Production of plant metabolites with applications in the food industry using engineered microorganisms

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Keywords

plant natural products, food applications, metabolic engineering, polyphenols, terpenes

Highlights

- Plant metabolites have numerous applications in the food industry
- Most plant metabolites with food applications are (poly)phenols and terpenoids
- Microorganisms can be engineered towards producing plant metabolites
- Precursor supply and product toxicity are key limitations during microbial production

Abstract

Secondary plant metabolites are extensively used in today's food industries, *e.g.* as coloring-, flavouring- or texturizing agents. In particular, metabolites with antioxidative properties find applications as preservatives or anti-browning agents. Today, extraction from plant material represents the major source of these metabolites, but progress in the field of metabolic engineering also enabled the microbial production of these valuable compounds as a more economic and ecological alternative. This review article presents the current state of metabolic engineering of microorganisms for production of plant metabolites with applications in the food industries. We focus on compounds, which are already used in food applications, discuss current limitations of microbial plant metabolite production, and outline strategies on how these challenges can be addressed in the future.

Introduction

Food additives typically improve the food quality, modify the food texture or structure, or increase the food´s shelf life. Quality as well as texture and structure are related to the visual impression or taste, whereas an increased shelf life results from protection against spoilage or contamination with microorganisms such as fungi and bacteria. Most of phytochemicals with applications in the food industries are secondary metabolites, which are not essential for plant growth and propagation, but enable interaction of the plant with its biotic and abiotic environment [1]. Natural functions of these compounds include protection against UV radiation, scavenging of radicals, defense against phytopathogenic bacteria, fungi or viruses, or attraction of pollinators [2]. Characteristics such as antioxidative and antimicrobial activities render plant metabolites interesting for food applications. Secondary plant metabolites can be grouped into three major classes, namely phenols, terpenoids and alkaloids. Compounds of the same secondary metabolite class are typically synthesized from the same set of precursors originating from the primary carbon metabolism. Plant phenols (including the large class of polyphenols) are derived from aromatic amino acids, whereas terpenoids are produced from intermediates of glycolysis (either acetyl-CoA or pyruvate/glyceraldehyde-3-phosphate) [3]. Alkaloids are a structurally more diverse class of *N*-heterocycles, which are either derived from the three aromatic amino acids or from glutamate, aspartate or glycine [4]. Since most of the plant-derived compounds relevant for food applications are either phenols (*e.g.* phenylpropanoids, hydroxybenzoic acids, flavonoids, coumarins, and curcuminoids) or terpenoids (*e.g.* monoterpenes, sesquiterpenes, or diterpenes), we focus on these to close and do not discuss alkaloids further. A small number of compounds, which cannot be assigned to one of these big groups, are glycosides, amino acids, proteins, or vitamins.

Today, many plant secondary metabolites are obtained by direct extraction them from plant material. This strategy is only economically feasible for a very small number of plant-derived compounds. Extraction from plant material as a general strategy for getting access to these

compounds is typically challenging because plants often harbor complex mixtures of chemically closely related secondary metabolites. I addition, not every desired compound is produced at all times and in all plant tissues, and product concentrations are low or subject to seasonal and geographical variations. In contrast, microorganisms engineered for plant metabolite production represent a promising alternative as they reach high growth rates and can be easily cultivated in cheap cultivation media yielding high biomass concentrations.

In this review article we describe the current state of metabolic engineering of microorganisms for production of plant metabolites with applications in the food industries. We focus on compounds, which are already used in food applications, discuss current limitations of microbial plant metabolite production, and outline strategies on how these challenges can be addressed in the future. In the context of food applications, plant secondary metabolites can be classified in two ways: (a) based on the same area of application or (b) according to the natural compound class they belong to. Since the metabolic engineering strategy during microbial strain development largely depends on the precursors, which need to be provided by the endogenous microbial metabolism, we organized the text according to the latter classification.

Phenolic compounds

Plant-derived phenols comprise a large family of aromatic compounds ranging from hydroxylated monocyclic benzoic acids to more complex polycyclic compounds such as stilbenes and flavonoids [5] (Fig. 1). Natural monocyclic aromatics, which are used as flavoring agents, include *e.g*. vanillin (4-hydroxy-3-methoxybenzaldehyde), benzaldehyde and raspberry ketone [4-(4-Hydroxyphenyl)-2-butanone]. Vanillin, the most important flavoring agent used world-wide, can in principle be extracted from beans of the vanilla orchid [6]. However, the low vanillin concentration in the natural plant producer renders large-scale vanillin extraction expensive. In fact, less than 1 % of globally produced vanillin is obtained from the vanilla orchid (*Vanilla planifolia)* today, whereas most vanillin is produced with engineered microorganisms [6]. Usually, vanillin is produced by biotransformation of

ferulic acid using *Escherichia coli*, *Pseudomonas fluorescens* or *Streptomyces sannanensis* as production hosts. To this end, microbial catabolic pathways for ferulic acid leading to vanillin are often exploited [7,8]. Alternatively, a combined cultivation process involving *Aspergillus niger* and *Pycnoporus cinnabarinus* [9-12] can be used. Noteworthy, the highest reported conversion yield of ferulic acid to vanillin was 75 % in *Amycolatopsis* sp. [13,14]. In contrast, vanillin production from cheap glucose could be successfully established in *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* [15•]. In this study, the natural aromatic amino acid-forming shikimate pathway was recruited for the production of the aromatic precursor protocatechuate, which was then converted to vanillin by reduction and subsequent *O*-methylation (Fig. 1). However, rather low product titers of 65 mg/L (0.42 mM) and 45 mg/L (0.30 mM), respectively, currently prohibit any large scale application of these strains. Interestingly, introduction of a glycosyltransferase from *Arabidopsis thaliana* into *S. cerevisiae*, enabled the production of up to 500 mg/L (1.59 mM) vanillin β-D-glucoside, which has similar applications as vanillin but turned out to be less toxic to the microbial host [16]. In 2014, it could be demonstrated that a single enzyme, the vanillin synthase of *V. planifolia*, is capable of converting ferulic acid to vanillin [17]. This enzyme is very interesting for future microbial biotransformations of ferulic acid as only one heterologous gene needs to be functionally expressed.

Benzaldehyde, a major constituent of almond oil, is an important flavoring compound for cakes and other baked goods [18]. Production of 0.79 g/L (7.5 mM) benzaldehyde could be achieved in the fungus *P. cinnabarinus* starting from supplemented L-phenylalanine [19]. To this end, the aromatic amino acid was first deaminated to the phenylpropanoid cinnamic acid. Subsequently, the same enzymes relevant for converting ferulic acid to vanillin in this microorganism were exploited for converting cinnamic acid to benzaldehyde.

The biosynthetic pathway leading to raspberry ketone [4-(4-Hydroxyphenyl)butan-2-one] as primary aroma compound in raspberries, was reconstructed in *S. cerevisiae*, which allowed for product titers of up to 7.5 mg/L (0.045 mM) from supplemented *p*-coumaric acid (Fig. 1)

[20]. Gallic acid (3,4,5-trihydroxybenzoic acid) represents the precursor for different gallate esters (food additives E310, E311, E312), which are used as antioxidants to protect oils and fats from oxidation [21]. Already in 2000, production of 20 g/L (118 mM) gallic acid was achieved in *E. coli* by deregulation of the shikimate pathway and oxidation of the pathway intermediate dehydroshikimic acid to gallic acid [22•].

More complex phenols with food applications include phenylpropanoid esters, coumarins, curcumins, and several members of the large flavonoid family, in particular anthocyanins. Production of antioxidants such as the phenylpropanoid ester rosmarinic acid and the coumarin umbelliferone was demonstrated in *E. coli* by functional introduction of the phenylpropanoid-converting enzymes rosmarinic acid synthase and *p*-coumarate 6'-hydroxylase, respectively [23,24]. In strains further engineered towards increased precursor supply, product titers of 130 mg/L (0.36 mM) rosmarinic acid and of 66 mg/L (0.41 mM) umbelliferone were obtained. Curcumin (E100) is a natural yellow colorant and flavor originally produced by turmeric (*Curcuma longa)*. It is synthesized via conjugation of the phenylpropanoid ferulic acid and a ferulic acid diketide catalyzed by curcuminoid synthases. Functional introduction of a curcuminoid synthase from rice (*Oryza sativa*) into *E. coli* enabled the accumulation of 60 mg/L (0.16 mM) curcumin in the culture supernatant [25]. Neohesperidin dihydrochalcone (E959), a rutinosylated chalcone found in citrus, is used as a natural sweetener in beverages, yoghurt and ice cream. Although this compound was not produced in engineered microbes so far, naringin dihydrochalcone, a very similar compound differing from neohesperidin dihydrochalcone only by a single *O*-methyl group, could be produced by an engineered *S. cerevisiae* strain [26•]. In addition, with a strain expressing heterologous genes coding for nine enzymes, *de novo* production of 12 mg/L (0.021 mM) naringin dihydrochalcone could be achieved.

