V. SUMMARY

To move away from agriculture and chemically derived products, we have created engineered cells for the bioproduction of flavonoids and anthocyanins. This approach provides a feasible route for the rapid, safe, economical, and sustainable production of a wide variety of important flavonoids.

Herein, a range of flavonoids and anthocyanins including naringenin, eriodictyol, taxifolin, dihydrokaempferol, (+)-catechin, cyanidin, and cyaninidin-3-glucoside are biomanufactured using a modified microbial host. Herein, the engineered cells include one or more genetic modifications that increase(s) flavonoid and anthocyanin bioproduction by increasing metabolic flux to flavonoid precursors and/or reducing carbon losses resulting from the production of byproducts.

Provided herein are cells engineered for the production of flavonoids, anthocyanins and other organic compounds, where the engineered cells include one or more genetic modifications that increase flavonoid production by increasing metabaolic flux to flavonoid precursors and/or reducing carbon losses resulting from the production of byproducts. As nonlimiting examples, a genetic modification can be a modification for over-expressing or under-expressing one or more endogenous genes in the engineered host cell or can be a modification for expressing one or more non-native genes in the engineered host cell. Engineered cells as provided herein can include multiple genetic modifications.

Also provided are cell cultures for producing one or more flavonoids or anthocyanins. The cell cultures include engineered cells as disclosed herein in a culture medium that includes a carbon source that can also be an energy source, such as glycerol, sugar, or an organic acid. In various examples, the culture medium can include at least one feed molecule such as but not limited to one or more organic acids or amino acids that can be converted into a flavonoid precursor (such as tyrosine, p-coumaroyl-CoA or malonyl-CoA). Examples of feed molecules include, but are not limited to, acetate, malonate, tyrosine, phenylalanine, pantothenate, coumarate, etc. In some examples, the feed molecules may be of reduced or low purity. For example, glycerol as a feed molecule may be crude glycerol, including a biomass comprising glycerol, for example, glycerol obtained as a byproduct of biodiesel processing. Alternatively, or in addition, the culture medium can include a supplemental compound that can be a cofactor or a

precursor of a cofactor used by an enzyme that functions in a flavonoid pathway, such as, for examples, bicarbonate, biotin, thiamine, pantothenate, alpha-ketoglutarate, ascorbate, or 5-aminolevulinic acid.

Further provided are methods for producing flavonoids and anthocyanins that include culturing a cell engineered for the production of flavonoids or anthocyanins as provided herein under conditions in which the cell produces flavonoids or anthocyanins. In some examples, the methods include culturing the engineered cells in a culture medium that includes at least one feed molecule or supplement such as but not limited to: tyrosine, phenylalanine, malonate, p-coumarate, bicarbonate, acetate, pantothenate, biotin, thiamine, alpha-ketoglutarate, ascorbate, and 5-aminolevulinic acid. The methods can further include recovering at least one of the flavonoids from culture medium, whole culture, or the cells.

In one aspect, the present disclosure provides an engineered host cell that comprises one or more genetic modifications resulting in production of flavonoid or anthocyanin from a carbon source that can also be an energy source, through multiple chemical intermediates, by the engineered host cell through enzymatic transformation, wherein: the carbon source is selected from a group consisting of: (i) glycerol, (ii) a sugar, (iii) an organic acid, (iv) an amino acid, (v) a biomass comprising glycerol; and (vi) any combination thereof; optionally the engineered host cell is cultured in a medium comprising molecules selected from a group consisting of: (i) glycerol, (ii) a sugar, (iii) an organic acid, (iv) an amino acid, (v) a biomass comprising glycerol, and (vi) any combination thereof; and optionally the engineered host cell is *E. coli.* In a first example, provided herein are cells engineered to produce one or more flavonoids or anthocyanins, wherein the cells include, in addition to nucleic acid sequences encoding either tyrosine ammonia lyase activity and/or phenylalanine ammonia lyase activity and cinnamate-4-hydroxylase activity, 4-coumarate-CoA ligase activity, chalcone synthase activity, chalcone isomerase activity, flavanone-3-hydroxylase activity, flavonoid 3'-hydroxylase activity or flavonoid 3'5'-hydroxylase activity, cytochrome P450 reductase activity, leucoanthocyanidin reductase activity, and dihydroflavonol-4- reductase activity, one or more genetic modifications for improving production of the flavonoids or anthocyanins. As set forth herein, a cell that is engineered to produce one or more of the flavonoids is engineered to include an exogenous nucleic acid sequence encoding tyrosine ammonia lyase activity that can form 4-coumaric acid

using tyrosine as substrate (e.g., tyrosine ammonia lyase TAL, EC: 4.3.1.25) or, alternatively or in addition, an exogenous nucleic acid sequence encoding phenylalanine ammonia lyase activity that can convert phenylalanine to trans-cinnamic acid and an exogenous nucleic acid sequence encoding cinnamate-4-hydroxylase activity that forms 4-coumaric acid from trans-cinnamic acid, an exogenous nucleic acid sequence encoding CoA ligase activity that forms p-coumaroyl-CoA from coumaric acid (e.g., 4-coumarate--CoA ligase, 4CL, EC:6.2.1.12), an exogenous nucleic acid sequence encoding polyketide synthase activity that forms naringenin chalcone using malonyl-CoA and p-coumaroyl-CoA as substrates (e.g., chalcone synthase, CHS, EC:2.3.1.74), an exogenous nucleic acid sequence encoding chalcone isomerase activity that forms naringenin

from naringenin chalcone via its cyclase activity (e.g., chalcone-flavonone isomerase, CHI, EC:5.5.1.6), an exogenous nucleic acid sequence encoding flavanone-3-hydroxylase activity that forms dihydrokaempferol from naringenin or forms taxifolin from eriodictyol (e.g., naringenin 3-dioxygenase, F3H, EC: 1.14.11.9), an exogenous nucleic acid sequence encoding flavonoid 3'-hydroxylase or flavonoid 3'5'-hydroxylase activity coupled with an exogenous nucleic acid sequence encoding cytochrome P450 reductase activity to form taxifolin or dihydromyricetin from dihydrokaempferol or to form eriodictyol or pentahydroxyflavone from naringenin (e.g., flavonoid 3'-monooxygenase, F3'H, EC: 1.14.13.21, EC: 1.14.14.82; cytochrome P450/NADPH--P450 reductase, EC:1.14.14.1; F3'5'H, EC:1.14.14.81), an exogenous nucleic acid sequence encoding dihydroflavonol-4-reductase activity that forms leucocyanidin from taxifolin, leucodelphinidin from dihydromyricetin, or leucopelargonidin from dihydrokaempferol (e.g., dihydroflavonol 4-reductase, EC:1.1.1), and an exogenous nucleic acid sequence encoding leucoanthocyanidin reductase activity that forms catechin from leucocyanidin (e.g., leucoanthocyanidin reductase, LAR, EC:1.17.1.3). Optionally, a cell that is engineered to produce anthocyanins is further engineered to include an exogenous nucleic acid sequence encoding anthocyanin synthase activity that forms cyanidin from catechin or leucocyanidin, forms delphinidin from leucodelphinidin, or forms pelargonidin from leucopelargonidin (e.g., anthocyanin synthase, ANS, EC:1.14.20.4) and to include an exogenous nucleic acid sequence encoding glucosyltransferase activity that forms cyanidin-3-O-beta-D-glucoside from cyanidin, delphinidin-3-O-beta-D-glucoside from delphinidin, or pelagonidin-3-O-beta-D-glucoside from pelagonidin (e.g., anthocyanidin 3-O-glucosyltransferase, 3GT, EC:2.4.1.115). The cells provided herein that are engineered to produce flavonoids or anthocyanins are further engineered to increase the production of flavonoids or anthocyanins product, for example by increasing metabolic flux to a flavonoid or anthocyanin pathway, or by decreasing byproduct formation.

A cell engineered to produce a flavonoid is further engineered to increase the supply of precursor malonyl-CoA. One strategy for increasing malonyl-CoA includes increasing acetyl-CoA carboxylase (ACC) activity. In various examples, the ACC enzyme, which in most eukaryotes, including fungi, is a large single chain polypeptide, and in plant and bacteria such as *E. coli* is a multi-subunit enzyme, is overexpressed in the host strain. Examples of acetyl-CoA carboxylase that can be expressed in a host cell engineered to produce a flavonoid or anthocyanin include, without limitation, the ACC genes of *Mucor circinelloides*, *Rhodotorula*

toruloides, *Lipomyces starkeyi*, *Ustilago maydis*, and orthologs of these ACCs in other species having at least 50% amino acid identity to these ACCs.

Additional strategies for increasing malonyl-CoA include increasing acetyl-CoA, which is converted to malonyl-CoA by acetyl-CoA carboxylase (ACC). In some examples, acetyl-CoA synthase (ACS) that converts acetate and CoA to acetyl-CoA is over-expressed in the host cells. Cultures of engineered host cells that include overexpressed nucleic acid sequence encoding ACS can optionally include acetate in the culture medium. Examples of acetyl-CoA synthase that can be expressed in a host cell engineered to produce a flavonoid or anthocyanin include, without limitation, the ACS gene of *E. coli*, the ACS of *Salmonella typhimurium*, orthologs of these ACSs in other species having at least 50% amino acid identity to these ACSs.

Also considered, in further examples, is an engineered host cell that overexpresses a gene encoding pyruvate dehydrogenase (PDH), which converts pyruvate to acetyl-CoA. Further, in *E. coli*, a variant of the Lpd subunit of PDH can be expressed that includes a mutation (E354K) that reduces inhibition of PDH by NADH.

Alternatively, or in addition to strategies for increasing ACC activity and strategies for increasing acetyl-CoA, strategies for increasing malonyl-CoA by mechanisms that do not rely on the activity of an ACC can be employed. In some examples, a cell engineered to produce a flavonoid, or an anthocyanin, is further engineered to increase the cell's supply of malonyl-CoA includes an exogenous nucleic acid sequence encoding a malonyl-CoA synthetase that generates malonyl-CoA from malonate. Examples of malonyl-CoA synthetases include the malonyl-CoA synthetases of *Streptomyces coelicolor*, *Rhodopseudomonas palustris*, or a malonyl-CoA synthetase having at least 50% identity to any of these or other naturally occurring malonyl-CoA synthetases. Malonate can optionally be added to the culture medium of a culture that includes a cell engineered to express a malonyl-CoA synthetase. An engineered cell that includes an exogenous gene encoding a malonyl-CoA synthetase can also include an exogenous nucleic acid sequence encoding a malonate transporter, such as a malonate transporter encoded by a matC gene, for example, of *Streptomyces coelicolor*, or a malonate transporter encoded by DctPQM of *Sinorhizobium medicae.*

In additional examples, a cell engineered to produce a flavonoid or an anthocyanin is further engineered to include an exogenous nucleic acid sequence encoding malonate CoA-

transferase that makes malonyl-CoA by direct transfer of the CoA from acetyl-CoA. Examples of malonate CoA-transferase that can be expressed in an engineered cell as provided herein include, without limitation, the alpha subunit (mdcA) of malonate decarboxylase from *Acinetobacter calcoaceticus*, *Geobacillus sp*, or a transferase having at least 50% identity to any of these or other naturally occurring malonate CoA-transferases.

