercetin 7-*O*-glucoside; Quercetin 4′-*O*-glucoside; Kaempferol 3-*O*-glucoside; Kaempferol 7-*O*-glucoside; Kaempferol 4′-*O*-glucoside; Kaempferol glucuronide; Isorhamnetin 3-*O*-glucoside; Isorhamnetin 7-*O*-glucoside; Isorhamnetin 4′-*O*-glucoside; Isorhamnetin glucuronide	[24,44]
Cherry 	Quercetin 3-*O*-rutinoside; Kaempferol 3-*O*-rutinoside; Quercetin 3-*O*-glucosil-rutinoside; Quercetin 3-*O*-rhamnoside; Quercetin 3-*O*-galactoside; Quercetin 3-*O*-glucoside; Quercetin 3-*O*-diglucoside; Kaempferol 3-*O*-glucoside; Kaempferol 3-*O*-rhamnoside; Isorhamnetin 3-*O*-rutinoside	[45]
Tomato 	Quercetin 3-*O*-rutinoside; Kaempferol 3-*O*-rutinoside; Quercetin glucosyl-glucoside rhamnoside; Quercetin 3-*O*-rutinoside-7-*O*-glucoside; Quercetin 3-*O*-glucoside; Quercetin 3,7-*O*-glucoside; Kaempferol glucosyl-glucoside rhamnoside; Kaempferol 3-*O*-glucoside; Kaempferol 3,7-*O*-glucoside; Kaempferol 3-*O*-rutinoside-7-*O*-glucoside	[46,47,48]
Mango 	Quercetin 3-*O*-galactoside; Quercetin 3-*O*-glucoside; Quercetin 3-*O*-xyloside; Quercetin diglycoside; Quercetin 3-*O*-arabinopyranoside; Quercetin 3-*O*-arabinofuranoside; Quercetin 3-*O*-rhamnoside; Rhamnetin 3-*O*-galactoside; Rhamnetin 3-*O*-glucoside; Rhamnetin 3-*O*-galactopyranoside; Rhamnetin 3-*O*-glucopyranoside; Kaempferol 3-*O*-glucoside; Quercetin; Isorhamnetin 3-*O*-glucoside	[26]
Litchi 	Quercetin rhamnosyl-rutinoside; Quercetin 3-*O*-rutinoside; Isorhamnetin rhamnosyl-rutinoside; Quercetin rhamnosyl-glucoside; Isomer of Quercetin rhamnosyl-glucoside; Quercetin 3-*O*-rutinoside-*O*-rhamnoside; Quercetin glucosyl-rutinoside; Quercetin rhamnosyl-glucosyl-rutinoside; Kaempferol rhamnosyl-rutinoside; Kaempferol 3-*O*-rutinoside-*O*-rhamnoside; Kaempferol 3-*O*-rutinoside; Keampferol rhamnosyl-glucosyl-rutinoside; Isorhamnetin 3-*O*-rutinoside; Isorhamnetin 3-*O*-rutinoside-*O*-rhamnoside; Isorhamnetin glucosyl-rutinoside; Myricetin rutinoside	[49,50]

Table 2 (continued)

Fruit species	Divergent flavonols	Reference
Citrus 	Quercetin 3-*O*-glucoside; Quercetin 3-*O*-rutinoside; Quercetin 7-*O*-glucoside; Quercetin 7-*O*-rutinoside; Quercetin 3-*O*-glucofuranoside; Kaempferol 3-*O*-glucoside; Kaempferol 3-*O*-rutinoside; Kaempferol 7-*O*-glucoside; Kaempferol 7-*O*-rutinoside	[51,52]
Kiwi fruit 	Quercetin 3-*O*-rutinoside; Quercetin 3-*O*-glucoside; Kaempferol 3-*O*-rutinoside; Quercetin 3-*O*-arabinofuranoside; Quercetin 3-*O*-rhamnoside; Quercetin 4′-*O*-glucoside; Kaempferol 3-*O*-galactoside; Kaempferol 3-*O*-rhamnoside; Kaempferol 3-*O*-robinobioside; Kaempferol 3,7-*O*-diglucoside 8-prenyl derivative; Myricetin 3-*O*-galactoside; Syringetin	[33]

known for accumulating quercetin glycosides and estimates obtained by comparing HPLC peak areas with standard curves indicate a content of about 150 mg kg^{-1} fresh weight (FW) quercetin 3-*O*-rhamnoside and 100 mg kg^{-1} FW quercetin 3-*O*-galactoside respectively[10]. Isorhamnetin and myricetin derivatives are less widespread flavonols compared to kaempferol and quercetin glycosides. It has been demonstrated that isorhamnetin metabolites are the major flavonols in pear with content of isorhamnetin 3-*O*-galactoside up to 65.15 mg kg^{-1} FW, quantified by comparing peak area with the standard curves using UPLC[21], and they have also been detected in peach[22], grape[23], strawberry (*Fragaria* × *ananassa*)[24] and blueberry[25]. Myricetin compounds are mainly distributed in berry fruits, especially Chinese bayberry (*Morella rubra*)[11], blueberry[25] and grape[18]. So far, little research has been carried out on less common flavonols in fruits. Rhamnetins have been detected mainly in mango (*Mangifera indica* L.)[26], and laricitrins and syringetins have been identified mainly in blueberry[25], while sexangularetins has been found only in hawthorn (*Crataegus laevigata*)[27]. However, the distribution pattern of flavonols in fruits depends on the degree of accessibility to previous illumination due to the fact that their formation is accelerated by light. For example, the content of flavonols is usually higher in the peel of peach and persimmon (*Diospyros kaki* Thunb.) than in the pulp[28,29]. Generally, flavonol glycosides are located mainly in the outer parts of fruits such as the peel and they decrease in concentration toward the central core.