For the microbial production of colorful plant anthocyanins (E163), *E. coli* has been extensively engineered in the last 15 years [27,28]. Until today, the highest titer of 350 mg/L cyanidin-3-*O*-glucoside (0.78 mM) was obtained in a biotransformation of catechin [29]. More

recently, production of 10 mg/L (0.023 mM) pelargonidin-3-*O*-glucoside was achieved by a polyculture of four engineered *E. coli* strains [30••]. *Corynebacterium glutamicum* and *S. cerevisiae* were used for the production of several other plant polyphenols, in particular stilbenes (*e.g.* resveratrol) and flavonoids (*e.g.* quercetin) [31•,32,33••]. The application of different metabolic engineering strategies for the microbial production of important stilbenes and other flavonoids has been reviewed in more detail recently [34].

Terpenoids

Terpenoids (also referred to as isoprenoids) are aliphatic or aromatic molecules, which are in most cases formed by cyclization of precursor molecules, which in turn are derived from condensation of two to eight units of isoprene (2-methyl-1,3-butadiene) [35] (Fig. 2). Terpenoid precursor molecules provided by the central metabolism are either acetyl-CoA (mevalonate pathway) or pyruvate and glyceraldehyde-3-phosphate (methylerythritol phosphate pathway, also referred to as non-mevalonate pathway) (Fig. 2) [36]. Monoterpenes (C_{10}), compounds derived from two isoprene units, are typically used as flavorings and odorants, *e.g.* linalool (lavandula-like odor), α-pinene (woody type flavor), terpineol (lilac/conifer type flavor), menthol (mint type flavor), thymol (characteristic smell of thyme) and carvacrol (pungent type flavor). Some of these compounds also have anti-microbial or antioxidative properties [37,38].

Microbial production of linalool was demonstrated in a wine yeast strain of *S. cerevisiae*. After functional introduction of a linalool synthase of the wildflower *Clarkia breweri* the recombinant strain accumulated 80 µg/L (0.52 µM) linalool from the geranyldiphophate percursor supplied by the endogenous yeast isoprenoid metabolism [39]. In a more recent study, a comparable product titer of 96 µg/L (0.62 µM) was reached when the linalool synthase gene from *Lavandula angustifolia* was functionally expressed in *S. cerevisiae* [40]. Furthermore, *E. coli* and *C. glutamicum* were exploited for the production of the monoterpene α-pinene (Fig. 2) [41•,42]. In both organisms heterologous genes coding for a geranyl

diphosphate synthase and a pinene synthase were co-expressed. During shaking flask cultivation of the engineered *C. glutamicum* strain up to 0.18 mg/L (1.3 µM) α-pinene accumulated in the supernatant [41], whereas a maximal product titer of 5.4 mg/L (40 µM) and 970 mg/L (7.1 mM) α-pinene could be determined during shaking flask- and fed-batch cultivations, respectively using an accordingly engineered *E. coli* strain [42]. Biotechnological production of the natural flavor α-terpineol could be realized in a biotransformation setup starting from supplemented limonene or with β-pinene employing yeast and fungi such as *Fusarium oxysporum* and *Penicillium digitatum* as host organisms [43-45]. These approaches led to overall product titers ranging from 1.8 - 4 g/L (12.1 - 26.0 mM). By following a similar strategy in *E. coli*, menthol was produced from pulegone or via hydrolysis of menthyl acetate [46,47].

Sesquiterpenes (C_{15}) are cyclic molecules derived from the precursor farnesyl diphosphate, which in turn is obtained from condensation of three isoprene units. Relevant sesquiterpenes for food applications are *e.g.* valencene and nootkatone. Valencene is an aroma compound from citrus and serves as a precursor for the production of nootkatone, the flavor-determining compound in grapefruit [48]. *C. glutamicum*, which naturally produces the C_{50} isoprenoid decaprenoxanthin, was engineered towards the production of valencene [49]. In initial experiments, heterologous expression of a gene coding for the valencene synthase from *Citrus sinensis* did not lead to valencene production. After elimination of competing endogenous prenyltransferase activity and by increasing the flux into the isoprenoid-supplying methylerythritol phosphate pathway 2.4 mg/L (0.011 mM) valencene was produced using an alternative valencene synthase from *Callitropsis nootkatensis* (Nootka cypress) [49]. The same enzyme was also functionally introduced into a *Rhodobacter sphaeroides* strain, which co-expressed heterologous genes coding for enzymes of the farnesyl diphosphate-forming mevalonate pathway [50]. This strategy led to the production of 352 mg/L (1.73 mM) valencene. Production of nootkatone was demonstrated in engineered *Pichia pastoris* [51]. The production strain expressed a gene coding for a truncated hydroxy-methylglutaryl-CoA

reductase, the rate-limiting enzyme of the mevalonate pathway, along with genes coding for the *C. nootkatensis* valencene synthase. For hydroxylation of valencene a remnaspirodiene oxygenase gene was additionally expressed and an endogenous alcohol dehydrogenase was capable of converting the resulting nootkatol to nootkatone [51]. The final strain was capable of producing up to 208 mg/L (0.95 mM) nootkatone from glucose. In a different approach, six microorganisms were analyzed with regard to an efficient conversion of valencene to nootkatone [52]. In these experiments, *Botryodiplodia theobromae* and *Yarrowia lipolytica* were identified as promising alternative host strains for nootkatone production.

More complex diterpenes (C_{20}) derived from four isoprene units include carnosic acid (E392) and carnosol (Fig. 2), both of which are potent antioxidants and putative anti-cancer agents found in rosemary extracts [53]. The complete pathway for the production of carnosol was elucidated and reconstructed in *S. cerevisiae* [54•]. Tetraterpene (C_{40}) precursors obtained from eight molecules of isoprene give rise to carotenoids such as β-carotene and lycopene (E160d) (Fig. 2), which are used as natural colorants or antioxidants [55]. An operon for β-carotene biosynthesis from the bacterium *Pantoea agglomerans* was functionally expressed in *E. coli* principally enabling the β-carotene synthesis in this microorganism [56••]. Subsequently, the activity of the central carbon metabolism (citrate cycle, pentose phosphate pathway, ATP synthesis) of the host strain was modulated and the impact on β-carotene synthesis was systematically analyzed. An optimized genetic background finally enabled the production of 2.1 g/L (3.9 mM) of β-carotene. In *S. cerevisiae*, expression of heterologous genes coding for enzymes of the β-carotene pathway in *Xanthophyllomyces dendrorhous* led to production of 5.9 mg (0.011 mM) β-carotene per gram dry weight [57]. Very similar metabolic engineering strategies enabling the microbial production of lycopene were reviewed recently [58]. Noteworthy, the highest reported lycopene titer of 1.44 g/L (2.7 mM) could be reached employing an engineered *E. coli* strain expressing genes from the bacterium *Pantoea ananatis* [59].

Apocarotenoids are terpene-like compounds, which are produced by oxidative cleavage of carotenoids (Fig. 2) [60]. β-Ionone, characteristic of the scent of violets, is an important flavoring agent produced at a scale of 8,000 tons per year [61]. A β-carotene-cleaving dioxygenase from *Petunia x hybrida* was functionally introduced into a β-carotene-producing *S. cerevisiae* strain, enabling the production of 5 mg/L (0.03 mM) β-ionone [62]. Similarly, heterologous introduction of a zeaxanthin-cleaving dioxygenase from *Crocus* together with an aldehyde dehydrogenase from *Synechocystis* sp PCC6803 in a zeaxanthin-producing *S. cerevisiae* strain resulted in synthesis of crocetin, one of the coloring components of saffron, at final product titers of 1.2 mg/L (0.004 mM) [63]. Up to 600 mg/L (2.1 mM) retinal, the aldehyde of retinol (vitamin A), was produced from a mixture of glucose, glycerol and arabinose in engineered *E. coli* after extensive optimization of fermentation conditions [64].