In some examples, a cell engineered to produce flavonoids or anthocyanins is further engineered to increase the supply of coenzyme A (CoA) to increase its availability for producing acetyl-CoA, malonyl-CoA, and/or p-coumaroyl-CoA. Strategies for increasing CoA supply include upregulating endogenous pantothenate kinase (PanK) (EC:2.7.1.33) that produces CoA from pantothenate. Alternatively, or in addition, a host cell can be engineered to include a nucleic acid sequence encoding type III pantothenate kinase that is not feedback inhibited by coenzyme A, such as CoaX gene of *pseudomonas aeruginosa* (EC:2.7.1.33). Cultures of cells engineered for the production of flavonoids or anthocyanins can in some examples include a medium that includes pantothenate, a precursor of CoA biosynthesis, and can optionally also include cysteine, used in the CoA biosynthesis.

Additional strategies to increase malonyl-CoA flux to the flavonoid pathway include mutation or downregulation of one or more genes that function in fatty acid biosynthesis. Without limiting the examples to any particular mechanism, limiting fatty acid biosynthesis can increase the malonyl-CoA supply available for flavonoid biosynthesis. In some examples, the gene beta-ketoacyl-ACP synthase II (*E. coli* fabF) can be disrupted to reduce fatty acid biosynthesis. Another example of a fatty acid biosynthesis gene of a host cell that may be mutated or downregulated is a gene encoding malonyl-CoA-ACP transacylase (*E. coli* fabD). Other fatty acid biosynthesis genes of the engineered host cell that can be downregulated include a beta-ketoacyl-ACP synthase I enzyme (*E. coli* fabB) and acyl carrier protein (*E. coli* acpP).

Additional genetic modifications that may be present in a host cell engineered to produce flavonoids or anthocyanins include downregulation, disruption, or deletion of genes encoding alcohol dehydrogenase, lactate dehydrogenase, pyruvate oxidase, acetyl phosphate transferase and acetate kinase. In an *E. coli* host cell, genes that are downregulated, disrupted, or deleted can include aldehyde-alcohol dehydrogenase (adhE), lactate dehydrogenase (ldhA), pyruvate oxidase (poxB), and enzyme acetate kinase phosphate acetyltransferase (ackA-pta).

Further, a cell engineered for the production of flavonoids or anthocyanins can have one or more genes encoding thioesterases downregulated, disrupted, or deleted to prevent hydrolysis of precursors malonyl-CoA, actetyl-CoA, and/or p-coumaryol-CoA. For example, in an *E. coli* host one or more of the thioesterase genes tesA, tesB, yciA, and ybgC can be downregulated, disrupted, or deleted.

Alternatively, or in addition, genes encoding enzymes of the tricarboxylic acid cycle (TCA), such as succinate dehydrogenase, can be disrupted or downregulated to increase alpha-ketoglutarate supply which serves as a cofactor for one or more of the flavonoid and anthocyanin pathway enzymes. Other TCA enzymes that can be downregulated include citrate synthase that converts acetyl-CoA to citrate.

Also considered, in further examples, is an engineered host cell for the production of flavonoids or anthocyanins to upregulate the endogenous biosynthesis of amino acid tyrosine. Tyrosine is one of the precursors for the flavonoid biosynthesis and its conversion to coumaric acid is the first committed step of the pathway. L-tyrosine is one of the three aromatic amino acids derived from the shikimate pathway. The initial step of the shikimate pathway is catalyzed by DAHP synthase isozymes and regulated through feedback-inhibition. Strategies to increase tyrosine production can include, without limitation, transcriptional deregulation, removing feedback inhibition, overexpression of rate-limiting enzymes, and/or deletion of the L-phenylalanine branch of the aromatic acid biosynthetic pathway. For example, in an *E. coli* host the tyrR gene can be disrupted, feedback-inhibition-resistant versions of the DAHP synthase (aroG) and chorismate mutase (tyrA) can be introduced, and/or rate-limiting enzymes, shikimate kinase (aroK or aroL) and quinate (QUIN)/shikimate dehydrogenase (ydiB) can be overexpressed. Further, the Phosphoenolpyruvate synthase (ppsA) and transketolase (tktA) can be exogenously introduced to enhance tyrosine production.

Also considered, in further examples, is an engineered host cell for the production of flavonoids or anthocyanins further engineered to upregulate the endogenous biosynthesis of cofactor heme. Cytochrome P450 (CYPs), one of the exogenous genes in the engineered cells provided herein, contain heme as a cofactor. Improving heme supply can be an effective strategy to increase flavonoid biosynthesis. 5-aminolevulinic acid (ALA) is the first committed precursor to the heme pathway. Strategies to increase heme supply include overexpression of the genes that

synthesize the precursor ALA. In an *E. coli* host, ALA is formed from the 5-carbon skeleton of glutamate (the C5 pathway). The three enzymes involved in ALA biosynthesis are glutamyl-tRNA synthetase (gltX), glutamyl-tRNA reductase (hemA), and glutamate-1-semialdehyde aminotransferase (hemL). In an *E. coli* host, the engineered cells provided herein can be further engineered to express or overexpress hemA or its variants, and/or hemL to increase the heme precursor ALA production. The nonlimiting examples of hemA gene that can be overexpressed include a mutated hemA (inserting two lysine residuals between Thr-2 and Leu-3 at N terminus of hemA gene from *Salmonella typhimurium* (EC:1.1.1.70). Alternatively, or in addition, a heterologous ALAS gene can be introduced to produce ALA via the C4 pathway (ALS is synthesized by the condensation of glycine and succinyl-CoA). Nonlimiting examples of heterologous ALAS that can be expressed in *E. coli* include ALAS of *Bradyrhizobium japonicum* (EC*:* 2.3.1.37), ALAS of *Rhodobacter capsulatus*, or an ALAS having at least 50% sequence identity to a naturally occurring ALAS. Further, one or more of the downstream genes (e.g., in *E. coli* hemB, hemC, hemD, hemE, hemF, hemG, hemI, or hemH) that catalyze the synthesis of heme from ALA can be overexpressed to drive the flux from ALA to heme production. Cultures of cells engineered for the production of flavonoids or anthocyanins can in some examples include a medium that includes succinate and/or glycine, precursors of heme biosynthesis via the C4 pathway.

In another example, provided herein are cell cultures that include engineered cells as provided herein in a culture medium, where the culture medium includes a carbon source that is also an energy source for the cells, where the carbon source can be, for example, glycerol, a sugar, or an organic acid, as nonlimiting examples. The culture medium can further include a feed molecule that is used to produce flavonoids or anthocyanins. A feed molecule can be, for example, acetate, malonate, tyrosine, pantothenate, coumarate, biotin, alpha-ketoglutarate, ascorbate, 5-aminolevulinic acid, succinate, or glycine. In some examples, the culture comprises a culture medium that includes a carbon source and at least one supplement that is a cofactor of an enzyme or is a precursor of an enzyme cofactor.

In yet another example, methods for producing flavonoids and anthocyanins that include incubating a culture of engineered host cell as provided herein to produce flavonoids or

anthocyanins. The methods can further include recovering at least one of the flavonoids from the cells, the culture medium, or the whole culture.

In yet another example, the disclosure provides an engineered host cell that comprises one or more genetic modifications resulting in production of flavonoid or anthocyanin from a carbon source that can also be an energy source, through multiple chemical intermediates, by the engineered host cell. In certain examples, the production of flavonoid or anthocyanin from glycerol occurs through enzymatic transformation. In certain examples, the production of flavonoid or anthocyanin from a carbon source that can also be an energy source occurs through enzymatic transformation. In certain examples, the carbon source is selected from a group consisting of: (i) glycerol, (ii) a sugar, (iii) an organic acid, (iv) an amino acid, (v) a biomass comprising glycerol; and (vi) any combination thereof. In certain examples, the engineered host cell is cultured in a medium comprising molecules selected from a group consisting of: (i) glycerol, (ii) a sugar, (iii) an organic acid, (iv) an amino acid, (v) a biomass comprising glycerol; and (vi) any combination thereof. In certain examples, one or more genetic modifications lead to increase metabolic flux to flavonoid precursors or cofactors. In certain examples, one or more genetic modifications cause reduction of formation of byproducts. In certain examples, one or more genetic modifications are selected from: (i) one or more modifications for over-expressing one or more endogenous genes in the engineered host cells; (ii) one or more modifications for under-expressing one or more endogenous genes in the engineered host cells; (iii) one or more genetic modification is expressing one or more non-native genes in the engineered host cells; and (iv) a combination thereof. In certain examples, the engineered host cell is cultured in a medium comprising molecules selected from: tyrosine, phenylalanine, malonate, p-coumarate, bicarbonate, acetate, pantothenate, biotin, thiamine, alpha-ketoglutarate, ascorbate, and 5-aminolevulinic acid, wherein one or more of the selected molecules are the chemical intermediates, including molecules in the biosynthesis pathway or cofactors. In certain examples, the engineered host cell comprises at least one or more nucleic acid sequences selected from: (i) nucleic acid sequences encoding tyrosine ammonia lyase activity; (ii) nucleic acid sequences encoding phenylalanine ammonia lyase activity; (iii) nucleic acid sequences encoding cinnamate 4-hydroxylase activity; (iv) nucleic acid sequences encoding 4-courmarate-CoA ligase (4CL) activity; and (v) any combination thereof. In certain examples, the engineered host cell comprises at least one or more peptides selected from: (i)