Advances in the biochemistry of the flavonol pathways

Biosynthesis of flavonol aglycones

The mechanisms of flavonol biosynthesis have been widely elucidated and a simplified flavonol metabolic pathway is shown in Fig. 2. Chalcone synthase (CHS) catalyzes the first step in flavonol biosynthesis by converting substrates *p*-coumaroyl-CoA and malonyl-CoA to product naringenin chalcone[53]. The following second catalytic reaction performed by chalcone isomerase (CHI) and chalcone reductase (CHR) is very important for the corresponding formation of 5,7-oxo and 5-deoxy flavonols. CHI was confirmed to catalyze the stereospecific cyclization of naringenin chalcone to naringenin[54], which is a general precursor for 5,7-oxo flavonols. This step can also proceed spontaneously. CHR, which catalyzes the production of 6′-deoxy chalcone (isoliquiritigenin) through its effects on CHS catalyzed reaction[55], is a key enzyme mediating 5-deoxy flavonol biosynthesis. Flavanone 3-hydroxylase (F3H), flavonoid 3′-hydroxylase (F3′H), flavonoid 3′5′-hydroxylase (F3′5′H), and flavonol synthase (FLS) cover the core metabolic grid of flavonol biosynthesis and the production of different flavonols (Fig. 2). F3H and FLS belong to the 2-oxoglutarate-dependent dioxygenases protein family and catalyze 3-hydroxylation and oxidation of carbon 2 and carbon 3 of flavonols on the C ring. F3′H and F3′5′H are members of the cytochrome P450 protein family and catalyze 3′4′-hydroxylation and 3′4′5′-hydroxylation on the B ring. Thus, the CHI-catalyzed compounds naringenin and liquiritigenin can be converted to the corresponding dihydrokaempferol and garbanzol by F3H[56,57], and then the dihydroflavonols dihydrokaempferol and garbanzol are converted to 5,7-oxo flavonol kaempferol and 5-deoxy flavonol resokaempferol by FLS[57,58]. The 5,7-oxo flavonols quercetin and myricetin are produced directly by FLS consuming the intermediates dihydroquercetin and dihydromyricetin[58,59], which are produced by two hydroxylases: flavonoid 3′-hydroxylase (F3′H) and flavonoid 3′5′-hydroxylase (F3′5′H) respectively[60,61]. Recently, a *F3′5′H* gene isolated from Chinese bayberry was postulated to be the important factor determining the accumulation of myricetin, because it drives pathway flux towards the trihydroxylated flavonol by hydroxylating kaempferol without the need for a dihydromyricetin specific FLS[62]. Isorhamnetin, a 5,7-oxo methylated flavonol, is produced by the addition of a methyl group to quercetin by *O*-methyltransferases (OMT)[63]. The 5-deoxy flavonol fisetin is produced by conversion from resokaempferol by F3′H[57].

Modification of flavonol aglycones

Glycosylation, hydroxylation, methylation and acylation are the major modification reactions resulting in the formation of a wide range of flavonol products. These modifications tend to alter the stability, solubility and cellular localization of the corresponding flavonol aglycones. In fruit species, a few genes have now been identified that are involved in catalyzing such decorations of flavonol derivatives.

Flavonols are largely glycosylated by uridine diphosphate glycosyltransferases (UGTs), which use uridine 5-diphosphate-sugars (UDP) such as UDP-glucoside, UDP-galactoside, UDP-rhamnoside as the donor molecule. Most fruit UGTs are reported to participate in the generation of 3-oxoglycosylated flavonols. For instance, the enzymes AY519364[64] from citrus (*Citrus sinensis*), AcF3GT2 from kiwifruit (*Actinidia chinensis*)[65], and MdUGT71B1 from apple[10] have been confirmed to catalyze the glucosylation of the 3-hydroxyl group of quercetin efficiently, while DkFGT from persimmon[66] and MdUGT75B1 from apple[10] preferentially galactosylated the 3-hydroxyl group of quercetin. In grapevine, VvGT5 was identified as a flavonol-3-*O*-glucuronosyltransferase that exhibited

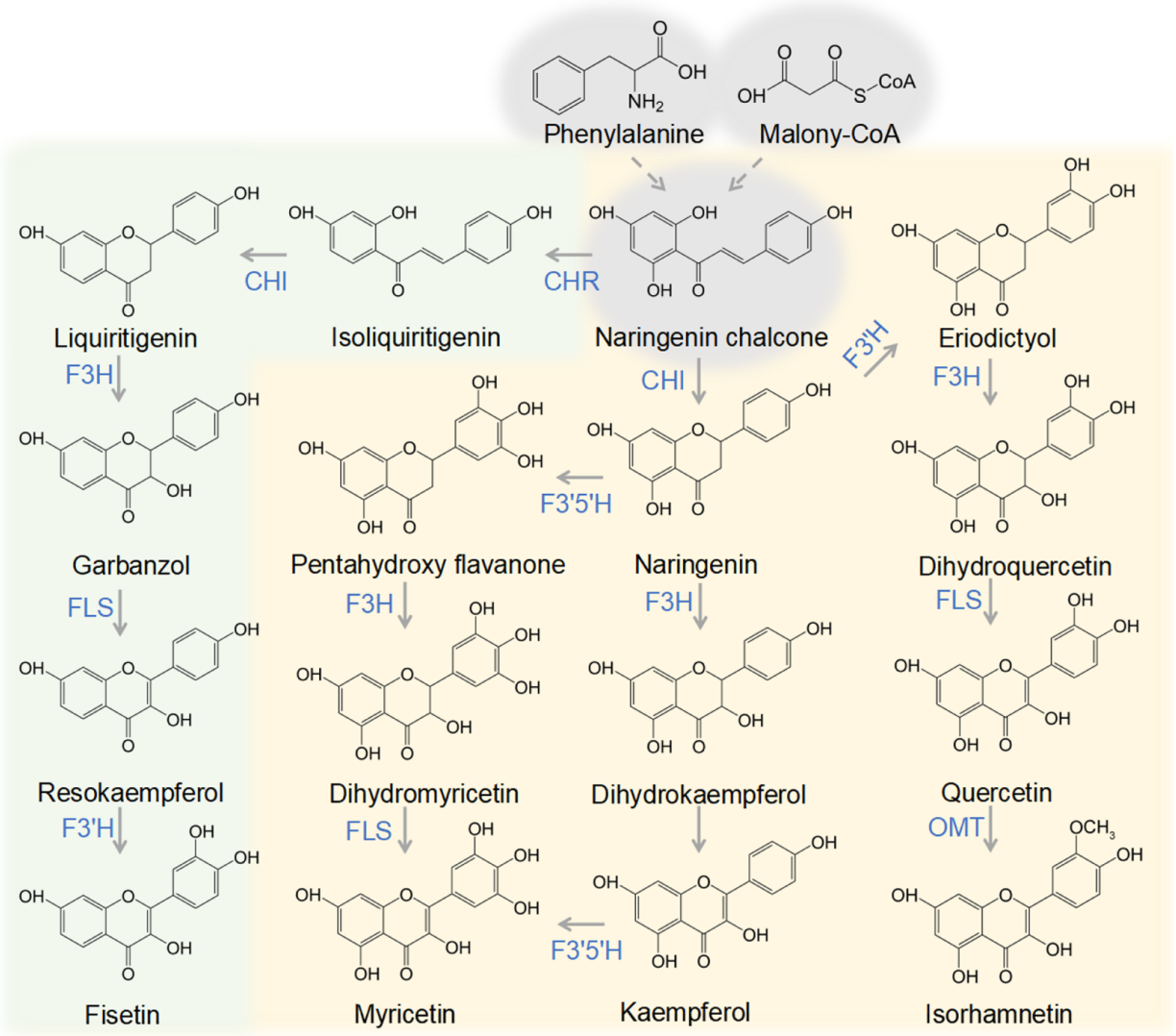

Fig. 2 Representative flavonol biosynthetic pathways. The pathways utilize naringenin chalcone, produced from phenylalanine and malonyl-CoA, highlighted in grey. The metabolic pathway of 5,7-oxo flavonols is highlighted in yellow, and biosynthetic pathways for 5-deoxy flavonols are highlighted in green. CHR: chalcone reductase; CHI: chalcone isomerase; F3H: flavanone 3-hydroxylase; F3'H: flavonoid 3'-hydroxylase; F3'5'H: flavonoid 3'5'-hydroxylase; OMT: *O*-methyltransferases; FLS: flavonol synthase.