Other compounds

Additional plant-derived compounds, which do not belong to one of the three major classes of plant secondary metabolites, do also find numerous applications in the food industries. These plant metabolites include non-proteinogenic amino acids, proteins, vitamins and fatty acid derivatives (Fig. 3). γ-Decalactone (4-decanolide) is the main volatile compound found in peaches and is used as a flavoring agent *e.g.* in beverages [65]. This natural lactone is a fatty acid derivative typically produced from ricinoleic acid or methyl ricinoleate, the main components of castor oil, using yeasts such as *Y. lipolytica, S. cerevisiae or Pichia etchelsii* [66]. During the biotransformation, the endogenous peroxisomal β-oxidation machinery of the host strains is recruited for chain-shortening of ricinoleic acid to γ-decalactone, which also led to the accumulation of several undesired side products [67]. Therefore, strain engineering focused on decreasing side product formation by altering the activity of the β-oxidation machinery in order to improve product yields [68]. Under optimized cultivation conditions 6.8 g/L (40 mM) γ-decalactone was produced from crude castor oil in *Y. lipolytica* [69], whereas

253 the yeast *Lindnera saturnus* produced 5.8 g/L (34 mM) γ-decalactone from crude glycerol
254 [70•].

255 The non-proteinogenic amino acid L-theanine (γ-glutamylethylamide), responsible for the
256 "*umami*" taste of (green) tea, is naturally produced by condensation of L-glutamate with
257 ethylamine (obtained from the decarboxylation of L-alanine) [71]. *In vitro* L-theanine
258 production from L-glutamate and ethylamine with maximal yields of more than 90% could be
259 succesfully demonstrated using purified bacterial γ-glutamyltranspeptidases from various
260 organisms [72-74]. In an *E. coli* strain overexpressing the native γ-glutamyltranspeptidase
261 gene *ggt*, a conversion yield of 95 % was achieved from supplemented glutamic acid γ-
262 methyl ester and ethylamine [75].

263 L-Ascorbic acid (vitamin C), a vitamin traditionally consumed with fruits and vegetables, is
264 also produced at the scale of more than 100,000 tons per year and added to foods,
265 beverages and pharmaceuticals [76]. Biotechnological production of L-ascorbic acid starts
266 from D-sorbitol or sorbose, which is first oxidized to 2-keto-L-gulonic acid by
267 *Gluconobacter oxydans* or *Ketogulonicigenium vulgare*, respectively and then chemically
268 converted to L-ascorbic acid.

269 Although artificial sweeteners are of commercial importance, their natural pendants increase
270 their market share continuously due to customers changing mind-set towards low-calorie
271 food and against synthetic ingredients. Intensively sweet-tasting proteins such as thaumatins
272 (E957), monellin and brazzein are applied as natural additives in sweets for diabetics, *e.g.* in
273 chocolate or chewing gums [77]. Thaumatin II (one of the six proteins in the natural mixture
274 of thaumatins) was obtained in protease-deficient *Aspergillus awamori* strains as well as in
275 *Bacillus subtilis* by expression of a codon-optimized thaumatin II gene and subsequent
276 protein secretion [78-80]. Similar strategies for protein production and secretion were
277 followed for establishing monellin production in *B. subtilis*, *S. cerevisiae*, *E. coli* and
278 *Candida utilis* [81-84]. Brazzein, which shows a higher thermo- and pH-stability compared to
279 thaumatins and monellin, was produced *e.g.* in *Lactococcus lactis*, *P. pastoris* and

Kluyveromyces lactis [85,86]. Concentrations ranging from 1.5 to 410 mg/L of active brazzein were obtained. In addition to thaumatin and steviol glycosides as sweeteners, sugar alcohols (*e.g.* erythritol and xylitol), and indigestible sugars such as isomaltulose are of increasing importance. Thus, more sustainable fermentative processes have been developed in addition to chemical syntheses [87-90]. In addition to the application as sweeteners, sugars or more precisely oligosaccharides may find other applications in the food industries due to their texture- and viscosity-modifying properties, or prebiotic properties in case of human milk saccharides [91].

Conclusions and Outlook

In the last years, predominantly *E. coli* and *S. cerevisiae* have been engineered towards plant natural product synthesis for food applications (Tab. 1). This is not surprising when considering the long history of these well-studied organisms in industrial biotechnology and the availability of molecular tools for their manipulation. However, more recently also other microorganisms proved to be valuable platforms for the synthesis of such compounds. In particular bacteria such as *C. glutamicum* and *Pseudomonas* sp., both characterized by a pronounced robustness against aromatic compounds, represent promising organisms *e.g.* for the production of compounds derived from the shikimate pathway [31,92-94]. *C. glutamicum* is also a suitable organism for terpenoid production as it naturally produces the C_{50} carotenoid decaprenoxanthin and thus harbors the required pathways for isoprenoid synthesis [95]. In addition, phototrophic bacteria such as cyanobacteria and purple α-proteobacteria of the genus *Synechocystis* and *Rhodobacter*, intrinsically producing and accumulating high amounts of carotenoids as photopigments, could achieve increasing importance as alternative hosts for the sustainable production of plant-derived terpenes in near future [96,97•].

Already today, modular approaches are followed during metabolic engineering in which separate genetic modules for precursor synthesis, product formation and product diversification are functionally introduced into the platform organisms. This allows for a

simple exchange of separate modules for the production of different compounds of the same class. By following this strategy, many plant secondary metabolites relevant for food applications can now be produced with bacteria and/or yeast, but in most cases low product titers prevent their microbial production at industrial scale (Tab. 1). Future efforts for increasing product titers may include additional rational strain construction using state-of-the-art molecular tools such as CRISPR/Cas9 [98], but also biosensor-based high-throughput screenings of genetically diverse production strain libraries might prove to be successful approaches [99]. Targets will be predominantly the endogenous microbial carbon metabolism to further improve the supply of relevant precursors for the functionally introduced plant pathways. With increasing product titers, engineering of product export and *in situ* product removal will also become increasingly important as most plant metabolites are potentially toxic for the producing microorganism.

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References

1. Dillard CJ, German JB: **Phytochemicals: nutraceuticals and human health**. *J. Sci. Food Agric.* 2000, **80**:1744-1756.

2. Singer AC, Crowley DE, Thompson IP: **Secondary plant metabolites in phytoremediation and biotransformation**. *Trends Biotechnol.* 2003, **21**:123-130.

3. Crozier A, Clifford MN, Ashihara H: *Plant secondary metabolites: occurrence, structure and role in the human diet*. John Wiley & Sons; 2008.

4. Pelletier SW: *Alkaloids: chemical and biological perspectives*. Springer; 1999.

5. Hollman PCH: **Evidence for health benefits of plant phenols: local or systemic effects?** *J. Sci. Food Agric.* 2001, **81**:842-852.

6. Gallage NJ, Møller BL: **Vanilla: The Most Popular Flavour**. In *Biotechnology of Natural Products*. Springer; 2018:3-24.

7. Gasson MJ, Kitamura Y, McLauchlan WR, Narbad A, Parr AJ, Parsons ELH, Payne J, Rhodes MJ, Walton NJ: **Metabolism of Ferulic Acid to Vanillin A bacterial gene of the enoyl-SCoA hydratase/isomerase superfamily encodes an enzyme for the hydration and cleavage of a hydroxycinnamic acid SCoA thioester**. *J. Biol. Chem.* 1998, **273**:4163-4170.

8. Narbad A, Gasson MJ: **Metabolism of ferulic acid via vanillin using a novel CoA-dependent pathway in a newly-isolated strain of *Pseudomonas fluorescens***. *Microbiology* 1998, **144**:1397-1405.

9. Chattopadhyay P, Banerjee G, Sen SK: **Cleaner production of vanillin through biotransformation of ferulic acid esters from agroresidue by *Streptomyces sannanensis***. *J. Clean. Prod.* 2018*,* **182**: 272-279

10. Barghini P, Di Gioia D, Fava F, Ruzzi M: **Vanillin production using metabolically engineered *Escherichia coli* under non-growing conditions**. *Microb. Cell Fact.* 2007, **6**:13.

11. Zheng L, Zheng P, Sun Z, Bai Y, Wang J, Guo X: **Production of vanillin from waste residue of rice bran oil by Aspergillus niger and Pycnoporus cinnabarinus**. *Bioresour. Technol.* 2007, **98**:1115-1119.

12. Di Gioia D, Luziatelli F, Negroni A, Ficca AG, Fava F, Ruzzi M: **Metabolic engineering of Pseudomonas fluorescens for the production of vanillin from ferulic acid**. *J. Biotechnol.* 2011, **156**:309-316.