chalcone isomerase; (ii) chalcone synthase; (iii) a fusion protein comprises a chalcone synthase and a chalcone isomerase; and (iv) any combination thereof. In certain examples, the engineered cell is *E. coli*. In certain examples, one or more genetic modifications decreases fatty acid biosynthesis. In certain examples, the engineered host cell comprises an exogenous nucleic acid sequence selected from: (i) nucleic acid sequence encoding tyrosine ammonia lyase, wherein the encoded tyrosine ammonia lyase forms 4-coumaric acid using tyrosine as a substrate; (ii) nucleic acid sequence encoding phenylalanine ammonia lyase, wherein the encoded phenylalanine ammonia lyase converts phenylalanine to trans-cinnamic acid; (iii) nucleic acid sequence encoding cinnamate-4-hydroxylase, wherein the cinnamate-4-hydroxylase produces 4-coumaric acid from trans-cinnamic acid; (iv) nucleic acid sequence encoding flavanone-3-hydroxylase, wherein flavanone-3-hydroxylase forms dihydrokaempferol from naringenin; and (v) any combinations thereof. In certain examples, the engineered host cell comprises an exogenous nucleic acid sequence selected from the group consisting of: (i) nucleic acid sequences encoding tyrosine ammonia lyase, wherein the encoded tyrosine ammonia lyase forms 4-coumaric acid using tyrosine as a substrate; (ii) nucleic acid sequence encoding phenylalanine ammonia lyase, wherein the encoded phenylalanine ammonia lyase converts phenylalanine to trans-cinnamic acid; (iii) nucleic acid sequence encoding cinnamate-4-hydroxylase, wherein the cinnamate-4-hydroxylase produces 4-coumaric acid from trans-cinnamic acid; (iv) nucleic acid sequence encoding 4-courmarate-CoA ligase activity, wherein 4-courmarate-CoA ligase forms p-coumaroyl-CoA from coumaric acid (v) nucleic acid sequence encoding chalcone synthase activity, wherein chalcone synthase forms naringenin chalcone from malonyl-CoA and p-coumaroyl-CoA; (vi) nucleic acid sequence encoding chalcone isomerase activity, wherein chalcone isomerase forms naringenin from naringenin chalcone; (vii) nucleic acid sequence encoding flavanone-3-hydroxylase, wherein flavanone-3-hydroxylase forms dihydrokaempferol from naringenin; and (viii) any combinations thereof. In certain examples, the flavonoid is catechin.

In another aspect, the present disclosure provides a method of increasing the production of flavonoids or anthocyanins, the method comprising: providing an engineered host cell that comprises one or more genetic modifications resulting in production of flavonoid or anthocyanin from a carbon source that can also be an energy source, through multiple chemical intermediates,

by the engineered host cell through enzymatic transformation, wherein the carbon source is selected from a group consisting of: (i) glycerol, (ii) a sugar, (iii) an organic acid, (iv) an amino acid, (v) a biomass comprising glycerol; and (vi) any combination thereof; optionally the engineered host cell is cultured in a medium comprising molecules selected from a group consisting of: (i) glycerol, (ii) a sugar, (iii) an organic acid, (iv) an amino acid, (v) a biomass comprising glycerol, and (vi) any combination thereof; and optionally the engineered host cell is *E. coli*. In yet another example, the disclosure provides a method of increasing the production of flavonoids or anthocyanins, the method comprising: providing an engineered host cell that comprises one or more genetic modifications resulting in production of flavonoid or anthocyanin from a carbon source that can also be an energy source, through multiple chemical intermediates, by the engineered host cell. In certain examples, the production of flavonoid or anthocyanin

from a carbon source that can also be an energy source occurs through enzymatic transformation. In certain examples, the carbon source is selected from a group consisting of: (i) glycerol, (ii) a sugar, (iii) an organic acid, (iv) an amino acid, (v) a biomass comprising glycerol; and (vi) any combination thereof. In certain examples, the engineered host cell is cultured in a medium comprising molecules selected from a group consisting of: (i) glycerol, (ii) a sugar, (iii) an organic acid, (iv) an amino acid, (v) a biomass comprising glycerol; and (vi) any combination thereof. In certain examples, one or more genetic modifications lead to increase metabolic flux to flavonoid precursors or cofactors. In certain examples, one or more genetic modifications cause increased metabolic flux to flavonoid precursors. In certain examples, one or more genetic modifications cause reduction in the formation of byproducts. In certain examples, one or more genetic modifications are selected from: (i) one or more modifications for over-expressing one or more endogenous genes in the engineered host cells; (ii) one or more modifications for under-expressing one or more endogenous genes in the engineered host cells; (iii) one or more genetic modification is expressing one or more non-native genes in the engineered host cells; and (iv) a combination thereof. In certain examples, the engineered host cell is cultured in a medium comprising molecules selected from: tyrosine, phenylalanine, malonate, p-coumarate, bicarbonate, acetate, pantothenate, biotin, thiamine, alpha-ketoglutarate, ascorbate, and 5-aminolevulinic acid, wherein one or more of the selected molecules are the chemical intermediates, including molecules in the biosynthesis pathway or cofactors. In certain examples, the engineered host cell comprises at least one or more nucleic acid sequences selected from: (i) a nucleic acid sequences encoding tyrosine ammonia lyase activity; (ii) a nucleic acid sequences encoding phenylalanine ammonia lyase activity; (iii) cinnamate 4-hydroxylase; and (iv) any combination thereof. In certain examples, the engineered host cell comprises at least one or more peptides selected from: (i) chalcone isomerase; (ii) chalcone synthase; (iii) a fusion protein comprises a chalcone synthase and a chalcone isomerase; and (iv) any combination thereof. In certain examples, the engineered cell is *E. Coli*. In certain examples, one or more genetic modifications decreases fatty acid biosynthesis. In certain examples, the engineered host cell comprises an exogenous nucleic acid sequence selected from: (i) nucleic acid sequences encoding tyrosine ammonia lyase, wherein the encoded tyrosine ammonia lyase forms 4-coumaric acid using tyrosine as a substrate; (ii) nucleic acid sequence encoding phenylalanine ammonia lyase, wherein the

encoded phenylalanine ammonia lyase converts phenylalanine to trans-cinnamic acid; (iii) nucleic acid sequence encoding cinnamate-4-hydroxylase, wherein the cinnamate-4-hydroxylase produces 4-coumaric acid from trans-cinnamic acid; (iv) nucleic acid sequence encoding flavanone-3-hydroxylase, wherein flavanone-3-hydroxylase forms dihydrokaempferol from naringenin; and (v) any combinations thereof. In certain examples, the engineered host cell comprises an exogenous nucleic acid sequence selected from the group consisting of: (i) nucleic acid sequence encoding tyrosine ammonia lyase, wherein the encoded tyrosine ammonia lyase forms 4-coumaric acid using tyrosine as a substrate; (ii) nucleic acid sequence encoding phenylalanine ammonia lyase, wherein the encoded phenylalanine ammonia lyase converts phenylalanine to trans-cinnamic acid; (iii) nucleic acid sequence encoding cinnamate-4-hydroxylase, wherein the cinnamate-4-hydroxylase produces 4-coumaric acid from trans-cinnamic acid; (iv) nucleic acid sequence encoding 4-courmarate-CoA ligase activity, wherein 4-courmarate-CoA ligase forms p-coumaroyl-CoA from coumaric acid (v) nucleic acid sequence encoding chalcone synthase activity, wherein chalcone synthase forms naringenin chalcone from malonyl-CoA and p-coumaroyl-CoA; (vi) nucleic acid sequence encoding chalcone isomerase activity, wherein chalcone isomerase forms naringenin from naringenin chalcone; (vii) nucleic acid sequence encoding flavanone-3-hydroxylase, wherein flavanone-3-hydroxylase forms dihydrokaempferol from naringenin; and (viii) any combinations thereof. In certain examples, the flavonoid is catechin.

In yet another aspect, the present disclosure provides a plurality of engineered host cells, wherein each of the plurality of the engineered host cells comprises one or more genetic modifications resulting in production of flavonoid or anthocyanin from a carbon source that can also be an energy source, through multiple chemical intermediates, by the engineered host cells through enzymatic transformation, wherein the carbon source is selected from a group consisting of: (i) glycerol, (ii) a sugar, (iii) an organic acid, (iv) an amino acid, (v) a biomass comprising glycerol, and (vi) any combination thereof; optionally the engineered host cell is cultured in a medium comprising molecules selected from a group consisting of: (i) glycerol, (ii) a sugar, (iii) an organic acid, (iv) an amino acid, (v) a biomass comprising glycerol, and (vi) any combination thereof; and optionally the engineered host cell is *E. coli.* In yet another example, the disclosure provides a plurality of engineered host cells, wherein each of the plurality of the engineered host cells comprises one or more genetic modifications resulting in production of flavonoid or

anthocyanin from a carbon source that can also be an energy source, through multiple chemical intermediates. In certain examples, the production of flavonoid or anthocyanin from a carbon source that can also be an energy source occurs through enzymatic transformation. In certain examples, the carbon source is selected from a group consisting of: (i) glycerol, (ii) a sugar, (iii) an organic acid, (iv) an amino acid, (v) a biomass comprising glycerol; and (vi) any combination thereof. In certain examples, the engineered host cell is cultured in a medium comprising molecules selected from a group consisting of: (i) glycerol, (ii) a sugar, (iii) an organic acid, (iv) an amino acid, (v) a biomass comprising glycerol; and (vi) any combination thereof. In certain examples, one or more genetic modifications lead to increase metabolic flux to flavonoid precursors or cofactors. In

certain examples, one or more genetic modifications lead to increase metabolic flux to flavonoid precursors or cofactors. In certain examples, one or more genetic modifications cause reduction of formation of byproducts. In certain examples, one or more genetic modifications are selected from: (i) one or more modifications for over-expressing one or more endogenous genes in the engineered host cells; (ii) one or more modifications for under-expressing one or more endogenous genes in the engineered host cells; (iii) one or more genetic modification is expressing one or more non-native genes in the engineered host cells; and (iv) a combination thereof. In certain examples, at least one of the engineered cells from the plurality of the engineered host cells is cultured in a medium comprising molecules selected from: tyrosine, phenylalanine, malonate, p-coumarate, bicarbonate, acetate, pantothenate, biotin, thiamine, alpha-ketoglutarate, ascorbate, and 5-aminolevulinic acid, wherein one or more of the selected molecules are the chemical intermediates, including molecules in biosynthesis pathway or cofactors. In certain examples, at least one of the engineered cells from the plurality of the engineered host cells comprise at least one or more nucleic acid sequences selected from: (i) nucleic acid sequences encoding tyrosine ammonia lyase activity; (ii) nucleic acid sequences encoding phenylalanine ammonia lyase activity; (iii) nucleic acid sequences encoding cinnamate 4-hydroxylase activity; (iv) nucleic acid sequences encoding 4-courmarate-CoA ligase (4CL) activity; and (v) any combination thereof. In certain examples, at least one of the engineered host cell from the plurality of engineered host cells comprise at least one or more peptides selected from: (i) chalcone isomerase; (ii) chalcone synthase; (iii) a fusion protein comprises a chalcone synthase and a chalcone isomerase; and (iv) any combination thereof. In certain examples, at least one the engineered host cell is *E. coli*. In certain examples, one or more genetic modifications decreases fatty acid biosynthesis. In certain examples, at least one of the engineered host cell from the plurality of the engineered host cells comprises an exogenous nucleic acid sequence selected from: (i) nucleic acid sequence encoding tyrosine ammonia lyase, wherein the encoded tyrosine ammonia lyase forms 4-coumaric acid using tyrosine as a substrate; (ii) nucleic acid sequence encoding phenylalanine ammonia lyase, wherein the encoded phenylalanine ammonia lyase converts phenylalanine to trans-cinnamic acid; (iii) nucleic acid sequence encoding cinnamate-4-hydroxylase, wherein the cinnamate-4-hydroxylase produces 4-coumaric acid from trans-cinnamic acid; (iv) nucleic acid sequence encoding flavanone-3-hydroxylase, wherein flavanone-3-hydroxylase forms dihydrokaempferol