a strong glucuronosyl transfer activity from UDP-glucuronic acid to kaempferol, quercetin and isorhamnetin. VvGT6 was demonstrated to be a bifunctional glycosyltransferase, which was capable of adding a UDP-glucose or UDP-galactose group to kaempferol, quercetin and isorhamnetin separately[67]. Strawberry UGTs have been reported to be capable of glycosylating at different hydroxyl positions[24]. Using recombinant enzymes, it was shown that both FaGT6 and FaGT7 were able to convert quercetin, kaempferol and isorhamnetin to the corresponding 3-*O*-glucosides, 7-*O*-glucosides, and 4'-*O*-glucosides, respectively. FaGT6 was capable of forming a 3'-*O*-monoglucoside and one diglucoside with quercetin as a substrate, while FaGT7 only formed 3'-*O*-monoglycoside but no diglucoside[24]. CsUGT76F1 from sweet orange has been shown to carry out glycosylation at the carbon 3 or 7 position of flavonoids, converting kaempferol and quercetin to the corresponding 3-*O*-glucosides, 7-*O*-glucosides, and 7-*O*-rhamnosides. However, the enzyme CsUGT76F1 was found to be capable of converting kaempferol to its 3,7-*O*-diglucoside but no quercetin 3,7-*O*-diglucoside product was formed with quercetin as a substrate[51]. In addition to showing preferences for different glycosylation positions, several fruit UGTs have been found to possess selectivity to receptor flavonol molecules. For example, citrus AY519364 glucosylated only the flavonol aglycones quercetin, kaempferol and myricetin[64], and strawberry UGT75T1 exhibited very strict substrate specificity and glucosylated only the flavonol galangin out of 33 compounds tested[68]. Thus, different fruit UGTs have obvious preferences for different flavonol aglycones and glycosylation sites.

Hydroxylation at carbon 3, 3' and 3'5' positions of flavonols is largely catalyzed by F3H, F3'H and F3'5'H discussed above, and hydroxylation at the carbon 6 and 8 positions is generally performed by flavonol 6-hydroxylase[69] and flavonoid 8-hydroxylase[70] separately. Methylation of flavonols is almost exclusively catalyzed by OMTs, and several fruit OMT genes

have been identified that methylate flavonols, for example from apple[71], tomato (*Solanum lycopersicon*)[72] and citrus[63]. However, no genes encoding enzymes functional in acylation have been verified in fruits so far. Future studies could address this issue and may reveal other target flavonol substrates and new decoration enzymes.

Regulation of flavonols biosynthesis

Regulatory genes in flavonol biosynthesis

The transcriptional control of flavonol biosynthesis genes is often regulated by myeloblastosis (MYB) transcription factors and has been extensively studied in fruits.

MYB genes belong to one of the largest transcription factor (TF) families in plants and modulate a number of different biological processes. In Arabidopsis, MYBs are divided into subgroups (SGs), according to sequence similarity and SG7 group members, including *MYB12*, *MYB11* and *MYB111*, have been confirmed as flavonol-specific factors[73,74]. In fruits, the SG7 MYBs, which have been identified as activators, have been comprehensively researched in grape, apple, pear, peach and other plants (Table 3). Generally, members of this subclade of MYBs, participate in flavonol accumulation by activating expression of structural genes encoding enzymes in the biosynthetic pathway. For example, apple MdMYB22 binds to the promoter of *FLS* directly to induce flavonol accumulation[75]. Overexpression of peach *PpMYB15* or *PpMYBF1*[22] or *Morella MrMYB12*[76] significantly induced the accumulation of flavonols in tobacco flowers. MYBs belonging to other subclasses, including SG4 (flavonoid repressors clade), SG5 (proanthocyanidin-related subclade), SG6 (anthocyanidin-related subclade) are also related to flavonol accumulation (Table 3). Different members of the SG4 subclass have been identified as both inhibitors and activators. For instance, strawberry FaMYB1 was identified as an inhibitor and heterologous expression of *FaMYB1* in tobacco resulted in a clear reduction in the levels of quercetin glycosides[77], while apple MdMYB3 was identified as an activator and higher levels of kaempferol and quercetin were observed in transgenic tobacco flowers overexpressing this gene than in wild type plants[78]. MYBs belong to the SG5 and SG6 subclasses have been shown to be activators, such as pear PbMYB9 (SG5)[8] and crabapple McMYB10 (SG6)[80].

In addition, several other transcription factors have been reported to be involved in the regulation of flavonol biosynthesis. The basic region/leucine zipper (bZIP) family transcription factors VvibZIPC22 and VvMYB114 from grape were identified as activators and shown to be involved in transcriptional regulation of flavonol metabolic pathway related genes[81,82]. Similarly, MdMYB8 from crabapple was confirmed as an active regulator of flavonol biosynthesis that activates the *MdFLS* promoter[83]. In apple, the promoter of *FLS* was activated by ELONGATED HYPOCOTYL 5 (HY5), which is involved in response to light and could be enhanced by the presence of MYB22[9]. Although studies on transcriptional regulation of flavonols have mostly been focused on MYBs, new regulatory mechanisms affecting the flavonol biosynthetic pathways should be given more attention.

Factors affecting the biosynthesis of flavonols

The biosynthesis of flavonols is determined by an intricate system of genetically controlled enzymes and influenced by extrinsic factors such as light in fruit species. Most research has shown that formation of flavonols is significantly accelerated by light. In grape, flavonols were shown to be the most drastically reduced flavonoid compounds following shading and leaf removal treatments, and this was related to *VvMYB12*-mediated reduction in expression of *VvFLS*. In contrast, exposure to sunlight substantially induced the accumulation of grape flavonols compared to shading[85]. Similarly, the content of flavonols in peels of apple exposed to sunlight were higher than shaded peels[90]. Further, flavonol accumulation in Cabernet Sauvignon grape was dramatically enhanced by increasing sunlight irradiance and exposure time[91]. However, the level of flavonols can be significantly changed in response to different shade treatments. In crabapple, for example, shading decreased the content of flavonols at 15 days after shading while it increased the level

Table 3. Summary of MYB and bZIP transcription factors characterized in a wide range of fruit species regulating flavonol accumulation.