13. Rabenhorst J, Hopp R: **Process for the preparation of vanillin and suitable microorganisms**. *European Patent* 1997, **761817**.

14. Müller B, Münch T, Muheim A, Wetli M: **Process for the production of vanillin**. *European Patent* 1998, **885968**.

• 15. Hansen EH, Møller BL, Kock GR, Bünner CM, Kristensen C, Jensen OR, Okkels FT, Olsen CE, Motawia MS, Hansen J: ***De novo* biosynthesis of vanillin in fission yeast (*Schizosaccharomyces pombe*) and baker's yeast (*Saccharomyces cerevisiae*)**. *Appl. Environ. Microbiol.* 2009, **75**:2765-2774.

16. Brochado AR, Matos C, Møller BL, Hansen J, Mortensen UH, Patil KR: **Improved vanillin production in baker's yeast through in silico design**. *Microb. Cell Fact.* 2010, **9**:84.

17. Gallage NJ, Hansen EH, Kannangara R, Olsen CE, Motawia MS, Jørgensen K, Holme I, Hebelstrup K, Grisoni M, Møller BL: **Vanillin formation from ferulic acid in *Vanilla planifolia* is catalysed by a single enzyme**. *Nature Commun.* 2014, **5**:4037.

18. Pozo-Bayón MA, Guichard E, Cayot N: **Feasibility and application of solvent assisted flavour evaporation and standard addition method to quantify the aroma compounds in flavoured baked matrices**. *Food Chem.* 2006, **99**:416-423.

19. Lomascolo A, Lesage-Meessen L, Labat M, Navarro D, Delattre M, Asther M: **Enhanced benzaldehyde formation by a monokaryotic strain of Pycnoporus cinnabarinus using a selective solid adsorbent in the culture medium**. *Can. J. Microbiol.* 1999, **45**:653-657.

20. Lee D, Lloyd ND, Pretorius IS, Borneman AR: **Heterologous production of raspberry ketone in the wine yeast *Saccharomyces cerevisiae* via pathway engineering and synthetic enzyme fusion**. *Microb. Cell Fact.* 2016, **15**:49.

21. Aruoma OI, Murcia A, Butler J, Halliwell B: **Evaluation of the antioxidant and prooxidant actions of gallic acid and its derivatives**. *J. Agric. Food Chem.* 1993, **41**:1880-1885.

• 22. Kambourakis S, Draths K, Frost J: **Synthesis of gallic acid and pyrogallol from glucose: replacing natural product isolation with microbial catalysis**. *J. Am. Chem. Soc.* 2000, **122**:9042-9043.

23. Bloch SE, Schmidt-Dannert C: **Construction of a chimeric biosynthetic pathway for the de novo biosynthesis of rosmarinic acid in *Escherichia coli***. *ChemBioChem* 2014, **15**:2393-2401.

24. Yang S-M, Shim GY, Kim B-G, Ahn J-H: **Biological synthesis of coumarins in Escherichia coli**. *Microb. Cell Fact.* 2015, **14**:65.

25. Katsuyama Y, Matsuzawa M, Funa N, Horinouchi S: **Production of curcuminoids by *Escherichia coli* carrying an artificial biosynthesis pathway**. *Microbiology* 2008, **154**:2620-2628.

• 26. Eichenberger M, Lehka BJ, Folly C, Fischer D, Martens S, Simón E, Naesby M: **Metabolic engineering of *Saccharomyces cerevisiae* for *de novo* production of dihydrochalcones with known antioxidant, antidiabetic, and sweet tasting properties**. *Metab. Eng.* 2017, **39**:80-89.

27. Yan Y, Li Z, Koffas MA: **High-yield anthocyanin biosynthesis in engineered *Escherichia coli***. *Biotechnol. Bioeng.* 2008, **100**:126-140.

28. Yan Y, Chemler J, Huang L, Martens S, Koffas MA: **Metabolic engineering of anthocyanin biosynthesis in *Escherichia coli***. *Appl. Environ. Microbiol.* 2005, **71**:3617-3623.

29. Lim CG, Wong L, Bhan N, Dvora H, Xu P, Venkiteswaran S, Koffas MA: **Development of a recombinant *Escherichia coli* strain for overproduction of the plant pigment anthocyanin**. *Appl. Environ. Microbiol.* 2015, **81**:6276-6284.

•• 30. Jones JA, Vernacchio VR, Collins SM, Shirke AN, Xiu Y, Englaender JA, Cress BF, McCutcheon CC, Linhardt RJ, Gross RA: **Complete biosynthesis of anthocyanins using *E. coli* polycultures**. *MBio* 2017, **8**:e00621-00617.

• 31. Kallscheuer N, Vogt M, Stenzel A, Gätgens J, Bott M, Marienhagen J: **Construction of a *Corynebacterium glutamicum* platform strain for the production of stilbenes and (2*S*)-flavanones**. *Metab. Eng.* 2016, **38**:47-55.

32. Kallscheuer N, Vogt M, Bott M, Marienhagen J: **Functional expression of plant-derived *O*-methyltransferase, flavanone 3-hydroxylase, and flavonol synthase in *Corynebacterium glutamicum* for production of pterostilbene, kaempferol, and quercetin**. *J. Biotechnol.* 2017, **258**:190-196.

•• 33. Li M, Kildegaard KR, Chen Y, Rodriguez A, Borodina I, Nielsen J: ***De novo* production of resveratrol from glucose or ethanol by engineered *Saccharomyces cerevisiae***. *Metab. Eng.* 2015, **32**:1-11.

34. Milke L, Aschenbrenner J, Marienhagen J, Kallscheuer N: **Production of plant-derived polyphenols in microorganisms: current state and perspectives**. *Appl. Microbiol. Biotechnol.* 2018, **102**:1575-1585.

35. Zwenger S, Basu C: **Plant terpenoids: applications and future potentials**. *Biotechnol. Mol. Biol. Rev.* 2008, **3**:1.

36. Rohmer M, Seemann M, Horbach S, Bringer-Meyer S, Sahm H: **Glyceraldehyde 3-phosphate and pyruvate as precursors of isoprenic units in an alternative non-mevalonate pathway for terpenoid biosynthesis**. *J. Am. Chem. Soc.* 1996, **118**:2564-2566.

37. Trombetta D, Castelli F, Sarpietro MG, Venuti V, Cristani M, Daniele C, Saija A, Mazzanti G, Bisignano G: **Mechanisms of antibacterial action of three monoterpenes**. *Antimicrob. Agents Chemother.* 2005, **49**:2474-2478.

38. Quiroga PR, Asensio CM, Nepote V: **Antioxidant effects of the monoterpenes carvacrol, thymol and sabinene hydrate on chemical and sensory stability of roasted sunflower seeds**. *J. Sci. Food Agric.* 2015, **95**:471-479.

39. Herrero Ó, Ramón D, Orejas M: **Engineering the *Saccharomyces cerevisiae* isoprenoid pathway for *de novo* production of aromatic monoterpenes in wine**. *Metab. Eng.* 2008, **10**:78-86.

40. Amiri P, Shahpiri A, Asadollahi MA, Momenbeik F, Partow S: **Metabolic engineering of *Saccharomyces cerevisiae* for linalool production**. *Biotechnol. Lett.* 2016, **38**:503-508.

• 41. Kang M-K, Eom J-H, Kim Y, Um Y, Woo HM: **Biosynthesis of pinene from glucose using metabolically-engineered *Corynebacterium glutamicum***. *Biotechnol. Lett.* 2014, **36**:2069-2077.

42. Yang J, Nie Q, Ren M, Feng H, Jiang X, Zheng Y, Liu M, Zhang H, Xian M: **Metabolic engineering of *Escherichia coli* for the biosynthesis of alpha-pinene**. *Biotechnol. Biofuels* 2013, **6**:60.

43. Prieto S. GA, Perea V. J, A, Ortiz L. C, C: **Microbial biotransformation of (*R*)-(+)-limonene by *Penicillium digitatum* DSM 62840 for producing (*R*)-(+)-terpineol**. *Vitae* 2011, **18**.

44. Rottava I, Cortina PF, Grando CE, Colla AR, Martello E, Cansian RL, Toniazzo G, Treichel H, Antunes OA, Oestreicher EG: **Isolation and screening of microorganisms for *R*-(+)-limonene and (−)-β-pinene biotransformation**. *Appl. Biochem. Biotechnol.* 2010, **162**:719-732.