from naringenin; and (v) any combinations thereof. In certain examples, the engineered host cell comprises an exogenous nucleic acid sequence selected from the group consisting of: (i) nucleic acid sequence encoding tyrosine ammonia lyase, wherein the encoded tyrosine ammonia lyase forms 4-coumaric acid using tyrosine as a substrate; (ii) nucleic acid sequence encoding phenylalanine ammonia lyase, wherein the encoded phenylalanine ammonia lyase converts phenylalanine to trans-cinnamic acid; (iii) nucleic acid sequence encoding cinnamate-4-hydroxylase, wherein the cinnamate-4-hydroxylase produces 4-coumaric acid from trans-cinnamic acid; (iv) nucleic acid sequence encoding 4-courmarate-CoA ligase activity, wherein 4-courmarate-CoA ligase forms p-coumaroyl-CoA from coumaric acid (v) nucleic acid sequence encoding chalcone synthase activity, wherein chalcone synthase forms naringenin chalcone from malonyl-CoA and p-coumaroyl-CoA; (vi) nucleic acid sequence encoding chalcone isomerase activity, wherein chalcone isomerase forms naringenin from naringenin chalcone; (vii) nucleic acid sequence encoding flavanone-3-hydroxylase, wherein flavanone-3-hydroxylase forms dihydrokaempferol from naringenin; and (viii) any combinations thereof. In certain examples, the flavonoid is catechin.

In yet another aspect, the present disclosure provides a method of increasing the production of flavonoids or anthocyanins, the method comprising: providing a plurality of engineered host cells, wherein each of the plurality of the engineered host cell comprises one or more genetic modifications that resulting in production of flavonoid or anthocyanin from a carbon source that can also be an energy source, through multiple chemical intermediates, through multiple chemical intermediates, by the engineered host cell through enzymatic transformation, wherein the carbon source is selected from a group consisting of: (i) glycerol, (ii) a sugar, (iii) an organic acid, (iv) an amino acid, (v) a biomass comprising glycerol, and (vi) any combination thereof; optionally the engineered host cell is cultured in a medium comprising molecules selected from a group consisting of: (i) glycerol, (ii) a sugar, (iii) an organic acid, (iv) an amino acid, (v) a biomass comprising glycerol, and (vi) any combination thereof; and optionally the engineered host cell is *E. coli.* In yet another example, the disclosure provides a method of increasing the production of flavonoids or anthocyanins, the method comprising: providing a plurality of engineered host cells, wherein each of the plurality of the engineered host cell comprises one or more genetic modifications resulting production of flavonoid or anthocyanin from a carbon source that can also be an energy source, through multiple chemical

intermediates, by the engineered host cell. In certain examples, the production of flavonoid or anthocyanin from a carbon source that can also be an energy source occurs through enzymatic transformation. In certain examples, the carbon source is selected from a group consisting of: (i) glycerol, (ii) a sugar, (iii) an organic acid, (iv) an amino acid, (v) a biomass comprising glycerol; and (vi) any combination thereof. In certain examples, the engineered host cell is cultured in a medium comprising molecules selected from a group consisting of: (i) glycerol, (ii) a sugar, (iii) an organic acid, (iv) an amino acid, (v) a biomass comprising glycerol; and (vi) any combination thereof. In certain examples, one or more genetic modifications lead to increase metabolic flux to flavonoid precursors or cofactors. In certain examples, one or more genetic modifications lead to increase metabolic flux to flavonoid precursors or cofactors. In certain examples, one or more genetic modifications cause reduction of formation of byproducts. In certain examples,

one or more genetic modifications are selected from: (i) one or more modifications for over-expressing one or more endogenous genes in the engineered host cells; (ii) one or more modifications for under-expressing one or more endogenous genes in the engineered host cells; (iii) one or more genetic modification is expressing one or more non-native genes in the engineered host cells; and (iv) a combination thereof. In certain examples, at least one of the engineered cells from the plurality of the engineered host cells is cultured in a medium comprising molecules selected from: tyrosine, phenylalanine, malonate, p-coumarate, bicarbonate, acetate, pantothenate, biotin, thiamine, alpha-ketoglutarate, ascorbate, and 5-aminolevulinic acid, wherein one or more of the selected molecules are the chemical intermediates, including molecules in biosynthesis pathway or cofactors. In certain examples, at least one of the engineered cells from the plurality of the engineered host cells comprise at least one or more nucleic acid sequences selected from: (i) nucleic acid sequences encoding tyrosine ammonia lyase activity; (ii) nucleic acid sequences encoding phenylalanine ammonia lyase activity; (iii) nucleic acid sequences encoding cinnamate 4-hydroxylase activity; (iv) nucleic acid sequences encoding 4-courmarate-CoA ligase (4CL) activity; and (v) any combination thereof. In certain examples, at least one of the engineered host cell from the plurality of engineered host cells comprise at least one or more peptides selected from: (i) chalcone isomerase; (ii) chalcone synthase; (iii) a fusion protein comprises a chalcone synthase and a chalcone isomerase; and (iv) any combination thereof. In certain examples, at least one the engineered host cell is *E. coli*. In certain examples, one or more genetic modifications decreases fatty acid biosynthesis. In certain examples, at least one of the engineered host cell from the plurality of the engineered host cells comprises an exogenous nucleic acid sequence selected from: (i) nucleic acid sequence encoding tyrosine ammonia lyase, wherein the encoded tyrosine ammonia lyase forms 4-coumaric acid using tyrosine as a substrate; (ii) nucleic acid sequence encoding phenylalanine ammonia lyase, wherein the encoded phenylalanine ammonia lyase converts phenylalanine to trans-cinnamic acid; (iii) nucleic acid sequence encoding cinnamate-4-hydroxylase, wherein the cinnamate-4-hydroxylase produces 4-coumaric acid from trans-cinnamic acid; (iv) nucleic acid sequence encoding flavanone-3-hydroxylase, wherein flavanone-3-hydroxylase forms dihydrokaempferol from naringenin; and (v) any combinations thereof. In certain examples, the engineered host cell comprises an exogenous nucleic acid sequence selected from the group consisting of: (i) nucleic acid sequence encoding tyrosine

ammonia lyase, wherein the encoded tyrosine ammonia lyase forms 4-coumaric acid using tyrosine as a substrate; (ii) nucleic acid sequence encoding phenylalanine ammonia lyase, wherein the encoded phenylalanine ammonia lyase converts phenylalanine to trans-cinnamic acid; (iii) nucleic acid sequence encoding cinnamate-4-hydroxylase, wherein the cinnamate-4-hydroxylase produces 4-coumaric acid from trans-cinnamic acid; (iv) nucleic acid sequence encoding 4-courmarate-CoA ligase activity, wherein 4-courmarate-CoA ligase forms p-coumaroyl-CoA from coumaric acid (v) nucleic acid sequence encoding chalcone synthase activity, wherein chalcone synthase forms naringenin chalcone from malonyl-CoA and p-coumaroyl-CoA; (vi) nucleic acid sequence encoding chalcone isomerase activity, wherein chalcone isomerase forms naringenin from naringenin chalcone; (vii) nucleic acid sequence encoding flavanone-3-hydroxylase, wherein flavanone-3-hydroxylase forms dihydrokaempferol from naringenin; and (viii) any combinations thereof. In certain examples, the flavonoid is catechin.

In yet another aspect, the present disclosure provides an engineered host cell, wherein the engineered host cell comprises one or more genetic modifications to increase the production and/or availability of malonyl-CoA wherein optionally the production and/or availability of malonyl-CoA is increased by transformation of acetyl-CoA to malonyl-CoA; and optionally the engineered host cell is *E. coli.* In yet another example, the engineered host cell comprises one or more genetic modifications to increase the production and/or availability of malonyl-CoA. In certain examples, the production and/or availability of malonyl-CoA is increased by transformation of acetyl-CoA to malonyl-CoA. In certain examples, the engineered host cell comprises one or more genetic modifications selected from: (i) expression of acetyl-CoA carboxylase (ACC); and (ii) overexpression of acetyl-CoA carboxylase. In another example, the engineered host cell is an *E. coli*. In certain examples, the *E. coli* cell further comprises genes from fungi. In certain examples, the acetyl-CoA carboxylase is from: *Mucor circinelloides*, *Rhodotorula toruloides*, *Lipomyces starkeyi*, and *Ustilago maydis*, and orthologs of acetyl-CoA carboxylase having at least 50% amino acid identity to the acetyl-CoA carboxylase of these aforementioned species. In certain examples, one or more genetic modification is deletion or attenuation of one or more fatty biosynthetic genes resulting in decrease in fatty acid biosynthesis. In certain examples, one or more genetic modification is overexpression of acetyl-CoA synthase (ACS). In certain examples, the acetyl-CoA synthase is selected from: acetyl-CoA

synthase gene of *E. coli*, acetyl-CoA synthase gene of *Salmonella typhimurium*, and orthologs of acetyl-CoA synthase gene in any other species having at least 50% amino acid identity to the acetyl-CoA synthase gene of *E. coli* and *Salmonella typhimurium*. In certain examples, one or more genetic modification is selected from a group consisting of: (i) overexpression a gene encoding

pyruvate dehydrogenase (PDH), wherein the PDH may include E354K mutation; (ii) exogenous nucleic acid sequence encoding a malonyl-CoA synthetase; (iii) upregulation of endogenous pantothenate kinase (PanK), wherein PanK is not feedback inhibited by coenzyme A; (iv) exogenous nucleic acid sequence encoding a malonate transporter; and (v) any combinations thereof. In certain examples, the malonyl-CoA synthetase is selected from of malonyl-CoA synthetases of *Streptomyces coelicolor*, *Rhodopseudomonas palustris*, or a malonyl-CoA synthetase having at least 50% identity to any of these or other naturally occurring malonyl-CoA synthetases. In certain examples, one or more genetic modifications to decrease fatty acid biosynthesis is selected from: (i) mutation or downregulation of a gene encoding malonyl-CoA-ACP transacylase (*E. coli* fabD); (ii) modifications to the gene beta-ketoacyl-ACP synthase II (*E. coli* fabF); (iii) downregulation of beta-ketoacyl-ACP synthase I enzyme (*E. coli* fabB); (iv) downregulation of acyl carrier protein (*E. coli* acpP); and (v) any combinations thereof. In certain examples, the engineered host cell comprises peptides selected from: (i) acetyl-CoA carboxylase (ACC) having an amino acid sequence at least 80% identical to the polypeptide set forth in SEQ ID NO: 15 or SEQ ID NO: 16; (ii) malonate CoA-transferase having an amino acid sequence at least 80% identical to the polypeptide set forth in SEQ ID NO: 19; (iii) acetyl-CoA synthase (ACS) having an amino acid sequence at least 80% identical to the polypeptide set forth in SEQ ID NO: 16; (iv) malonyl-CoA synthase having an amino acid sequence at least 80% identical SEQ ID NO: 77, SEQ ID NO: 78, or SEQ ID NO: 79; (v) malonate transporter having an amino acid sequence at least 80% identical to SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, or SEQ ID NO: 87; (vi) pantothenate kinase having an amino acid sequence at least 80% identical to SEQ ID NO: 88, SEQ ID NO: 89, or SEQ ID NO: 90; and (vii) any combinations thereof.