Species	Genes	Metabolites	Subgroup	Reference
Fragaria ananasa	*FaMYB1*	Flavonol, Anthocyanin	SG4	[76]
Vitis vinifera	*VvMYB5a*	Flavonol, Anthocyanin	SG6	[84,85]
	VvMYBF1	Flavonol	SG7	[86]
	VvMYB12	Flavonol	SG7	[85]
	VvibZIPC22	Flavonol, Anthocyanin	bZIPC	[81]
	VvMYBA2	Flavonol, Anthocyanin	SG6	[87]
	VvMYB114	Flavonol, Anthocyanin	Unknown	[82]
Malus domestica	*MdMYB3*	Flavonol, Anthocyanin	SG4	[78]
	MdMYB22	Flavonol	SG7	[75]
Malus crabapple	*McMYB10*	Flavonol, Anthocyanin	SG6	[80]
	MdMYB8	Flavonol	Unknown	[83]
Pyrus bretschneideri	*PbMYB9*	Flavonol, Anthocyanin	SG5	[79]
	PbMYB12b	Flavonol	SG7	[37]
	PbMYB17	Flavonol	SG7	[36]
Prunus persica	*PpMYB15, PpMYBF1*	Flavonol	SG7	[22]
Morella rubra	*MrMYB12*	Flavonol	SG7	[77]
Solanum lycopersicum	*SlMYB12*	Flavonol	SG7	[88]
Citrus sinensis	*CsMYBF1*	Flavonol	SG7	[89]

of flavonols at 35 and 50 days after shading[92].

Flavonols are considered as effective UV-absorbing compounds, and are generally induced by UV light, particularly damaging UVB radiation. In grape, supplementing UV with white light treatment drastically increased the accumulation of flavonols by inducing the expression of *VvCHS2*, *VvCHS3*, *VvCHI1*, *VvF3H2*, *VvF3'5'H*, *VvFLS4*, *VvMYB12*, and *VvHY5* genes[93,94]. Conversely, the concentration of grape flavonols was greatly reduced in response to exclusion of UVB[95]. Similarly, lower levels of flavonols occurred in UVB-excluded apples compared to solar UVB-exposed fruits[9]. In several berry fruits such as blueberry[96], grape[97]and strawberry[98], it has been reported that UVC treatment significantly enhanced the content of flavonols.

The accumulation of flavonols in fruits is affected by other abiotic factors. Blackberries treated with methyl jasmonate (0.01 and 0.1 mM) had higher quercetin 3-*O*-glucoside and quercetin 3-*O*-rhamnoside content[99]. High medium pH values induced the content of flavonols in crabapple leaves, and this was related to up-regulation of *McFLS* transcript levels[100]. The plant growth regulator 24-epibrassinolide and 5-aminolevulinic acid up-regulated the expression of the structural gene *MdFLS*, which was decreased by brassinazole[101]. High nitrogen treatment reduced the overall content of total flavonoids in apple by 19.01%, although kaempferol-3-*O*-arabinoside increased while quercetin and rhamnetin derivatives decreased[35]. Temperature had little effect on the flavonol content of grape berry skins, although lower temperature (15 °C) increased the content with white and supplementary UV light conditions[93]. In apple, however, lower temperatures (10 °C) inhibited the accumulation of quercetin glycosides compared with 20 °C under both UVB and visible light irradiation[102].

Metabolic engineering of flavonol compounds

To our knowledge, dietary flavonols with potent bioactivity and good biosafety are regarded as natural health metabolites and are derived primarily from fruit sources. Engineering of fruits to enrich for desirable flavonols has recently become the focus of scientific attention. The directed manipulation of target gene expression is regarded as a useful tool to induce the accumulation of flavonol constituents especially in model fruit, such as tomatoes, which are consumed in large volumes. Overexpression of petunia *CHI* in tomato variety FM6203 produced 16.52 mg g^{-1} dry weight (DW) quercetin and 2.05 mg g^{-1} DW kaempferol, indicating increases of 66- and 57-fold over control peel extracts, respectively[103]. Subsequently, Luo et al.[46] introduced *AtMYB12* into the tomato MicroTom and Money Maker background separately and the contents of flavonols in transgenic fruits were increased to 72 mg g^{-1} DW and 48 mg g^{-1} DW on a whole-fruit basis, representing increases of up to 65-fold compared to control fruits. Based on the *AtMYB12* mediated genetic background, a crossed phenotype termed Indigo (anthocyanin-enrich *Del/Ros*1 parent × flavonol-enrich *AtMYB12* parent) tomato had even greater content of flavonols in fruits, approximately 3-fold more than parental AtMYB12 tomatoes[104]. In addition, introducing *AtMYB11* into tomato resulted in increased flavonol levels in fruit peels but showed a smaller effect on flavonols compared to *AtMYB12*[48]. With the continuous development and improvement of experimental technology, the prospects of enhancing the accumulation of flavonols in non-model fruits by altering transcript levels of genes related to flavonol metabolic pathway looks promising. Overexpression of either *MdMYB22* or *MdMYB8* in 'Orin' apple callus significantly promoted flavonol accumulation[75,83]. The concentrations of most flavonol metabolites were up-regulated by overexpressing *PbMYB12b* in pear fruits, except for quercetin 3-*O*-arabinoside[37].

Biotechnological production of flavonol compounds using microorganisms could possibly meet the increasing market demand for fruit flavonols. For instance, vectors containing citrus *F3H* and *FLS* genes were introduced into *E. coli* resulting in production of 15.1 mg L^{-1} kaempferol with tyrosine supplement and 1.1 mg L^{-1} galangin with phenylalanine supplement[105]. Fisetin has also been produced at a concentration of 0.3 mg L^{-1} by overexpressing flavonol biosynthesis-related genes in *E. coli* with 0.5 mM L-tyrosine supplement[57]. In recent years, *de novo* production of kaempferol, myricetin, quercetin using the actinomycete *Streptomyces coelicolor*, and fisetin in the host yeast *Saccharomyces cerevisiae* grown on a cheap carbon source has been described[106].

Conclusions and perspective

Flavonols with their extensive double bonds and polyphenolic nature are important secondary metabolites and have diverse functions in animals, plants, and microorganisms. In this article, we have attempted to summarize recent advances in the understanding of the structure, distribution, biosynthesis, regulation and metabolic engineering of fruit flavonols. Recent development in metabolomics, particularly the widespread adoption of high-resolution mass spectrometry, have considerably improved detection and identification of flavonol metabolites. The increasing development of functional genomics and transcriptomics and improvement of experimental systems for modifying gene expression have given a significant boost to studies on the biosynthesis, regulatory mechanisms and modification of flavonol content. Whilst most research on regulation of flavonol production to date has focused on MYB transcription factors, there is a need to better understand how environmental and stress responses affect the production of flavonols and identify other participating transcription factors. Furthering our understanding of the factors affecting the structure, accumulation and distribution of fruit flavonols will facilitate production of metabolically engineered plants containing desirable bioactive compounds and promote consumption of healthier fruit.