45. Bicas JL, de Quadros CP, Néri-Numa IA, Pastore GM: **Integrated process for co-production of alkaline lipase and *R*-(+)-α-terpineol by *Fusarium oxysporum***. *Food Chem.* 2010, **120**:452-456.

46. Zheng G-W, Pan J, Yu H-L, Ngo-Thi M-T, Li C-X, Xu J-H: **An efficient bioprocess for enzymatic production of L-menthol with high ratio of substrate to catalyst using whole cells of recombinant *E. coli***. *J. Biotechnol.* 2010, **150**:108-114.

47. Toogood HS, Cheallaigh AN, Tait S, Mansell DJ, Jervis A, Lygidakis A, Humphreys L, Takano E, Gardiner JM, Scrutton NS: **Enzymatic menthol production: one-pot approach using engineered *Escherichia coli***. *ACS synthetic biology* 2015, **4**:1112-1123.

48. Del Rio JA, Ortuno A, Garcia-Puig D, Porras I, Garcia-Lidon A, Sabater F: **Variations of nootkatone and valencene levels during the development of grapefruit**. *J. Agric. Food Chem.* 1992, **40**:1488-1490.

49. Frohwitter J, Heider SA, Peters-Wendisch P, Beekwilder J, Wendisch VF: **Production of the sesquiterpene (+)-valencene by metabolically engineered *Corynebacterium glutamicum***. *J. Biotechnol.* 2014, **191**:205-213.

50. Beekwilder J, Houwelingen A, Cankar K, Dijk AD, Jong RM, Stoopen G, Bouwmeester H, Achkar J, Sonke T, Bosch D: **Valencene synthase from the heartwood of Nootka cypress (*Callitropsis nootkatensis*) for biotechnological production of valencene**. *Plant Biotechnol. J.* 2014, **12**:174-182.

51. Wriessnegger T, Augustin P, Engleder M, Leitner E, Müller M, Kaluzna I, Schürmann M, Mink D, Zellnig G, Schwab H: **Production of the sesquiterpenoid (+)-nootkatone by metabolic engineering of *Pichia pastoris***. *Metab. Eng.* 2014, **24**:18-29.

52. Palmerín-Carreño D, Rutiaga-Quiñones O, Calvo JV, Prado-Barragán A, Huerta-Ochoa S: **Screening of microorganisms for bioconversion of (+)-valencene to (+)-nootkatone**. *LWT-Food Sci. Technol.* 2015, **64**:788-793.

53. Aruoma O, Halliwell B, Aeschbach R, Löligers J: **Antioxidant and pro-oxidant properties of active rosemary constituents: carnosol and carnosic acid**. *Xenobiotica* 1992, **22**:257-268.

• 54. Scheler U, Brandt W, Porzel A, Rothe K, Manzano D, Božić D, Papaefthimiou D, Balcke GU, Henning A, Lohse S: **Elucidation of the biosynthesis of carnosic acid and its reconstitution in yeast**. *Nature Commun.* 2016, **7**:12942.

55. Shi J, Maguer ML: **Lycopene in tomatoes: chemical and physical properties affected by food processing**. *Crit. Rev. Food Sci. Nutr.* 2000, **40**:1-42.

•• 56. Zhao J, Li Q, Sun T, Zhu X, Xu H, Tang J, Zhang X, Ma Y: **Engineering central metabolic modules of *Escherichia coli* for improving β-carotene production**. *Metab. Eng.* 2013, **17**:42-50.

57. Verwaal R, Wang J, Meijnen J-P, Visser H, Sandmann G, van den Berg JA, van Ooyen AJ: **High-level production of beta-carotene in *Saccharomyces cerevisiae* by successive transformation with carotenogenic genes from *Xanthophyllomyces dendrorhous***. *Appl. Environ. Microbiol.* 2007, **73**:4342-4350.

58. Hernández-Almanza A, Montañez J, Martínez G, Aguilar-Jiménez A, Contreras-Esquivel JC, Aguilar CN: **Lycopene: Progress in microbial production**. *Trends Food Sci. Technol.* 2016, **56**:142-148.

59. Zhu F, Lu L, Fu S, Zhong X, Hu M, Deng Z, Liu T: **Targeted engineering and scale up of lycopene overproduction in *Escherichia coli***. *Process Biochem.* 2015, **50**:341-346.

60. Rodríguez-Bustamante E, Sánchez S: **Microbial production of C13-norisoprenoids and other aroma compounds via carotenoid cleavage**. *Crit. Rev. Microbiol.* 2007, **33**:211-230.

61. Beekwilder J, van Rossum HM, Koopman F, Sonntag F, Buchhaupt M, Schrader J, Hall RD, Bosch D, Pronk JT, van Maris AJ: **Polycistronic expression of a β-carotene biosynthetic pathway in *Saccharomyces cerevisiae* coupled to β-ionone production**. *J. Biotechnol.* 2014, **192**:383-392.

62. López J, Essus K, Kim I-k, Pereira R, Herzog J, Siewers V, Nielsen J, Agosin E: **Production of β-ionone by combined expression of carotenogenic and plant CCD1 genes in *Saccharomyces cerevisiae***. *Microb. Cell Fact.* 2015, **14**:84.

63. Chai F, Wang Y, Mei X, Yao M, Chen Y, Liu H, Xiao W, Yuan Y: **Heterologous biosynthesis and manipulation of crocetin in *Saccharomyces cerevisiae***. *Microb. Cell Fact.* 2017, **16**:54.

64. Lee J-H, Choi J-G, Kim Y-S, Kim K-R, Kim S-W, Oh D-K: **Enhancement of retinal production by supplementing the surfactant Span 80 using metabolically engineered *Escherichia coli***. *J. Biosci. Bioeng.* 2012, **113**:461-466.

65. Ravid U, Elkabetz M, Zamir C, Cohen K, Larkov O, Aly R: **Authenticity assessment of natural fruit flavour compounds in foods and beverages by auto-HS–SPME stereoselective GC–MS**. *Flavour Fragrance J.* 2010, **25**:20-27.

66. Pagot Y, Endrizzi A, Nicaud JM, Belin JM: **Utilization of an auxotrophic strain of the yeast *Yarrowia lipolytica* to improve γ-decalactone production yields**. *Lett. Appl. Microbiol.* 1997, **25**:113-116.

67. Waché Y, Aguedo M, Choquet A, Gatfield IL, Nicaud J-M, Belin J-M: **Role of β-oxidation enzymes in γ-decalactone production by the yeast *Yarrowia lipolytica***. *Appl. Environ. Microbiol.* 2001, **67**:5700-5704.

68. Waché Y, Aguedo M, LeDall M-T, Nicaud J-M, Belin J-M: **Optimization of *Yarrowia lipolytica*'s β-oxidation pathway for γ-decalactone production**. *J. Mol. Catal. B Enzym.* 2002, **19**:347-351.

69. Gomes N, Teixeira JA, Belo I: **Fed-batch versus batch cultures of *Yarrowia lipolytica* for γ-decalactone production from methyl ricinoleate**. *Biotechnol. Lett.* 2012, **34**:649-654.

• 70. Soares GP, Souza KS, Vilela LF, Schwan RF, Dias DR: **γ-Decalactone production by *Yarrowia lipolytica* and *Lindnera saturnus* in crude glycerol**. *Prep. Biochem. Biotechnol.* 2017, **47**:633-637.

71. Juneja LR, Chu D-C, Okubo T, Nagato Y, Yokogoshi H: **L-theanine—a unique amino acid of green tea and its relaxation effect in humans**. *Trends Food Sci. Technol.* 1999, **10**:199-204.

72. Suzuki H, Izuka S, Miyakawa N, Kumagai H: **Enzymatic production of theanine, an "umami" component of tea, from glutamine and ethylamine with bacterial γ-glutamyltranspeptidase**. *Enzyme Microb. Technol.* 2002, **31**:884-889.

73. Chen X, Su L, Wu D, Wu J: **Application of recombinant *Bacillus subtilis* γ-glutamyltranspeptidase to the production of L-theanine**. *Process Biochem.* 2014, **49**:1429-1439.

74. Shuai Y, Zhang T, Jiang B, Mu W: **Development of efficient enzymatic production of theanine by γ-glutamyltranspeptidase from a newly isolated strain of *Bacillus subtilis*, SK11. 004**. *J. Sci. Food Agric.* 2010, **90**:2563-2567.