In yet another aspect, the present disclosure provides a method of increasing the production of flavonoids comprising an engineered host cell, wherein the one or more engineered host cells comprise one or more genetic modifications to increase the production and/or availability of malonyl-CoA wherein optionally the production and/or availability of malonyl-CoA is increased by transformation of acetyl-CoA to malonyl-CoA; and optionally the engineered host cell is *E. coli.* In another example, the disclosure provides a method of increasing the production of flavonoids comprising an engineered host cell, wherein the one or more engineered host cells comprise one or more genetic modifications to increase the

production and/or availability of malonyl-CoA. In certain examples, the production and/or availability of malonyl-CoA is increased by transformation of acetyl-CoA to malonyl-CoA. In certain examples, the engineered host cell comprises one or more genetic modifications selected from: (i) expression of acetyl-CoA carboxylase (ACC); and (ii) overexpression of acetyl-CoA carboxylase. In another example, the engineered host cell is an *E. coli*. In certain examples, the *E. coli* cell

further comprises genes from fungi. In certain examples, the acetyl-CoA carboxylase is from: *Mucor circinelloides*, *Rhodotorula toruloides*, *Lipomyces starkeyi*, and *Ustilago maydis*, and orthologs of acetyl-CoA carboxylase having at least 50% amino acid identity to the acetyl-CoA carboxylase of these aforementioned species. In certain examples, one or more genetic modification is deletion or attenuation of one or more fatty biosynthetic genes resulting in decrease in fatty acid biosynthesis. In certain examples, one or more genetic modification is overexpression of acetyl-CoA synthase (ACS). In certain examples, the acetyl-CoA synthase is selected from: acetyl-CoA synthase gene of *E. coli*, acetyl-CoA synthase gene of *Salmonella typhimurium*, and orthologs of acetyl-CoA synthase gene in any other species having at least 50% amino acid identity to the acetyl-CoA synthase gene of *E. coli* and *Salmonella typhimurium*. In certain examples, one or more genetic modification is selected from a group consisting of: (i) overexpression a gene encoding pyruvate dehydrogenase (PDH), wherein the PDH may include E354K mutation; (ii) exogenous nucleic acid sequence encoding a malonyl-CoA synthetase; (iii) upregulation of endogenous pantothenate kinase (PanK), wherein PanK is not feedback inhibited by coenzyme A; (iv) exogenous nucleic acid sequence encoding a malonate transporter; and (v) any combinations thereof. In certain examples, the malonyl-CoA synthetase is selected from of malonyl-CoA synthetases of *Streptomyces coelicolor*, *Rhodopseudomonas palustris*, or a malonyl-CoA synthetase having at least 50% identity to any of these or other naturally occurring malonyl-CoA synthetases. In certain examples, one or more genetic modifications to decrease fatty acid biosynthesis is selected from: (i) mutation or downregulation of a gene encoding malonyl-CoA-ACP transacylase (*E. coli* fabD); (ii) modifications to the gene beta-ketoacyl-ACP synthase II (*E. coli* fabF); (iii) downregulation of beta-ketoacyl-ACP synthase I enzyme (*E. coli* fabB); (iv) downregulation of acyl carrier protein (*E. coli* acpP); and (v) any combinations thereof. In certain examples, the engineered host cell comprises peptides selected from: (i) acetyl-CoA carboxylase (ACC) having an amino acid sequence at least 80% identical to the polypeptide set forth in SEQ ID NO: 15 or SEQ ID NO: 16; (ii) malonate CoA-transferase having an amino acid sequence at least 80% identical to the polypeptide set forth in SEQ ID NO: 19; (iii) acetyl-CoA synthase (ACS) having an amino acid sequence at least 80% identical to the polypeptide set forth in SEQ ID NO: 16; (iv) malonyl-CoA synthase having an amino acid sequence at least 80% identical SEQ ID NO: 77, SEQ ID NO: 78, or SEQ ID NO: 79; (v) malonate transporter having an amino acid sequence at least 80%

identical to SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, or SEQ ID NO: 87; (vi) pantothenate kinase having an amino acid sequence at least 80% identical to SEQ ID NO: 88, SEQ ID NO: 89, or SEQ ID NO: 90; and (vii) any combinations thereof.

In yet another aspect, the present disclosure provides an engineered host cell, wherein the engineered host cell comprises one or more genetic modifications to increase transformation of leucocyanidin or catechin to cyanidin-3-glucoside (Cy3G), wherein the one more genetic modification is selected from the group consisting of overexpression of anthocyanin synthase, wherein optionally the anthocyanin synthase is selected from a group consisting of: (i) anthocyanin synthase of *Carica papaya* (SEQ. ID NO:13); (ii) the anthocyanin synthase has an amino acid sequence at least 80% identical to SEQ. ID NO: 66, SEQ. ID NO: 67, SEQ. ID NO: 68, or SEQ. ID NO: 69; (iii) the anthocyanin synthase has an amino acid sequence at least 80% identical to SEQ. ID NO: 13; and (iv) any combinations thereof; and expression of flavonoid-3-glucosyl transferase (3GT), wherein flavonoid-3-glucosyl transferase is selected from a group consisting of: (i) flavonoid-3-glucosyl transferase in *Vitis labrusca* (SEQ. ID NO:14); (ii) the flavonoid-3-glucosyl transferase has an amino acid sequence at least 80% identical to SEQ. ID NO: 70, SEQ. ID NO: 71, SEQ. ID NO: 72, or SEQ. ID NO: 73; and (iii) any combinations thereof. In another example, the disclosure provides an engineered host cell, wherein the engineered host cell comprises one or more genetic modifications to increase endogenous biosynthesis of tyrosine. In certain examples, one or more genetic modifications comprises upregulation of 3-deoxy-D-arabino-heptulosonate synthase. In certain examples, one or more genetic modifications are selected from: (i) upregulation of 13horismite mutase; (ii) upregulation of prephenate dehydrogenase; (iii) overexpression of shikimate kinase; (iv) overexpression of shikimate dehydrogenase; and (v) any combinations thereof. In certain examples, one or more genetic modifications comprises downregulation of L-phenylalanine biosynthetic pathway. In certain examples, one or more genetic modifications comprises expression of exogenous phosphoenolpyruvate synthase (ppsA). In certain examples, one or more genetic modifications comprises expression of exogenous transketolase (tktA). In certain examples, wherein the one or more genetic modifications comprises disruption of tyrR gene. In certain examples, one or more genetic modifications are selected from a group consisting of: (i) expression or overexpression of (D146N) variant of 13horismi-2-dehydro-3-deoxyheptonate aldolase; (ii) expression or

overexpression of variant of 3-dehydroquinate synthase (aroB); (iii) overexpression of transketolase tktA; (iv) deletion of shikimate kinase (aroK); (v) deletion of tyrR; (vi) expression or overexpression of A354V variant of 13horismite mutase (tyrA); (vi) and any combination thereof.

In yet another aspect, the present disclosure provides a method of increasing the production of flavonoids comprising an engineered host cell, wherein the engineered host cell comprises one or more genetic modifications to increase transformation of leucocyanidin or catechin to cyanidin-3-glucoside (Cy3G), wherein the one more genetic modification is selected from the group consisting of overexpression of anthocyanin synthase, wherein optionally the anthocyanin synthase is selected from a group consisting of: (i) anthocyanin synthase of *Carica papaya* (SEQ. ID NO:13); (ii) the anthocyanin synthase has an amino acid sequence at least 80% identical to SEQ. ID NO: 66, SEQ. ID NO: 67, SEQ. ID NO: 68, or SEQ. ID NO: 69; (iii) the anthocyanin synthase has an amino acid sequence at least 80% identical to SEQ. ID NO: 13; and (iv) any combinations thereof; expression of flavonoid-3-glucosyl transferase (3GT), wherein flavonoid-3-glucosyl transferase is selected from a group consisting of: (i) flavonoid-3-glucosyl transferase in *Vitis labrusca* (SEQ. ID NO:14); (ii) the flavonoid-3-glucosyl transferase has an amino acid sequence at least 80% identical to SEQ. ID NO: 70, SEQ. ID NO: 71, SEQ. ID NO: 72, or SEQ. ID NO: 73; and (iii) any combinations thereof. In another example, the disclosure provides a method of increasing endogenous biosynthesis of tyrosine comprising an engineered cell, wherein the engineered host cell comprises one or more genetic modifications to increase endogenous biosynthesis of tyrosine. In certain examples, one or more genetic modifications comprises upregulation of 3-deoxy-D-arabino-heptulosonate synthase. In certain examples, one or more genetic modifications are selected from: (i) upregulation of chorismate mutase; (ii) upregulation of prephenate dehydrogenase; (iii) overexpression of shikimate kinase; (iv) overexpression of shikimate dehydrogenase; and (v) any combinations thereof. In certain examples, one or more genetic modifications comprises

downregulation of L-phenylalanine biosynthetic pathway. In certain examples, one or more genetic modifications comprises expression of exogenous phosphoenolpyruvate synthase (ppsA). In certain examples, one or more genetic modifications comprises expression of exogenous transketolase (tktA). In certain examples, wherein the one or more genetic modifications comprises disruption of tyrR gene. In certain examples, one or more genetic modifications are selected from a group consisting of: (i) expression or overexpression of (D146N) variant of phospho-2-dehydro-3-deoxyheptonate aldolase; (ii) expression or overexpression of variant of 3-dehydroquinate synthase (aroB); (iii) overexpression of transketolase tktA; (iv) deletion of shikimate kinase (aroK); (v) deletion of tyrR; (vi) expression or overexpression of A354V variant of chorismate mutase (tyrA); (vi) and any combination thereof.