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Conflict of interest

The authors declare that they have no conflict of interest.

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REFERENCES

1. Hung HC, Joshipura KJ, Jiang R, Hu FB, Hunter D, et al. 2004. Fruit and vegetable intake and risk of major chronic disease. *Journal of the National Cancer Institute* 96:1577–84
2. Aune D, Giovannucci E, Boffetta P, Fadnes LT, Keum N, et al. 2017. Fruit and vegetable intake and the risk of cardiovascular disease, total cancer and all-cause mortality—a systematic review and dose-response meta-analysis of prospective studies. *International Journal of Epidemiology* 46:1029–56
3. Slavin JL, Lloyd B. 2012. Health benefits of fruits and vegetables. *Advances in Nutrition* 3:506–516
4. Martin C, Li J. 2017. Medicine is not health care, food is health care: plant metabolic engineering, diet and human health. *New Phytologist* 216:699–719
5. Thakur N, Raigond P, Singh Y, Mishra T, Singh B, et al. 2020. Recent updates on bioaccessibility of phytonutrients. *Trends in Food Science & Technology* 97:366–80
6. Kuhn BM, Geisler M, Bigler L, Ringli C. 2011. Flavonols accumulate asymmetrically and affect auxin transport in Arabidopsis. *Plant Physiology* 156:585–95
7. Muhlemann JK, Younts TLB, Muday GK. 2018. Flavonols control pollen tube growth and integrity by regulating ROS homeostasis during high-temperature stress. *Proceedings of the National Academy of Sciences USA* 115:E11188–E11197
8. Silva-Navas J, Moreno-Risueno MA, Manzano C, Téllez-Robledo B, Navarro-Neila S, et al. 2016. Flavonols mediate root phototropism and growth through regulation of proliferation-to-differentiation transition. *The Plant Cell* 28:1372–87
9. Henry-Kirk RA, Plunkett B, Hall M, McGhie T, Allan AC, et al. 2018. Solar UV light regulates flavonoid metabolism in apple (*Malus* × *domestica*). *Plant, Cell & Environment* 41:675–88
10. Xie L, Cao Y, Zhao Z, Ren C, Xing M, et al. 2020. Involvement of *MdUGT75B1* and *MdUGT71B1* in flavonol galactoside/glucoside biosynthesis in apple fruit. *Food Chemistry* 312:126124
11. Zhang X, Huang H, Zhang Q, Fan F, Xu C, et al. 2015. Phytochemical characterization of Chinese bayberry (*Myrica rubra* Sieb. et Zucc.) of 17 cultivars and their antioxidant properties. *International Journal of Molecular Sciences* 16:12467–81
12. Phillips PA, Sangwan V, Borja-Cacho D, Dudeja V, Vickers SM, et al. 2011. Myricetin induces pancreatic cancer cell death via the induction of apoptosis and inhibition of the phosphatidylinositol 3-kinase (PI3K) signaling pathway. *Cancer Letters* 308:181–88
13. Williams LK, Zhang X, Caner S, Tysoe C, Nguyen NT, et al. 2015. The amylase inhibitor montbretin A reveals a new glycosidase inhibition motif. *Nature Chemical Biology* 11:691–96
14. Kothari D, Lee WD, Kim SK. 2020. *Allium* flavonols: Health benefits, molecular targets, and bioavailability. *Antioxidants* 9:888
15. Barreca D, Trombetta D, Smeriglio A, Mandalari G, Romeo O, et al. 2021. Food flavonols: Nutraceuticals with complex health benefits and functionalities. *Trends in Food Science & Technology* In Press
16. Aherne SA, O'Brien NM. 2002. Dietary flavonols: chemistry, food content, and metabolism. *Nutrition* 18:75–81
17. Jin Q, Yang J, Ma L, Cai J, Li J. 2015. Comparison of polyphenol profile and inhibitory activities against oxidation and α-glucosidase in mulberry (Genus *Morus*) cultivars from China. *Journal of Food Science* 80:C2440–C2451
18. Vrhovsek U, Masuero D, Palmieri L, Mattivi F. 2012. Identification and quantification of flavonol glycosides in cultivated blueberry cultivars. *Journal of Food Composition and Analysis* 25:9–16
19. Fang F, Tang K, Huang W. 2013. Changes of flavonol synthase and flavonol contents during grape berry development. *European Food Research and Technology* 237:529–40
20. Pavlović AV, Dabić DČ, Momirović NM, Dojčinović BP, Milojković-Opsenica DM, et al. 2013. Chemical composition of two different extracts of berries harvested in Serbia. *Journal of Agricultural and Food ChemIstry* 61:4188–94
21. Kolniak-Ostek J. 2016. Identification and quantification of polyphenolic compounds in ten pear cultivars by UPLC-PDA-Q/TOF-MS. *Journal of Food Composition and Analysis* 49:65–77
22. Cao Y, Xie L, Ma Y, Ren C, Xing M, et al. 2019. *PpMYB15* and *PpMYBF1* transcription factors are involved in regulating flavonol biosynthesis in peach Fruit. *Journal of Agricultural and Food Chemistry* 67:644–52
23. Castillo-Muñoz N, Gómez-Alonso S, García-Romero E, Hermosín-Gutiérrez I. 2010. Flavonol profiles of *Vitis vinifera* white grape cultivars. *Journal of Food Composition and Analysis* 23:699–705
24. Griesser M, Vitzthum F, Fink B, Bellido ML, Raasch C, et al. 2008. Multi-substrate flavonol *O*-glucosyltransferases from strawberry (*Fragaria* × *ananassa*) achene and receptacle. *Journal of Experimental Botany* 59:2611–25
25. De Rosso M, Panighel A, Vedova AD, Gardiman M, Flamini R. 2015. Characterization of non-anthocyanic flavonoids in some hybrid red grape extracts potentially interesting for industrial uses. *Molecules* 20:18095–106
26. Berardini N, Fezer R, Conrad J, Beifuss U, Carle R, et al. 2005. Screening of mango (*Mangifera indica* L.) cultivars for their contents of flavonol *O*- and xanthone *C*-glycosides, anthocyanins, and pectin. *Journal of Agricultural and Food Chemistry* 53:1563–70
27. Prinz S, Ringl A, Huefner A, Pemp E, Kopp B. 2007. 4‴-Acetylvitexin-2″-*O*-rhamnoside, isoorientin, orientin, and 8-methoxykaempferol-3-*O*-glucoside as markers for the differentiation of *Crataegus monogyna* and *Crataegus pentagyna* from *Crataegus laevigata* (Rosaceae). *Chemistry & Biodiversity* 4:2920–31
28. Scordino M, Sabatino L, Muratore A, Belligno A, Gagliano G. 2012. Phenolic characterization of Sicilian yellow flesh peach (*Prunus persica* L.) cultivars at different ripening stages. *Journal of Food Quality* 35:255–62
29. Smrke T, Persic M, Veberic R, Sircelj H, Jakopic J. 2019. Influence of reflective foil on persimmon (*Diospyros kaki* Thunb.) fruit peel colour and selected bioactive compounds. *Scientific Reports* 9:19069
30. Aoyama H, Sakagami H, Hatano T. 2018. Three new flavonoids, proanthocyanidin, and accompanying phenolic constituents from *Feijoa sellowiana*. *Bioscience, Biotechnology, and Biochemistry* 82:31–41
31. Yang X, Kang SM, Jeon BT, Kim YD, Ha JH, et al. 2011. Isolation and identification of an antioxidant flavonoid compound from citrus-processing by-product. *Journal of the Science of Food and Agriculture* 91:1925–1927
32. Itoigawa M, Takeya K, Furukawa H. 1994. Cardiotonic flavonoids from *Citrus* plants (Rutaceae). *Biological and Pharmaceutical Bulletin* 17:1519–21