75. Zhang F, Zheng Q-Z, Jiao Q-C, Liu J-Z, Zhao G-H: **Synthesis of theanine from glutamic acid γ-methyl ester and ethylamine catalyzed by *Escherichia coli* having γ-glutamyltranspeptidase activity**. *Biotechnol. Lett.* 2010, **32**:1147-1150.

76. Pappenberger G, Hohmann H-P: **Industrial production of L-ascorbic acid (Vitamin C) and D-isoascorbic acid**. In *Biotechnology of food and feed additives*. Springer; 2013:143-188.

77. Kant R: **Sweet proteins–potential replacement for artificial low calorie sweeteners**. *Nutr. J.* 2005, **4**:5.

78. Moralejo F-J, Cardoza R-E, Gutierrez S, Martin JF: **Thaumatin production in *Aspergillus awamori* by use of expression cassettes with strong fungal promoters and high gene dosage**. *Appl. Environ. Microbiol.* 1999, **65**:1168-1174.

79. Moralejo FJ, Cardoza RE, Gutierrez S, Lombrana M, Fierro F, Martín JF: **Silencing of the aspergillopepsin B (*pepB*) gene of *Aspergillus awamori* by antisense RNA**

expression or protease removal by gene disruption results in a large increase in thaumatin production. *Appl. Environ. Microbiol.* 2002, **68**:3550-3559.

80. Illingworth C, Larson G, Hellekant G: **Secretion of the sweet-tasting plant protein thaumatin by *Bacillus subtilis***. *Biotechnol. Lett.* 1988, **10**:587-592.

81. Chen Z, Li Z, Yu N, Yan L: **Expression and secretion of a single-chain sweet protein, monellin, in *Saccharomyces cerevisiae* by an α-factor signal peptide**. *Biotechnol. Lett.* 2011, **33**:721-725.

82. Chen Z, Cai H, Lu F, Du L: **High-level expression of a synthetic gene encoding a sweet protein, monellin, in *Escherichia coli***. *Biotechnol. Lett.* 2005, **27**:1745-1749.

83. Kondo K, Miura Y, Sone H, Kobayashi K: **High-level expression of a sweet protein, monellin, in the food yeast *Candida utilis***. *Nat. Biotechnol.* 1997, **15**:453.

84. Chen Z, Heng C, Li Z, Liang X, Xinchen S: **Expression and secretion of a single-chain sweet protein monellin in *Bacillus subtilis* by *sacB* promoter and signal peptide**. *Appl. Microbiol. Biotechnol.* 2007, **73**:1377-1381.

85. Berlec A, Štrukelj B: **Large increase in brazzein expression achieved by changing the plasmid/strain combination of the NICE system in *Lactococcus lactis***. *Lett. Appl. Microbiol.* 2009, **48**:750-755.

86. Poirier N, Roudnitzky N, Brockhoff A, Belloir C, Maison M, Thomas-Danguin T, Meyerhof W, Briand L: **Efficient production and characterization of the sweet-tasting brazzein secreted by the yeast *Pichia pastoris***. *J. Agric. Food Chem.* 2012, **60**:9807-9814.

87. Regnat K, Mach RL, Mach-Aigner AR: **Erythritol as sweetener—wherefrom and whereto?** *Appl. Microbiol. Biotechnol.* 2018, **102**:587-595.

88. Philippe RN, De Mey M, Anderson J, Ajikumar PK: **Biotechnological production of natural zero-calorie sweeteners**. *Curr. Opin. Biotechnol.* 2014, **26**:155-161.

89. Jain H, Mulay S: **A review on different modes and methods for yielding a pentose sugar: xylitol**. *Int. J. Food Sci. Nutr.* 2014, **65**:135-143.

90. Park J-Y, Jung J-H, Seo D-H, Ha S-J, Yoon J-W, Kim Y-C, Shim J-H, Park C-S: **Microbial production of palatinose through extracellular expression of a sucrose isomerase from *Enterobacter* sp. FMB-1 in *Lactococcus lactis* MG1363**. *Bioresour. Technol.* 2010, **101**:8828-8833.

91. Bode L, Contractor N, Barile D, Pohl N, Prudden AR, Boons GJ, Jin YS, Jennewein S: **Overcoming the limited availability of human milk oligosaccharides: challenges and opportunities for research and application**. *Nutr. Rev.* 2016, **74**:635-644.

92. Wynands B, Lenzen C, Otto M, Koch F, Blank LM, Wierckx N: **Metabolic engineering of *Pseudomonas taiwanensis* VLB120 with minimal genomic modifications for high-yield phenol production**. *Metab. Eng.* 2018, **47**:121-133.

93. Kallscheuer N, Marienhagen J: **Corynebacterium glutamicum as platform for the production of hydroxybenzoic acids**. *Microb. Cell Fact.* 2018, **17**:70.

591 94. Loeschcke A, Thies S: ***Pseudomonas putida*—a versatile host for the production of**
592 **natural products**. *Appl. Microbiol. Biotechnol.* 2015, **99**:6197-6214.

593 95. Henke NA, Wichmann J, Baier T, Frohwitter J, Lauersen KJ, Risse JM, Peters-Wendisch
594 P, Kruse O, Wendisch VF: **Patchoulol production with metabolically engineered**
595 ***Corynebacterium glutamicum***. *Genes* 2018, **9**:219.

596 96. Heck A, Drepper T: **Engineering Photosynthetic α-Proteobacteria for the Production**
597 **of Recombinant Proteins and Terpenoids**. In *Modern Topics in the Phototrophic*
598 *Prokaryotes*. Springer; 2017:395-425.

599 • 97. Loeschcke A, Dienst D, Wewer V, Hage-Hülsmann J, Dietsch M, Kranz-Finger S,
600 Hüren V, Metzger S, Urlacher VB, Gigolashvili T: **The photosynthetic bacteria**
601 ***Rhodobacter capsulatus* and *Synechocystis* sp. PCC 6803 as new hosts for cyclic**
602 **plant triterpene biosynthesis**. *PloS One* 2017, **12**:e0189816.

603 98. Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Wu X, Jiang W, Marraffini
604 L: **Multiplex genome engineering using CRISPR/Cas systems**. *Science* 2013:1231143.

605 99. Schallmey M, Frunzke J, Eggeling L, Marienhagen J: **Looking for the pick of the**
606 **bunch: high-throughput screening of producing microorganisms with biosensors**.
607 *Curr. Opin. Biotechnol.* 2014, **26**:148-154.

608 100. Jiang J, Bi H, Zhuang Y, Liu S, Liu T, Ma Y: **Engineered synthesis of rosmarinic acid**
609 **in *Escherichia coli* resulting production of a new intermediate, caffeoyl-phenyllactate**.
610 *Biotechnol. Lett.* 2016, **38**:81-88.

611 101. Jiang G-Z, Yao M-D, Wang Y, Zhou L, Song T-Q, Liu H, Xiao W-H, Yuan Y-J:
612 **Manipulation of GES and ERG20 for geraniol overproduction in *Saccharomyces***
613 ***cerevisiae***. *Metab. Eng.* 2017, **41**:57-66.

Publications of special interest

• 15. Hansen EH, Møller BL, Kock GR, Bünner CM, Kristensen C, Jensen OR, Okkels FT, Olsen CE, Motawia MS, Hansen J: ***De novo* biosynthesis of vanillin in fission yeast (*Schizosaccharomyces pombe*) and baker's yeast (*Saccharomyces cerevisiae*)**. *Appl. Environ. Microbiol.* 2009, **75**:2765-2774.

The authors combined genes from various sources (plants, bacteria) and human genes for converting the shikimate pathway intermediate 3-dehydroshikimate to protocatechuate and ultimately to vanillin. *De novo* production of vanillin from glucose was demonstrated for the first time.

• 22. Kambourakis S, Draths K, Frost J: **Synthesis of gallic acid and pyrogallol from glucose: replacing natural product isolation with microbial catalysis**. *J. Am. Chem. Soc.* 2000, **122**:9042-9043.

The shikimate pathway in *E. coli* was exploited for the microbial production of gallic acid. The final titer was sufficient for rendering the production strain a promising alternative for natural product isolation.

• 26. Eichenberger M, Lehka BJ, Folly C, Fischer D, Martens S, Simón E, Naesby M: **Metabolic engineering of *Saccharomyces cerevisiae* for *de novo* production of dihydrochalcones with known antioxidant, antidiabetic, and sweet tasting properties**. *Metab. Eng.* 2017, **39**:80-89.