In yet another aspect, the present disclosure provides an engineered host cell, wherein the engineered host cell comprises one or more genetic modifications to increase the production of dihydroquercetin (DHQ), dihydromyricein (DHM), eriodictoyl (EDL), and/or pentahydroxyflayaone (PHF), wherein the engineered host cell comprises cytochrome P450 reductase (CPR) and at least one of flavanone-3-hydroxylase (F3H), flavanone-3'-hydroxylase (F3'H), or flavonoid 3',5'-hydroxylase (F3'5'H), wherein optionally: the flavonoid 3'-hydroxylase (F3'H) is truncated to remove the N-terminal leader sequence; the flavonoid 3',5'-hydroxylase (F3'5'H) is truncated to remove the N-terminal leader sequence; cytochrome P450 reductase (CPR) is truncated to remove the N-terminal leader sequence; flavonoid 3'-hydroxylase (F3'H) is fused with cytochrome P450 reductase (CPR); and/or flavonoid 3',5'-hydroxylase (F3'5'H) is fused with cytochrome P450 reductase (CPR). In another example, the disclosure provides an engineered host cell, wherein the engineered host cell comprises one or more genetic modifications to increase transformation of leucocyanidin or catechin to cyanidin-3-glucoside (Cy3G). In certain examples, one or more genetic modifications comprises overexpression of anthocyanin synthase. In certain examples, the anthocyanin synthase is selected from: (i) anthocyanin synthase of *Carica papaya* (SEQ. ID NO:13); (ii) the anthocyanin synthase has an amino acid sequence at least 80% identical to SEQ. ID NO: 66, SEQ. ID NO: 67, SEQ. ID NO: 68, or SEQ. ID NO: 69; (iii) the anthocyanin synthase has an amino acid sequence at least 80% identical to SEQ. ID NO: 13; and (iv) any combinations thereof. In certain examples, one or more engineered host cells comprises flavonoid-3-glucosyl transferase (3GT). In certain examples, flavonoid-3-glucosyl transferase is selected from: (i) flavonoid-3-glucosyl

transferase in *Vitis labrusca* (SEQ. ID NO:14); (ii) the flavonoid-3-glucosyl transferase has an amino acid sequence at least 80% identical to SEQ. ID NO: 70, SEQ. ID NO: 71, SEQ. ID NO: 72, or SEQ. ID NO: 73; and (iii) any combinations thereof. In certain examples, one or more genetic modifications comprises overexpression of anthocyanin synthase and flavonoid-3-glucosyl transferase (3GT). In certain examples, the one or more genetic modifications comprises overexpression of anthocyanin synthase and flavonoid-3-glucosyl transferase (3GT). In certain examples, the one or more genetic modifications are selected from a group consisting of: (i) anthocyanin synthase, (ii) flavonoid-3-glucosyl transferase (3GT), and (iii) a combination thereof.

In a further aspect, the present disclosure provides a method of increasing the production of dihydroquercetin (DHQ), dihydromyricein (DHM), eriodictoyl (EDL), and/or pentahydroxyflayaone (PHF) comprising an engineered host cell, wherein the engineered host cell comprises cytochrome P450 reductase (CPR) and at least one of flavanone-3-hydroxylase (F3H), flavanone-3'-hydroxylase (F3'H), or flavonoid 3',5'-hydroxylase (F3'5'H), wherein optionally the flavonoid 3'-hydroxylase (F3'H) is truncated to remove the N-terminal leader sequence; the flavonoid 3',5'-hydroxylase (F3'5'H) is truncated to remove the N-terminal leader sequence; cytochrome P450 reductase (CPR) is truncated to remove the N-terminal leader sequence; flavonoid 3'-hydroxylase (F3'H) is fused with cytochrome P450 reductase (CPR); and/or flavonoid 3',5'-hydroxylase (F3'5'H) is fused with cytochrome P450 reductase (CPR). In another example, the disclosure provides a method for increasing the production of flavonoids comprising an engineered host cell, wherein the engineered host cell comprises one or more genetic modifications to increase transformation of leucocyanidin or catechin to cyanidin-3-glucoside (Cy3G). In certain examples, one or more genetic modifications comprises overexpression of anthocyanin synthase. In certain examples, the anthocyanin synthase is selected from: (i) anthocyanin synthase of *Carica papaya* (SEQ. ID NO:13); (ii) the anthocyanin synthase has an amino acid sequence at least 80% identical to SEQ. ID NO: 66, SEQ. ID NO: 67, SEQ. ID NO: 68, or SEQ. ID NO: 69; (iii) the anthocyanin synthase has an amino acid sequence at least 80% identical to SEQ. ID NO: 13; and (iv) any combinations thereof. In certain examples, one or more engineered host cells comprises flavonoid-3-glucosyl transferase (3GT). In certain examples, flavonoid-3-glucosyl transferase is selected from: (i) flavonoid-3-glucosyl transferase in *Vitis labrusca* (SEQ. ID NO:14); (ii) the flavonoid-3-glucosyl transferase has an amino acid sequence at least 80% identical to SEQ. ID NO: 70, SEQ. ID NO: 71, SEQ. ID NO: 72, or SEQ. ID NO: 73; and (iii) any combinations thereof. In certain examples, one or more genetic modifications comprises overexpression of anthocyanin synthase and flavonoid-3-glucosyl transferase (3GT). In certain examples, the one or more genetic modifications comprises overexpression of anthocyanin synthase and flavonoid-3-glucosyl transferase (3GT). In certain examples, the one or more genetic modifications are selected from a group consisting of: (i) anthocyanin synthase, (ii) flavonoid-3-glucosyl transferase (3GT), and (iii) a combination thereof.

In another example, the disclosure provides a method of increasing the transformation of leucocyanidin or catechin to cyanidin-3-glucoside (Cy3G), delphinidin or gallocatechin to delphindin-3-glucoside (De3G), or afzelechin or pelargonidin to pelargonidin-3-glucoside (Pe3G) comprising anthocyanin synthase, wherein the anthocyanin synthase is selected from: (i) anthocyanin synthase of *Carica papaya* (SEQ. ID NO:13); (ii) the anthocyanin synthase has an amino acid sequence at least 80% identical to SEQ. ID NO: 66, SEQ. ID NO: 67, SEQ. ID NO: 68, or SEQ. ID NO: 69; (iii) the anthocyanin synthase has an amino acid sequence at least 80% identical to SEQ. ID NO: 13; and (iv) any combinations thereof. In certain examples, one or more genetic modifications comprises overexpression of anthocyanin synthase and flavonoid-3-glucosyl transferase (3GT). In certain examples, the one or more genetic modifications are

selected from a group consisting of: (i) anthocyanin synthase, (ii) flavonoid-3-glucosyl transferase (3GT), and (iii) a combination thereof.

In another example, the disclosure provides a method of increasing the transformation of cyanidin to cyanidin-3-glucoside (Cy3G), delphindin to delphindin-3-glucoside (De3G), or pelargonidin to pelagonidin-3-glucoside (Pe3G), comprising flavonoid-3-glucosyl transferase (3GT), wherein the flavonoid-3-glucosyl transferase is selected from: (i) flavonoid-3-glucosyl transferase in *Vitis labrusca* (SEQ. ID NO:14); (ii) the flavonoid-3-glucosyl transferase has an amino acid sequence at least 80% identical to SEQ. ID NO: 70, SEQ. ID NO: 71, SEQ. ID NO: 72, or SEQ. ID NO: 73; and (iii) any combinations thereof.

In another example, the disclosure provides an engineered host cell comprises one or more genetic modifications to increase the production of dihydroquercetin (DHQ), dihydromyricein (DHM), eriodictoyl (EDL), and/or pentahydroxyflayaone (PHF), wherein the engineered host cell comprises cytochrome P450 reductase (CPR) and at least one of flavanone-3-hydroxylase (F3H), flavanone-3'-hydroxylase (F3'H), or flavonoid 3',5'-hydroxylase (F3'5'H). In certain examples, the precursor for increase in production of dihydroquercetin (DHQ), dihydromyricein (DHM), eriodictoyl (EDL), and/or pentahydroxyflayanone (PHF) is naringenin and/or dihydrokaempferol (DHK). In certain examples, the engineered host cell further comprises peptides selected from a group consisting of: (i) flavonoid 3'-hydroxylase (F3'H); (ii) cytochrome P450 reductase (CPR); and (iii) any combination thereof. In certain examples, the engineered host cell produces eriodictyol or taxifolin. In certain examples, the engineered host cell further comprises flavonoid 3',5'-hydroxylase (F3'5'H). In certain examples, the engineered host cell produces pentahydroxyflavone or dihydromyricetin. In certain examples, flavonoid 3'-hydroxylase (F3'H) is truncated to remove the N-terminal leader sequence. In certain examples, cytochrome P450 reductase (CPR) is truncated to remove the N-terminal leader sequence. In certain examples, flavonoid 3'-hydroxylase (F3'H) is fused with cytochrome P450 reductase (CPR). In certain examples, flavonoid 3',5'-hydroxylase (F3'5'H) is fused with cytochrome P450 reductase (CPR). In certain examples, flavanone-3-hydroxylase (F3H) has an amino acid sequence at least 80% identical to the polypeptide set forth in SEQ ID NO. 7. In certain examples, flavanone-3'-hydroxylase (F3'H) has an amino acid sequence at least 80% identical to the polypeptide set forth in SEQ ID NO. 8. In certain

examples, cytochrome P450 reductase (CPR) has an amino acid sequence at least 80% identical to the polypeptide set forth in SEQ ID NO. 9. In certain examples, flavonoid 3',5'-hydroxylase (F3'5'H) has an amino acid sequence at least 80% identical to the polypeptides selected from a group consisting of: (i) SEQ ID NO. 10, (ii) SEQ ID NO. 56, and (iii) SEQ ID NO. 57. In certain examples, the engineered host cell further comprises cytochrome b_5. In certain examples, cytochrome b_5 has an amino acid sequence at least 80% identical to the polypeptide set forth in SEQ ID NO. 98. In certain examples, wherein the flavanone-3-hydroxylase (F3H) has an amino acid sequence at least 80% identical to the polypeptides selected from a group consisting of: (i) SEQ ID NO. 7, (ii) SEQ ID NO. 45, (iii) SEQ ID NO. 46, (iv) SEQ ID NO. 47, and (v) SEQ ID NO. 48.