33. Yu M, Man Y, Lei R, Lu X, Wang Y. 2020. Metabolomics study of flavonoids and anthocyanin-related gene analysis in kiwifruit (*Actinidia chinensis*) and kiwiberry (*Actinidia arguta*). *Plant Molecular Biology Reporter* 38:353–69
34. Dauguet J, Bert M, Dolley J, Bekaert A, Lewin G. 1993. 8-Methoxykaempferol 3-neohesperidoside and other flavonoids from bee pollen of *Crataegus monogyna*. *Phytochemistry* 33:1503–05
35. Wang F, Ge S, Xu X, Xing Y, Du X, et al. 2021. Multiomics analysis reveals new insights into the apple fruit quality decline under high nitrogen conditions. *Journal of Agricultural and Food Chemistry* 69:5559–5572
36. Premathilake AT, Ni J, Bai S, Tao R, Ahmad M, et al. 2020. R2R3-MYB transcription factor PpMYB17 positively regulates flavonoid biosynthesis in pear fruit. *Planta* 252:59
37. Zhai R, Zhao Y, Wu M, Yang J, Li X, et al. 2019. The MYB transcription factor PbMYB12b positively regulates flavonol biosynthesis in pear fruit. *BMC Plant Biology* 19:85
38. Wang X, Cao X, Shang Y, Bu H, Wang T, et al. 2020. Preharvest application of prohydrojasmon affects color development, phenolic metabolism, and pigment-related gene expression in red pear (*Pyrus ussuriensis*). *Journal of the Science of Food and Agriculture* 100:4766–75
39. Ferreres F, Gomes D, Valentão P, Gonçalves R, Pio R, et al. 2009. Improved loquat (*Eriobotrya japonica* Lindl.) cultivars: Variation of phenolics and antioxidative potential. *Food Chemistry* 114:1019–27
40. Zhang W, Zhao X, Sun C, Li X, Chen K. 2015. Phenolic composition from different loquat (*Eriobotrya japonica* Lindl.) cultivars grown in China and their antioxidant properties. *Molecules* 20:542–55
41. Chen H, Yang J, Deng X, Lei Y, Xie S, et al. 2020. Foliar-sprayed manganese sulfate improves flavonoid content in grape berry skin of Cabernet Sauvignon (*Vitis vinifera* L.) growing on alkaline soil and wine chromatic characteristics. *Food Chemistry* 314:126182
42. Yang N, Qiu R, Yang S, Zhou K, Wang C, et al. 2019. Influences of stir-frying and baking on flavonoid profile, antioxidant property, and hydroxymethylfurfural formation during preparation of blueberry-filled pastries. *Food Chemistry* 287:167–75
43. Lyu Q, Wen X, Liu Y, Sun C, Chen K, et al. 2021. Comprehensive profiling of phenolic compounds in white and red Chinese bayberries (*Morella rubra* Sieb. et Zucc.) and their developmental variations using tandem mass spectral molecular networking. *Journal of Agricultural and Food Chemistry* 69:741–49
44. Simirgiotis MJ, Schmeda-Hirschmann G. 2010. Determination of phenolic composition and antioxidant activity in fruits, rhizomes and leaves of the white strawberry (*Fragaria chiloensis* spp. *chiloensis* form *chiloensis*) using HPLC-DAD-ESI-MS and free radical quenching techniques. *Journal of Food Composition and Analysis* 23:545–53
45. Cao J, Jiang Q, Lin J, Li X, Sun C, et al. 2015. Physicochemical characterisation of four cherry species (*Prunus* spp.) grown in China. *Food Chemistry* 173:855–63
46. Luo J, Butelli E, Hill L, Parr A, Niggeweg R, et al. 2008. AtMYB12 regulates caffeoyl quinic acid and flavonol synthesis in tomato: expression in fruit results in very high levels of both types of polyphenol. *The Plant Journal* 56:316–26
47. Lijima Y, Nakamura Y, Ogata Y, Tanaka K, Sakurai N, et al. 2008. Metabolite annotations based on the integration of mass spectral information. *The Plant Journal* 54:949–62
48. Li Y, Chen M, Wang S, Ning J, Ding X, et al. 2015. *AtMYB11* regulates caffeoylquinic acid and flavonol synthesis in tomato and tobacco. *Plant Cell, Tissue and Organ Culture* 122:309–319
49. Lv Q, Si M, Yan Y, Luo F, Hu G, et al. 2014. Effects of phenolic-rich litchi (*Litchi chinensis* Sonn.) pulp extracts on glucose consumption in human HepG2 cells. *Journal of Functional Foods* 7:621–629
50. Lyu Q, Kuo T, Sun C, Chen K, Hsu CC, et al. 2019. Comprehensive structural characterization of phenolics in litchi pulp using tandem mass spectral molecular networking. *Food Chemistry* 282:9–17
51. Liu X, Lin C, Ma X, Tan Y, Wang J, et al. 2018. Functional characterization of a flavonoid glycosyltransferase in sweet orange (*Citrus sinensis*). *Frontiers in Plant Science* 9:166
52. Wang F, Chen L, Chen H, Chen S, Liu Y. 2019. Analysis of Flavonoid metabolites in citrus peels (*Citrus reticulata* "Dahongpao") using UPLC-ESI-MS/MS. *Molecules* 24:2680
53. Pandith SA, Dhar N, Rana S, Bhat WW, et al. 2016. Functional promiscuity of two divergent paralogs of type III plant polyketide synthases. *Plant Physiology* 171:2599–19
54. Zhao C, Liu X, Gong Q, Cao J, Shen W, et al. 2021. Three AP2/ERF family members modulate flavonoid synthesis by regulating type IV chalcone isomerase in citrus. *Plant Biotechnology Journal* 19:671–688
55. Mameda R, Waki T, Kawai Y, Takahashi S, Nakayama T. 2018. Involvement of chalcone reductase in the soybean isoflavone metabolon: identification of GmCHR5, which interacts with 2-hydroxyisoflavanone synthase. *The Plant Journal* 96:56–74
56. Kim BG, Kim JH, Kim J, Lee C, Ahn JH. 2008. Accumulation of flavonols in response to ultraviolet-B irradiation in soybean is related to induction of flavanone 3-beta-hydroxylase and flavonol synthase. *Molecules and Cells* 25:247–52
57. Stahlhut SG, Siedler S, Malla S, Harrison SJ, Maury J, et al. 2015. Assembly of a novel biosynthetic pathway for production of the plant flavonoid fisetin in *Escherichia coli*. *Metabolic Engineering* 31:84–93
58. Falcone Ferreyra ML, Rius S, Emiliani J, Pourcel L, Feller A, et al. 2010. Cloning and characterization of a UV-B-inducible maize flavonol synthase. *The Plant Journal* 62:77–91
59. Toh HC, Wang SY, Chang ST, Chu FH. 2013. Molecular cloning and characterization of flavonol synthase in *Acacia confusa*. *Tree Genetics & Genomes* 9:85–92
60. Thill J, Miosic S, Gotame TP, Mikulic-Petkovsek M, Gosch C, et al. 2013. Differential expression of flavonoid 3′-hydroxylase during fruit development establishes the different B-ring hydroxylation patterns of flavonoids in *Fragaria* × *ananassa* and *Fragaria vesca*. *Plant Physiology and Biochemistry* 72:72–78
61. Olsen KM, Hehn A, Jugdé H, Slimestad R, Larbat R, et al. 2010. Identification and characterisation of CYP75A31, a new flavonoid 3′5′-hydroxylase, isolated from *Solanum lycopersicum*. *BMC Plant Biology* 10:21
62. Xing M, Cao Y, Ren C, Liu Y, Li J, et al. 2021. Elucidation of myricetin biosynthesis in *Morella rubra* of the Myricaceae. *The Plant Journal* 108:411–25
63. Liu X, Zhao C, Gong Q, Wang Y, Cao J, et al. 2020. Characterization of a caffeoyl-CoA *O*-methyltransferase-like enzyme involved in biosynthesis of polymethoxylated flavones in *Citrus reticulata*. *Journal of Experimental Botany* 71:3066–79
64. Owens DK, McIntosh CA. 2009. Identification, recombinant expression, and biochemical characterization of a flavonol 3-*O*-glucosyltransferase clone from *Citrus paradisi*. *Phytochemistry* 70:1382–91
65. Montefiori M, Espley RV, Stevenson D, Cooney J, Datson PM, et al. 2011. Identification and characterisation of F3GT1 and F3GGT1, two glycosyltransferases responsible for anthocyanin biosynthesis in red-fleshed kiwifruit (*Actinidia chinensis*). *The Plant Journal* 65:106–18