The authors identified a native enzyme in *S. cerevisiae* which is capable of converting *p*-coumaroyl-CoA to *p*-dihydrocoumaryl-CoA. This enzyme was applied for the production of the glycosylated naringin dihydrochalcone from glucose by additionally deregulating the aromatic amino acid-forming shikimate pathway.

•• 30. Jones JA, Vernacchio VR, Collins SM, Shirke AN, Xiu Y, Englaender JA, Cress BF, McCutcheon CC, Linhardt RJ, Gross RA: **Complete biosynthesis of anthocyanins using *E. coli* polycultures**. *MBio* 2017, **8**:e00621-00617.

The entire pathway for anthocyanin production from glucose was reconstructed in four *E. coli* strains collectively expressing 15 heterologous genes. The polyculture of the four strains was capable of producing the glycosylated anthocyanin callistephin.

• 31. Kallscheuer N, Vogt M, Stenzel A, Gätgens J, Bott M, Marienhagen J: **Construction of a *Corynebacterium glutamicum* platform strain for the production of stilbenes and (2*S*)-flavanones**. *Metab. Eng.* 2016, **38**:47-55.

The authors performed deletion of 21 genes in four gene clusters in *C. glutamicum* for abolishing peripheral and central catabolic pathways for aromatic compounds. The constructed strain was subsequently used for heterologous production of plant stilbenes and flavanones, but also represents a promising platform for the production of additional commercially relevant aromatic compounds.

•• 33. Li M, Kildegaard KR, Chen Y, Rodriguez A, Borodina I, Nielsen J: ***De novo* production of resveratrol from glucose or ethanol by engineered *Saccharomyces cerevisiae***. *Metab. Eng.* 2015, **32**:1-11.

The authors rationally engineered *S. cerevisiae* for production of the stilbene resveratrol. In addition to the functional introduction of the resveratrol biosynthesis pathway, resveratrol production was further optimized by increasing the flux leading to the precursor metabolites L-tyrosine and malonyl-CoA. The final strain was capable of producing 0.53 g/L resveratrol from glucose.

• 41. Kang M-K, Eom J-H, Kim Y, Um Y, Woo HM: **Biosynthesis of pinene from glucose using metabolically-engineered *Corynebacterium glutamicum***. *Biotechnol. Lett.* 2014, **36**:2069-2077.

The natural capability of *C. glutamicum* for isoprenoid production was exploited for the production of the plant-derived terpene pinene. The constructed strain background is the basis for further increasing the porfolio of microbially accessible portfolio based on *C. glutamicum*.

• 54. Scheler U, Brandt W, Porzel A, Rothe K, Manzano D, Božić D, Papaefthimiou D, Balcke GU, Henning A, Lohse S: **Elucidation of the biosynthesis of carnosic acid and its reconstitution in yeast**. *Nature Commun.* 2016, **7**:12942.

Carnosic acid, a diterpene present in rosemary extract, has interesting applications as antioxidant or preservative, but the biosynthetic pathway for this compound has not been elucidated in detail. In this study, the authors identified key enzymes in the pathway leading to carnosic acid and initiated the pathway reconstruction in *S. cerevisiae*.

•• 56. Zhao J, Li Q, Sun T, Zhu X, Xu H, Tang J, Zhang X, Ma Y: **Engineering central metabolic modules of *Escherichia coli* for improving β-carotene production**. *Metab. Eng.* 2013, **17**:42-50.

Metabolic modules in the heterologous β-carotene biosynthetic pathway and in the central carbon metabolism were separately engineered for establishing and improving β-carotene in *E. coli*. Optimization of the cultivation conditions led to the production of β-carotene in the g/L scale.

• 70. Soares GP, Souza KS, Vilela LF, Schwan RF, Dias DR: **γ-Decalactone production by *Yarrowia lipolytica* and *Lindnera saturnus* in crude glycerol**. *Prep. Biochem. Biotechnol.* 2017, **47**:633-637.

The authors present microbial production of γ-decalactone as an alternative strategy for the chemical production of this compound. In this study the endogenous peroxisomal β-oxidation machinery in two different yeast species was exploited for γ-decalactone production from crude glycerol.

• 97. Loeschcke A, Dienst D, Wewer V, Hage-Hülsmann J, Dietsch M, Kranz-Finger S, Hüren V, Metzger S, Urlacher VB, Gigolashvili T: **The photosynthetic bacteria *Rhodobacter capsulatus* and *Synechocystis* sp. PCC 6803 as new hosts for cyclic plant triterpene biosynthesis**. *PloS One* 2017, **12**:e0189816.

Cyclic triterpenes are a very diverse group of compounds and exhibit versatile bioactivities. The results obtained in this study show that engineered photosynthetic bacteria are promising host strains for the production of plant triterpenes.

Figure legends

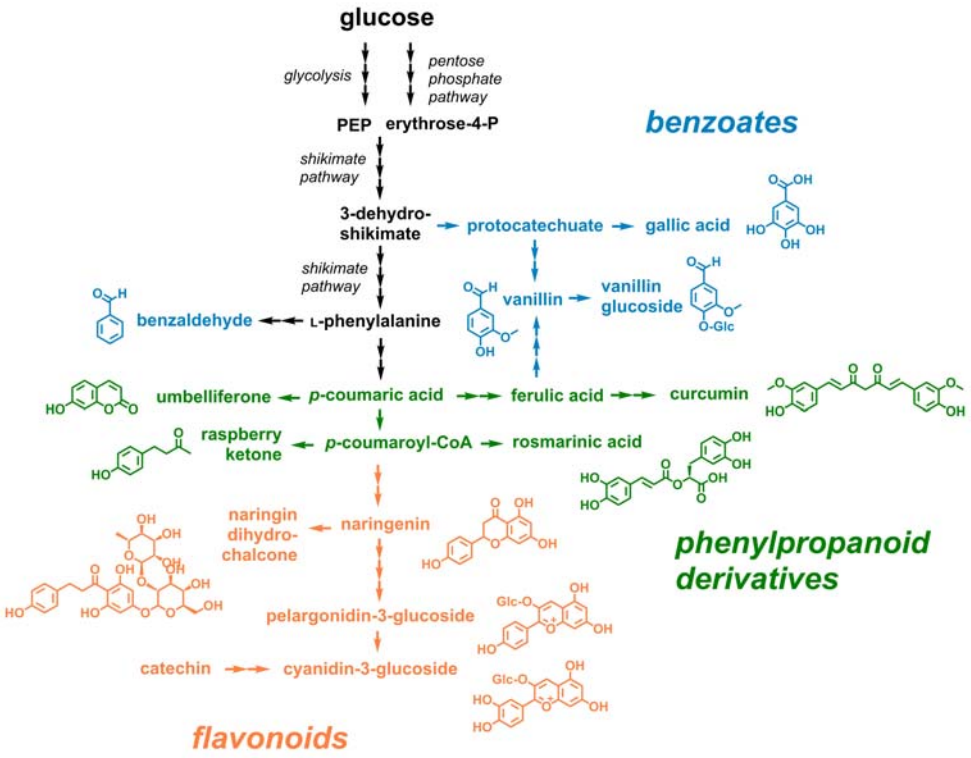
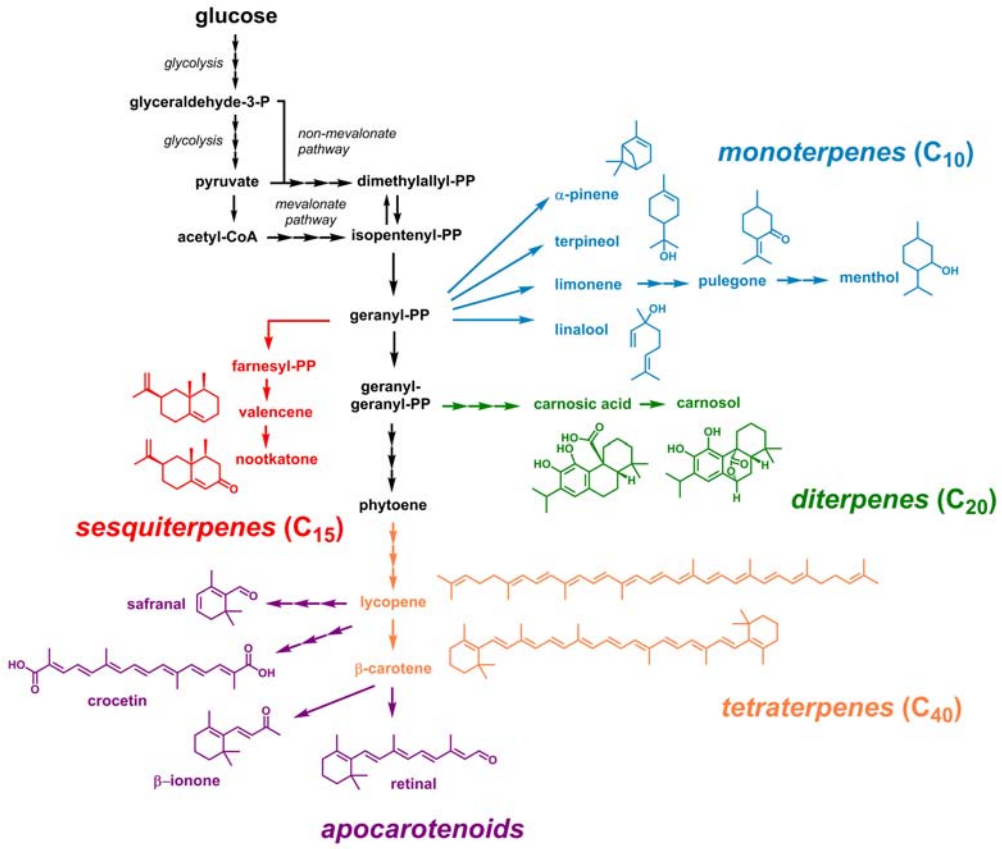
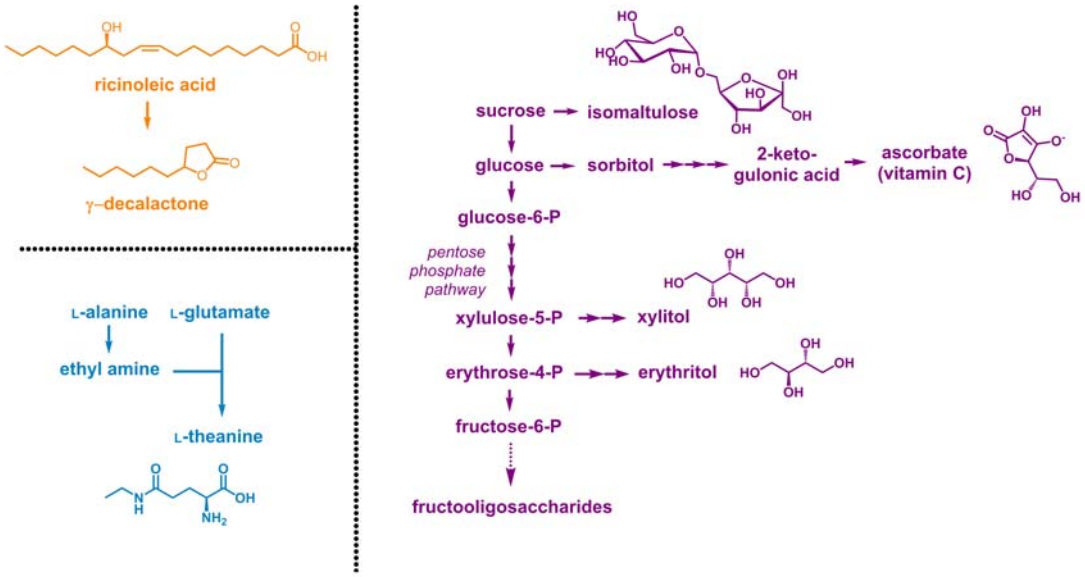