In another example, the disclosure provides method of increasing the production of dihydroquercetin (DHQ), dihydromyricein (DHM), eriodictoyl (EDL), and/or pentahydroxyflayaone (PHF) comprising an engineered host cell, wherein the engineered host cell comprises cytochrome P450 reductase (CPR) and at least one of flavanone-3-hydroxylase (F3H), flavanone-3'-hydroxylase (F3'H), or flavonoid 3',5'-hydroxylase (F3'5'H). In certain examples, the precursor for increase in production of dihydroquercetin (DHQ), dihydromyricein (DHM), eriodictoyl (EDL), and/or pentahydroxyflayanone (PHF) is naringenin and/or dihydrokaempferol (DHK). In certain examples, the engineered host cell further comprises peptides selected from a group consisting of: (i) flavonoid 3'-hydroxylase (F3'H); (ii) cytochrome P450 reductase (CPR); and (iii) any combination thereof. In certain examples, the engineered host cell produces eriodictyol or taxifolin. In certain examples, the engineered host cell further comprises flavonoid 3',5'-hydroxylase (F3'5'H). In certain examples, the engineered host cell produces pentahydroxyflavone or dihydromyricetin. In certain examples, flavonoid 3'-hydroxylase (F3'H) is truncated to remove the N-terminal leader sequence. In certain examples, cytochrome P450 reductase (CPR) is truncated to remove the N-terminal leader sequence. In certain examples, flavonoid 3'-hydroxylase (F3'H) is fused with cytochrome P450 reductase (CPR). In certain examples, flavonoid 3',5'-hydroxylase (F3'5'H) is fused with cytochrome P450 reductase (CPR). In certain examples, flavanone-3-hydroxylase (F3H) has an amino acid sequence at least 80% identical to the polypeptide set forth in SEQ ID NO. 7. In certain examples, flavanone-3'-hydroxylase (F3'H) has an amino acid sequence at least 80% identical to the polypeptide set forth in SEQ ID NO. 8. In certain

examples, cytochrome P450 reductase (CPR) has an amino acid sequence at least 80% identical to the polypeptide set forth in SEQ ID NO. 9. In certain examples, flavonoid 3',5'-hydroxylase (F3'5'H) has an amino acid sequence at least 80% identical to the polypeptides selected from a group consisting of: (i) SEQ ID NO. 10, (ii) SEQ ID NO. 56, and (iii) SEQ ID NO. 57. In certain examples, the engineered host cell further comprises cytochrome b_5. In certain examples, cytochrome b_5 has an amino acid sequence at least 80% identical to the polypeptide set forth in SEQ ID NO. 98. In certain examples, wherein the flavanone-3-hydroxylase (F3H) has an amino acid sequence at least 80% identical to the polypeptides selected from a group consisting of: (i) SEQ ID NO. 7, (ii) SEQ ID NO. 45, (iii) SEQ ID NO. 46, (iv) SEQ ID NO. 47, and (v) SEQ ID NO. 48.

VI. BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWING(S)

FIG. 1 shows the metabolic pathway of flavonoid and anthocyanin bioproduction in engineered cells and methods of preparing anthocyanins described herein.

FIG. 2 shows structures of the flavonoid and anthocyanin molecules that may be produced using engineered cells and methods of preparing anthocyanins described herein.

FIG. 3 shows HPLC spectra showing peaks corresponding to the molecules prepared using engineered cells and methods of preparing anthocyanins described herein.

FIG. 4 shows the pathway of flavonoid and anthocyanin bioproduction in engineered cells and methods of preparing anthocyanins described herein.

VII. DETAILED DESCRIPTION OF THE INVENTION

The present application provides engineered cells for producing one or more flavonoids, cultures that include the engineered cells, and methods of producing one or more flavonoids, or at least one anthocyanin. The terms "flavonoid", "flavonoid product", or "flavonoid compound" are used herein to refer to a member of a diverse group of phytonutrients found in almost all fruits and vegetables. As used herein, the terms "flavonoid", "flavonoid product", or "flavonoid compound" are used interchangeably to refer a molecule containing the general structure of a 15-carbon skeleton, which consists of two phenyl rings (A and B) and a heterocyclic ring. Flavonoids may include, but are not limited to, isoflavone type (e.g., genistein), flavone type (e.g., apigenin), flavonol type (e.g., kaempferol), flavanone type (e.g., naringenin), chalcone type

CLAIMS

1. An engineered host cell that comprises one or more genetic modifications resulting in production of flavonoid or anthocyanin from a carbon source that can also be an energy source, through multiple chemical intermediates, by the engineered host cell through enzymatic transformation, wherein:

   the carbon source is selected from a group consisting of: (i) glycerol, (ii) a sugar, (iii) an organic acid, (iv) an amino acid, (v) a biomass comprising glycerol; and (vi) any combination thereof;

   optionally the engineered host cell is cultured in a medium comprising molecules selected from a group consisting of: (i) glycerol, (ii) a sugar, (iii) an organic acid, (iv) an amino acid, (v) a biomass comprising glycerol, and (vi) any combination thereof; and

   optionally the engineered host cell is *E. coli*.

2. The engineered host cell of claim 1, wherein the one or more genetic modifications lead to increase in metabolic flux to flavonoid precursors or cofactors, and optionally cause reduction of formation of byproducts.

3. The engineered host cell of claim 1, wherein the engineered host cell is cultured in a medium comprising molecules selected from a group consisting of tyrosine, phenylalanine, malonate, p-coumarate, bicarbonate, acetate, pantothenate, biotin, thiamine, alpha-ketoglutarate, ascorbate, and 5-aminolevulinic acid.

4. The engineered host cell of claim 1, wherein the one or more genetic modifications decrease fatty acid biosynthesis.

5. The engineered host cell of claim 1, wherein the engineered host cell comprises an exogenous nucleic acid sequence selected from the group consisting of: (i) nucleic acid sequence encoding tyrosine ammonia lyase, wherein the encoded tyrosine ammonia lyase forms 4-coumaric acid using tyrosine as a substrate; (ii) nucleic acid sequence encoding phenylalanine ammonia lyase, wherein the encoded phenylalanine ammonia lyase converts phenylalanine to trans-cinnamic acid; (iii) nucleic acid sequence encoding cinnamate-4-

hydroxylase, wherein the cinnamate-4-hydroxylase produces 4-coumaric acid from trans-cinnamic acid; (iv) nucleic acid sequence encoding 4-courmarate-CoA ligase activity, wherein 4-courmarate-CoA ligase forms p-coumaroyl-CoA from coumaric acid (v) nucleic acid sequence encoding chalcone synthase activity, wherein chalcone synthase forms naringenin chalcone from malonyl-CoA and p-coumaroyl-CoA; (vi) nucleic acid sequence encoding chalcone isomerase activity, wherein chalcone isomerase forms naringenin from naringenin chalcone; (vii) nucleic acid sequence encoding flavanone-3-hydroxylase, wherein flavanone-3-hydroxylase forms dihydrokaempferol from naringenin; and (viii) any combinations thereof.

6. The engineered host cell of claim 1, wherein the flavonoid is catechin.

7. A method of increasing the production of flavonoids or anthocyanins, the method comprising: providing an engineered host cell that comprises one or more genetic modifications resulting in production of flavonoid or anthocyanin from a carbon source that can also be an energy source, through multiple chemical intermediates, by the engineered host cell through enzymatic transformation, wherein

the carbon source is selected from a group consisting of: (i) glycerol, (ii) a sugar, (iii) an organic acid, (iv) an amino acid, (v) a biomass comprising glycerol; and (vi) any combination thereof;

optionally the engineered host cell is cultured in a medium comprising molecules selected from a group consisting of: (i) glycerol, (ii) a sugar, (iii) an organic acid, (iv) an amino acid, (v) a biomass comprising glycerol, and (vi) any combination thereof; and

optionally the engineered host cell is *E. coli*.

8. A plurality of engineered host cells, wherein each of the plurality of the engineered host cells comprises one or more genetic modifications resulting in production of flavonoid or anthocyanin from a carbon source that can also be an energy source, through multiple chemical intermediates, by the engineered host cells through enzymatic transformation, wherein

the carbon source is selected from a group consisting of: (i) glycerol, (ii) a sugar, (iii) an organic acid, (iv) an amino acid, (v) a biomass comprising glycerol, and (vi) any combination thereof;

optionally the engineered host cell is cultured in a medium comprising molecules selected from a group consisting of: (i) glycerol, (ii) a sugar, (iii) an organic acid, (iv) an amino acid, (v) a biomass comprising glycerol, and (vi) any combination thereof; and

optionally the engineered host cell is *E. coli*.

9. The plurality of engineered host cells of claim 8, wherein the one or more genetic modifications lead to increase in metabolic flux to flavonoid precursors or cofactors, and/or reduction in the formation of by products, and optionally reducing the fatty acid biosynthesis.

10. The plurality of engineered host cells of claim 8, wherein at least one of the engineered host cell is cultured in a medium comprising molecules selected from a group consisting of tyrosine, phenylalanine, malonate, p-coumarate, bicarbonate, acetate, pantothenate, biotin, thiamine, alpha-ketoglutarate, ascorbate, and 5-aminolevulinic acid.

11. The plurality of engineered host cells of claim 8, wherein at least one of the engineered host cell comprises an exogenous nucleic acid sequence selected from the group consisting of: (i) nucleic acid sequence encoding tyrosine ammonia lyase, wherein the encoded tyrosine ammonia lyase forms 4-coumaric acid using tyrosine as a substrate; (ii) nucleic acid sequence encoding phenylalanine ammonia lyase, wherein the encoded phenylalanine ammonia lyase converts phenylalanine to trans-cinnamic acid; (iii) nucleic acid sequence encoding cinnamate-4-hydroxylase, wherein the cinnamate-4-hydroxylase produces 4-coumaric acid from trans-cinnamic acid; (iv) nucleic acid sequence encoding 4-courmarate-CoA ligase activity, wherein 4-courmarate-CoA ligase forms p-coumaroyl-CoA from coumaric acid (v) nucleic acid sequence encoding chalcone synthase activity, wherein chalcone synthase forms naringenin chalcone from malonyl-CoA and p-coumaroyl-CoA; (vi) nucleic acid sequence encoding chalcone isomerase activity, wherein chalcone isomerase forms naringenin from naringenin chalcone; (vii) nucleic acid sequence encoding flavanone-3-hydroxylase, wherein flavanone-3-hydroxylase forms dihydrokaempferol from naringenin; and (viii) any combinations thereof, and optionally the flavonoid is catechin.