66. Ikegami A, Akagi T, Potter D, Yamada M, Sato A, et al. 2009. Molecular identification of 1-Cys peroxiredoxin and anthocyanidin/flavonol 3-*O*-galactosyltransferase from proanthocyanidin-rich young fruits of persimmon (*Diospyros kaki* Thunb). *Planta* 230:841
67. Ono E, Homma Y, Horikawa M, Kunikane-Doi S, Imai H, et al. 2010. Functional differentiation of the glycosyltransferases that contribute to the chemical diversity of bioactive flavonol glycosides in grapevines (*Vitis vinifera*). *The Plant Cell* 22:2856–71
68. Song C, Gu L, Liu J, Zhao S, Hong X, et al. 2015. Functional characterization and substrate promiscuity of UGT71 glycosyltransferases from strawberry (*Fragaria* × *ananassa*). *The Plant Cell Physiology* 56:2478–93
69. Halbwirth H, Forkmann G, Stich K. 2004. The A-ring specific hydroxylation of flavonols in position 6 in *Tagetes* sp. is catalyzed by a cytochrome P450 dependent monooxygenase. *Plant Science* 167:129–35
70. Halbwirth H, Stich K. 2006. An NADPH and FAD dependent enzyme catalyzes hydroxylation of flavonoids in position 8. *Phytochemistry* 67:1080–87
71. Macheix JJ, Ibrahim RK. 1984. The *O*-methyltransferase system of apple fruit cell suspension culture. *Biochemie und Physiologie der Pflanzen* 179:659–64
72. Gomez Roldan MV, Outchkourov N, van Houwelingen A, Lammers M, Romero de la Fuente I, et al. 2014. An *O*-methyltransferase modifies accumulation of methylated anthocyanins in seedlings of tomato. *The Plant Journal* 80:695–708
73. Mehrtens F, Kranz H, Bednarek P, Weisshaar B. 2005. The Arabidopsis transcription factor MYB12 is a flavonol-specific regulator of phenylpropanoid biosynthesis. *Plant Physiology* 138:1083–96
74. Stracke R, Ishihara H, Huep G, Barsch A, Mehrtens F, et al. 2007. Differential regulation of closely related R2R3-MYB transcription factors controls flavonol accumulation in different parts of the *Arabidopsis thaliana* seedling. *The Plant Journal* 50:660–77
75. Wang N, Xu H, Jiang S, Zhang Z, Lu N, et al. 2017. MYB12 and MYB22 play essential roles in proanthocyanidin and flavonol synthesis in red-fleshed apple (*Malus sieversii* f. *niedzwetzkyana*). *The Plant Journal* 90:276–292
76. Cao Y, Jia H, Xing M, Jin R, Grierson D, et al. 2021. Genome-wide analysis of MYB gene family in Chinese bayberry (*Morella rubra*) and identification of members regulating flavonoid biosynthesis. *Frontiers in Plant Science* 12:691384
77. Aharoni A, De Vos CHR, Wein M, Sun Z, Greco R, et al. 2001. The strawberry *FaMYB1* transcription factor suppresses anthocyanin and flavonol accumulation in transgenic tobacco. *The Plant Journal* 28:319–32
78. Vimolmangkang S, Han Y, Wei G, Korban SS. 2013. An apple MYB transcription factor, *MdMYB3*, is involved in regulation of anthocyanin biosynthesis and flower development. *BMC Plant Biology* 13:176
79. Zhai R, Wang Z, Zhang S, Meng G, Song L, et al. 2016. Two MYB transcription factors regulate flavonoid biosynthesis in pear fruit (*Pyrus bretschneideri* Rehd.). *Journal of Experimental Botany* 67:1275–84
80. Lu BY, Hao S, Bu Y, Yang S, Zhang J, et al. 2017. McMYB10 regulates anthocyanins and quercetin accumulation during the fruit development of crabapples. *The Journal of Horticultural Science and Biotechnology* 92:358–66
81. Malacarne G, Coller E, Czemmel S, Vrhovsek U, Engelen K, Goremykin V, Bogs J, Moser C. 2016. The grapevine VvibZIPC22 transcription factor is involved in the regulation of flavonoid biosynthesis. *Journal of Experimental Botany* 67:3509–22
82. Tirumalai V, Swetha C, Nair A, Pandit A, Shivaprasad PV. 2019. miR828 and miR858 regulate VvMYB114 to promote anthocyanin and flavonol accumulation in grapes. *Journal of Experimental Botany* 70:4775–92
83. Li H, Li Y, Yu J, Wu T, Zhang J, Tian J, et al. 2020. MdMYB8 is associated with flavonol biosynthesis via the activation of the *MdFLS* promoter in the fruits of *Malus* crabapple. *Horticulture Research* 7:19
84. Deluc L, Barrieu F, Marchive C, Lauvergeat V, Decendit A, et al. 2006. Characterization of a grapevine R2R3-MYB transcription factor that regulates the phenylpropanoid pathway. *Plant Physiology* 140:499–511
85. Matus JT, Loyola R, Vega A, Peña-Neira A, Bordeu E, et al. 2009. Post-veraison sunlight exposure induces MYB-mediated transcriptional regulation of anthocyanin and flavonol synthesis in berry skins of *Vitis vinifera*. *Journal of Experimental Botany* 60:853–67
86. Czemmel S, Stracke R, Weisshaar B, Cordon N, Harris NN, et al. 2009. The grapevine R2R3-MYB transcription factor VvMYBF1 regulates flavonol synthesis in developing grape berries. *Plant Physiology* 151:1513–30
87. Niu TQ, Gao ZD, Zhang PF, Zhang XJ, Gao MY, et al. 2016. *MYBA2* gene involved in anthocyanin and flavonol biosynthesis pathways in grapevine. *Genetics and Molecular Research* 15:gmr15048922
88. Ballester AR, Molthoff J, de Vos R, Hekkert BtL, Orzaez D, et al. 2010. Biochemical and molecular analysis of pink tomatoes: deregulated expression of the gene encoding transcription factor SlMYB12 leads to pink tomato fruit color. *Plant Physiology* 152:71–84
89. Liu C, Long J, Zhu K, Liu L, Yang W, et al. 2016. Characterization of a citrus R2R3-MYB transcription factor that regulates the flavonol and hydroxycinnamic acid biosynthesis. *Scientific Reports* 6:25352
90. Feng F, Li M, Ma F, Cheng L. 2013. Phenylpropanoid metabolites and expression of key genes involved in anthocyanin biosynthesis in the shaded peel of apple fruit in response to sun exposure. *Plant Physiology and Biochemistry* 69:54–61
91. Sun R, Cheng G, Li Q, He Y, Wang Y, et al. 2017. Light-induced variation in phenolic compounds in Cabernet Sauvignon grapes (*Vitis vinifera* L.) involves extensive transcriptome reprogramming of biosynthetic enzymes, transcription factors, and phytohormonal regulators. *Frontiers in Plant Science* 8:547
92. Wang Y, Lu Y, Hao S, Zhang M, Zhang J, et al. 2015. Different coloration patterns between the red- and white-fleshed fruits of *malus* crabapples. *Scientia Horticulturae* 194:26–33
93. Azuma A, Yakushiji H, Koshita Y, Kobayashi S. 2012. Flavonoid biosynthesis-related genes in grape skin are differentially regulated by temperature and light conditions. *Planta* 236:1067–80
94. Liu L, Gregan SM, Winefield C, Jordan B. 2018. Comparisons of controlled environment and vineyard experiments in Sauvignon blanc grapes reveal similar UV-B signal transduction pathways for flavonol biosynthesis. *Plant Science* 276:44–53
95. Liu L, Gregan S, Winefield C, Jordan B. 2015. From UVR8 to flavonol synthase: UV-B-induced gene expression in Sauvignon blanc grape berry. *Plant, Cell & Environment* 38:905–19
96. Wang CY, Chen CT, Wang SY. 2009. Changes of flavonoid content and antioxidant capacity in blueberries after illumination with UV-C. *Food Chemistry* 117:426–31
97. Crupi P, Pichierri A, Basile T, Antonacci D. 2013. Postharvest stilbenes and flavonoids enrichment of table grape cv Redglobe (*Vitis vinifera* L.) as affected by interactive UV-C exposure and storage conditions. *Food Chemistry* 141:802–8