Figure 1. Biosynthetic pathways for plant-derived phenols relevant for in food applications. Abbreviation: CoA: coenzyme A, PEP: phosphoenolpyruvate, Glc: glucose



700

701 **Figure 2. Biosynthetic pathways leading to plant-derived terpenes relevant for food**
702 **applications.** Abbreviation: PP: diphosphate



703

704 **Figure 3. Biosynthetic pathways leading to other plant-derived compounds with**
705 **applications in food industries.**

706 **Table 1. Microbially produced plant metabolites with applications in food industries.**

Product	Product titer		Precursor	Precursor titer		Yield	Organism	Reference
	mg/L	mM		mg/L	mM	mol/mol		
phenols								
vanillin	2,520	16.6	ferulic acid	4,462	23.0	0.72	*E. coli*	[10]
vanillin	710	4.7	ferulic acid esters in wheat bran	--	--	--	*S. sannanensis*	[9]
vanillin	1,280	8.4	ferulic acid	2,328	12	0.70	*P. fluorescens*	[12]
vanillic acid	2,200	13.1	ferulic acid	4,000	20.6	0.64	*A. niger*	[11]
vanillin	65	0.42	none, from glucose	--	--	--	*S. pombe*	[15]
vanillin β-D-glucoside	500	1.59	none, from glucose	--	--	--	*S. cerevisiae*	[16]
benzaldehyde	790	7.5	L-phenylalanine	3,000	18.2	0.41	*P. cinnabarinus*	[19]
raspberry ketone	7.5	0.045	*p*-coumaric acid	492	3	0.01	*S. cerevisiae*	[20]
raspberry ketone	2.8	0.017	none, from glucose	--	--	--	*S. cerevisiae*	[20]
gallic acid	20,000	118	none, from glucose	--	--	--	*E. coli*	[22]
rosmarinic acid	130	0.36	caffeic acid	360	2	0.18	*E. coli*	[100]
rosmarinic acid	0.65	0.002	none, from glucose	--	--	--	*E. coli*	[23]
umbelliferone	66	0.41	none, from glucose	--	--	--	*E. coli*	[24]
curcumin	60	0.16	ferulic acid in rice bran pitch	--	--	--	*E. coli*	[25]
quercetin	10	0.033	caffeic acid	900	5	0.01	*C. glutamicum*	[32]
resveratrol	158	0.69	*p*-coumaric acid	820	5	0.14	*C. glutamicum*	[31]
naringin dihydrochalcone	12	0.021	none, from glucose	--	--	--	*S. cerevisiae*	[26]
cyanidin-3-*O*-glucoside	350	0.78	catechin	725	2.5	0.31	*E. coli*	[29]
pelargonidin-3-*O*-glucoside	10	0.023	none, from glucose	--	--	--	*E. coli*	[30]
terpenoids								
geraniol	1,680	10.9	none, from glucose / ethanol	--	--	--	*S. cerevisiae*	[101]
linalool	0.08	<0.001	none, from glucose	--	--	--	*S. cerevisiae*	[39]
linalool	0.096	<0.001	none, from galactose	--	--	--	*S. cerevisiae*	[40]
α-pinene	0.18	<0.001	none, from glucose	--	--	--	*C. glutamicum*	[41]
α-pinene	970	7.1	none, from glucose	--	--	--	*E. coli*	[42]
α-terpineol	4,000	26.0	limonene	5,000	36.8	0.71	*F. oxysporum*	[45]
α-terpineol	1,864	12.1	limonene	2,000	14.7	0.82	*P. digitatum*	[43]
menthol	53	0.34	pulegone	152	1	0.34	*E. coli*	[47]
valencene	2.4	0.011	none, from glucose	--	--	--	*C. glutamicum*	[49]
valencene	352	1.73	none, from glucose	--	--	--	*R. sphaeroides*	[50]
nootkatone	208	0.95	none, from glucose / methanol	--	--	--	*P. pastoris*	[51]
nootkatone	240	1.1	valencene	1,031	5	0.22	*B. theobromae*	[52]
β-carotene	2,100	3.9	none, from glucose	--	--	--	*E. coli*	[56]
lycopene	1,440	2.7	none, from glycerol	--	--	--	*E. coli*	[59]
β-ionone	5	0.026	none, from glucose	--	--	--	*S. cerevisiae*	[62]
crocetin	1.2	0.004	none, from glucose	--	--	--	*S. cerevisiae*	[63]
retinal	600	2.1	none, from glucose/arabinose	--	--	--	*E. coli*	[64]
other compounds								
γ-decalactone	6,800	40	methyl ricinoleate	30,000	96	0.42	*Y. lipolytica*	[69]
γ-decalactone	5,800	34	crude glycerol	--	--	--	*L. saturnus*	[70]
L-theanine	16,550	95	glutamic acid γ-methyl ester	16,100	100	0.95	*E. coli*	[75]
thaumatin	13	(*)	none, from glucose	--	--	--	*A. awamori*	[79]
monellin	410	(*)	none, from glucose	--	--	--	*S. cerevisiae*	[81]
brazzein	90	(*)	none, from glucose	--	--	--	*P. pastoris*	[86]

(*) in the case of proteins no concentrations are given in mM