12. A method of increasing the production of flavonoids or anthocyanins, the method comprising: providing a plurality of engineered host cells, wherein each of the plurality of the engineered host cell comprises one or more genetic modifications that resulting in production of flavonoid or anthocyanin from a carbon source that can also be an energy source, through multiple chemical intermediates, through multiple chemical intermediates, by the engineered host cell through enzymatic transformation, wherein

the carbon source is selected from a group consisting of: (i) glycerol, (ii) a sugar, (iii) an organic acid, (iv) an amino acid, (v) a biomass comprising glycerol, and (vi) any combination thereof;

optionally the engineered host cell is cultured in a medium comprising molecules selected from a group consisting of: (i) glycerol, (ii) a sugar, (iii) an organic acid, (iv) an amino acid, (v) a biomass comprising glycerol, and (vi) any combination thereof; and

optionally the engineered host cell is *E. coli*.

13. An engineered host cell, wherein the engineered host cell comprises one or more genetic modifications to increase the production and/or availability of malonyl-CoA wherein

optionally the production and/or availability of malonyl-CoA is increased by transformation of acetyl-CoA to malonyl-CoA; and

optionally the engineered host cell is *E. coli*.

14. The engineered host cell of claim 13, wherein the engineered host cell comprises one or more genetic modifications selected from a group consisting of: (i) expression or overexpression of acetyl-CoA carboxylase (ACC); and (ii) overexpression of acetyl-CoA synthase (ACS), wherein

optionally the acetyl-CoA carboxylase is from a species selected from the group consisting of *Mucor circinelloides*, *Rhodotorula toruloides*, *Lipomyces starkeyi*, and *Ustilago maydis*, and orthologs of acetyl-CoA carboxylase having at least 50% amino acid identity to the acetyl-CoA carboxylase of said species; and

the acetyl-CoA synthase is selected from the group consisting of, acetyl-CoA synthase gene of *E. coli*, acetyl-CoA synthase gene of *Salmonella typhimurium*, and orthologs of acetyl-CoA synthase gene in any other species having at least 50% amino acid identity to the acetyl-CoA synthase gene of *E. coli* and *Salmonella typhimurium*.

15. The engineered host cell of claim 13, wherein the *E. coli* further comprises genes from fungi.

16. The engineered host cell of claim 13, wherein the one or more genetic modification is selected from a group consisting of: (i) overexpression a gene encoding pyruvate dehydrogenase (PDH), wherein the PDH may include E354K mutation; (ii) exogenous nucleic acid sequence encoding a malonyl-CoA synthetase; (iii) upregulation of endogenous pantothenate kinase (PanK), wherein PanK is not feedback inhibited by coenzyme A; (iv) exogenous nucleic acid sequence encoding a malonate transporter; and (v) any combinations thereof.

17. The engineered host cell of claim 16, wherein the malonyl-CoA synthetase is selected from the group consisting of malonyl-CoA synthetases of *Streptomyces coelicolor*, *Rhodopseudomonas palustris*, or a malonyl-CoA synthetase having at least 50% identity to any of these or other naturally occurring malonyl-CoA synthetases.

18. The engineered host cell of claim 13, wherein the one or more genetic modifications results in decrease fatty acid biosynthesis, wherein the one or more genetic modifications are selected from the group consisting of: (i) mutation or downregulation of a gene encoding malonyl-CoA-ACP transacylase (*E. coli* fabD); (ii) modifications to the gene beta-ketoacyl-ACP synthase II (*E. coli* fabF); (iii) downregulation of beta-ketoacyl-ACP synthase I enzyme (*E. coli* fabB); (iv) downregulation of acyl carrier protein (*E. coli* acpP); and (v) any combinations thereof.

19. The engineered host cell of claim 13, wherein the engineered host cell comprises peptides selected from a group consisting of: (i) acetyl-CoA carboxylase (ACC) having an amino acid sequence at least 80% identical to the polypeptide set forth in SEQ ID NO: 15 or SEQ ID NO: 16; (ii) malonate CoA-transferase having an amino acid sequence at least 80% identical to the polypeptide set forth in SEQ ID NO: 19; (iii) acetyl-CoA synthase (ACS) having an amino acid sequence at least 80% identical to the polypeptide set forth in SEQ ID NO: 16; (iv) malonyl-CoA synthase having an amino acid sequence at least 80% identical SEQ ID NO: 77, SEQ ID NO: 78, or SEQ ID NO: 79; (v) malonate transporter having an amino acid sequence at least 80% identical to SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, or SEQ ID NO: 87; (vi) pantothenate kinase having an amino acid sequence at least 80% identical to SEQ ID NO: 88, SEQ ID NO: 89, or SEQ ID NO: 90; and (vii) any combinations thereof.

20. A method of increasing the production of flavonoids comprising an engineered host cell, wherein the one or more engineered host cells comprise one or more genetic modifications to increase the production and/or availability of malonyl-CoA wherein optionally the production and/or availability of malonyl-CoA is increased by transformation of acetyl-CoA to malonyl-CoA; and optionally the engineered host cell is *E. coli*.

21. An engineered host cell, wherein the engineered host cell comprises one or more genetic modifications to increase transformation of leucocyanidin or catechin to cyanidin-3-glucoside (Cy3G), wherein the one more genetic modification is selected from the group consisting of

overexpression of anthocyanin synthase, wherein optionally the anthocyanin synthase is selected from a group consisting of: (i) anthocyanin synthase of *Carica papaya* (SEQ. ID NO:13); (ii) the anthocyanin synthase has an amino acid sequence at least 80% identical to SEQ. ID NO: 66, SEQ. ID NO: 67, SEQ. ID NO: 68, or SEQ. ID NO: 69; (iii) the anthocyanin synthase has an amino acid sequence at least 80% identical to SEQ. ID NO: 13; and (iv) any combinations thereof; and

expression of flavonoid-3-glucosyl transferase (3GT), wherein flavonoid-3-glucosyl transferase is selected from a group consisting of: (i) flavonoid-3-glucosyl

transferase in *Vitis labrusca* (SEQ. ID NO:14); (ii) the flavonoid-3-glucosyl transferase has an amino acid sequence at least 80% identical to SEQ. ID NO: 70, SEQ. ID NO: 71, SEQ. ID NO: 72, or SEQ. ID NO: 73; and (iii) any combinations thereof.

22. A method of increasing the production of flavonoids comprising an engineered host cell, wherein the engineered host cell comprises one or more genetic modifications to increase transformation of leucocyanidin or catechin to cyanidin-3-glucoside (Cy3G), wherein the one more genetic modification is selected from the group consisting of

overexpression of anthocyanin synthase, wherein optionally the anthocyanin synthase is selected from a group consisting of: (i) anthocyanin synthase of *Carica papaya* (SEQ. ID NO:13); (ii) the anthocyanin synthase has an amino acid sequence at least 80% identical to SEQ. ID NO: 66, SEQ. ID NO: 67, SEQ. ID NO: 68, or SEQ. ID NO: 69; (iii) the anthocyanin synthase has an amino acid sequence at least 80% identical to SEQ. ID NO: 13; and (iv) any combinations thereof;

expression of flavonoid-3-glucosyl transferase (3GT), wherein flavonoid-3-glucosyl transferase is selected from a group consisting of: (i) flavonoid-3-glucosyl transferase in *Vitis labrusca* (SEQ. ID NO:14); (ii) the flavonoid-3-glucosyl transferase has an amino acid sequence at least 80% identical to SEQ. ID NO: 70, SEQ. ID NO: 71, SEQ. ID NO: 72, or SEQ. ID NO: 73; and (iii) any combinations thereof.

23. An engineered host cell, wherein the engineered host cell comprises one or more genetic modifications to increase the production of dihydroquercetin (DHQ), dihydromyricein (DHM), eriodictoyl (EDL), and/or pentahydroxyflayaone (PHF), wherein the engineered host cell comprises cytochrome P450 reductase (CPR) and at least one of flavanone-3-hydroxylase (F3H), flavanone-3'-hydroxylase (F3'H), or flavonoid 3',5'-hydroxylase (F3'5'H), wherein optionally:

the flavonoid 3'-hydroxylase (F3'H) is truncated to remove the N-terminal leader sequence;

the flavonoid 3',5'-hydroxylase (F3'5'H) is truncated to remove the N-terminal leader sequence;

cytochrome P450 reductase (CPR) is truncated to remove the N-terminal leader sequence;

flavonoid 3'-hydroxylase (F3'H) is fused with cytochrome P450 reductase (CPR); and/or

flavonoid 3',5'-hydroxylase (F3'5'H) is fused with cytochrome P450 reductase (CPR).

24. The cell of claim 23, wherein the precursor for increase in production of dihydroquercetin (DHQ), dihydromyricein (DHM), eriodictoyl (EDL), and/or pentahydroxyflayanone (PHF) is naringenin and/or dihydrokaempferol (DHK).

25. The engineered host cell of claim 23, wherein the flavanone-3'-hydroxylase (F3'H) has an amino acid sequence at least 80% identical to the polypeptide set forth in SEQ ID NO. 8; the cytochrome P450 reductase (CPR) has an amino acid sequence at least 80% identical to the polypeptide set forth in SEQ ID NO. 9; flavanone-3-hydroxylase (F3H) has an amino acid sequence at least 80% identical to the polypeptides selected from a group consisting of: (i) SEQ ID NO. 7, (ii) SEQ ID NO. 45, (iii) SEQ ID NO. 46, (iv) SEQ ID NO. 47, and (v) SEQ ID NO. 48; and/or the flavonoid 3',5'-hydroxylase (F3'5'H) has an amino acid sequence at least 80% identical to the polypeptides selected from a group consisting of: (i) SEQ ID NO. 10, (ii) SEQ ID NO. 56, and (iii) SEQ ID NO. 57.

26. The engineered host cell of any one of claims 23-25, wherein the engineered host cell further comprises cytochrome b_5, wherein optionally cytochrome b_5 has an amino acid sequence at least 80% identical to the polypeptide set forth in SEQ ID NO. 98.

27. A method of increasing the production of dihydroquercetin (DHQ), dihydromyricein (DHM), eriodictoyl (EDL), and/or pentahydroxyflayaone (PHF) comprising an engineered host cell, wherein the engineered host cell comprises cytochrome P450 reductase (CPR) and at least one of flavanone-3-hydroxylase (F3H), flavanone-3'-hydroxylase (F3'H), or flavonoid 3',5'-hydroxylase (F3'5'H), wherein optionally

the flavonoid 3'-hydroxylase (F3'H) is truncated to remove the N-terminal leader sequence;

the flavonoid 3',5'-hydroxylase (F3'5'H) is truncated to remove the N-terminal leader sequence;

cytochrome P450 reductase (CPR) is truncated to remove the N-terminal leader sequence;

flavonoid 3'-hydroxylase (F3'H) is fused with cytochrome P450 reductase (CPR); and/or

flavonoid 3',5'-hydroxylase (F3'5'H) is fused with cytochrome P450 reductase (CPR).