98. Xu Y, Charles MT, Luo Z, Mimee B, Veronneau PY, et al. 2017. Preharvest ultraviolet C irradiation increased the level of polyphenol accumulation and flavonoid pathway gene expression in strawberry fruit. *Journal of Agricultural and Food Chemistry* 65:9970–79
99. Wang SY, Bowman L, Ding M. 2008. Methyl jasmonate enhances antioxidant activity and flavonoid content in blackberries (*Rubus* sp.) and promotes antiproliferation of human cancer cells. *Food Chemistry* 107:1261–69
100. Zhang Y, Zhang J, Song T, Li J, Tian J, et al. 2014. Low medium pH value enhances anthocyanin accumulation in *Malus* crabapple leaves. *PLoS One* 9:e97904
101. Zheng J, An Y, Wang L. 2018. 24-Epibrassinolide enhances 5-ALA-induced anthocyanin and flavonol accumulation in calli of 'Fuji' apple flesh. *Plant Cell, Tissue and Organ Culture* 134:319–30
102. Reay PF, Lancaster JE. 2001. Accumulation of anthocyanins and quercetin glycosides in 'Gala' and 'Royal Gala' apple fruit skin with UV-B-visible irradiation: modifying effects of fruit maturity, fruit side, and temperature. *Scientia Horticulturae* 90:57–68
103. Muir SR, Collins GJ, Robinson S, Hughes S, Bovy A, et al. 2001. Overexpression of petunia chalcone isomerase in tomato results in fruit containing increased levels of flavonols. *Nature Biotechnology* 19:470–74
104. Zhang Y, Butelli E, Alseekh S, Tohge T, Rallapalli G, et al. 2015. Multi-level engineering facilitates the production of phenylpropanoid compounds in tomato. *Nature Communications* 6:8635
105. Miyahisa I, Funa N, Ohnishi Y, Martens S, Moriguchi T, et al. 2006. Combinatorial biosynthesis of flavones and flavonols in *Escherichia coli*. *Applied Microbiology and Biotechnology* 71:53–58
106. Rodriguez A, Strucko T, Stahlhut SG, Kristensen M, Svenssen DK, et al. 2017. Metabolic engineering of yeast for fermentative production of flavonoids. *Bioresource Technology* 245:1645–54