Microbial Production of Natural Flavors and Fragrances

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Abstract

Due to global warming, with climate changes and significantly decreasing petrochemical resources, an urgent need has arisen to produce bio-based products in a renewable manner. Chemical-based products also cause a serious impact on health and the environment. Chemical synthesis and extraction of products from plants are non-sustainable. The global demand for natural flavor and fragrance is continuously increasing and has shown a high interest in the aroma industry. Plants and microorganisms are the major sources of flavor and fragrance. Due to production in smaller concentrations, isolation and extraction of such value-added chemicals become expensive. These natural products are terpenoids, aldehydes, methyl ketones, etc., which are used in a wide range of domestic products including cosmetics, soaps, fresheners, candles, and foods. Microbial production is an alternative way of synthesis by modifying natural biosynthetic pathways or inserting a novel pathway into hosts. The present

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A. Kumar et al. (eds.), *Recent Advances in Food Biotechnology*,
https://doi.org/10.1007/978-981-16-8125-7_7

chapter highlights important chemicals for flavor and fragrance and their engineered production.

Keywords

Flavors · Fragrance · Metabolic engineering · Biosynthetic pathway

7.1 Introduction

Currently, global demand of natural flavor and fragrance is high, and the market is exponentially growing. The global flavors and fragrances market size was valued at USD 20.75 billion in 2018. It is expected to increase at a compound annual growth rate (CAGR) of 4.7%. These are used in food, beverages, perfumes, scent, cosmetics, toiletries, and many more. In India, the flavor market was valued at USD 284.2 million in 2011, which rose to USD 380.6 million in 2014, and is expected to grow at 10% annually. Noh et al. (2019) reported the use of butyl butyrate as a flavoring and fragrance agent in different products such as beverage, perfumes, food, and cosmetics.

Recently, Nies et al. (2020) produced methyl ketones that have been used as flavor, pharmaceutical, fragrance, and agrochemicals. They engineered *Pseudomonas taiwanensis* VLB120 for the production of methyl ketone. The strain was further improved by eliminating the competitive pathway and a yield of 9.8 g Laq^{-1}. This was fourfold higher than the initial strain. A wide range of microorganisms has the ability to produce natural flavors and fragrances. However, these microorganisms cannot be further manipulated due to the lack of molecular biology tools. Therefore, well-studied microorganisms can be used for the production of these chemicals by installing the complete biosynthesis pathway at an industrial scale. Microbial engineering requires a wide range of approaches including synthetic biology (Patel et al. 2018; Bhattacharjee et al. 2020), metabolic engineering (Singh et al. 2020; Gohil et al. 2017), and fermentation technology for large-scale production at competitive prices. This chapter highlights several microorganisms that have the ability to produce flavor and fragrance to fulfill the rising demand of market globally.

7.2 Production of Flavors and Fragrances

7.2.1 Diacetyl

Diacetyl, also known as butanedione or butane-2,3-dione, is an organic compound. It is a yellow- or green-colored liquid with buttery flavor. It is used in alcoholic beverages and also added in foods for buttery flavor. Natural flavor of yoghurt is achieved by lipolysis of milk fat and transformation of lactose and citrate. A number of compounds such as alcohols, acids, aromatic compounds, heterocyclic compounds, esters, hydrocarbons, and many more are available in yoghurt at low to trace amounts. Other than lactic acid, the natural flavor compounds in yoghurt are acetaldehyde, acetone, diacetyl, acetoin, and 2-butanone (Cheng 2010). Rincon-Delgadillo et al. (2012) used the starter distillates (SDL), which is a major ingredient for the formulation of a wide range of products including cottage cheese, vegetable oil spreads, margarine, processed cheese, and sour cream to enhance the buttery aroma in the products. The buttery aroma is possible because of the presence of vicinal dicarbonyl and diacetyl which is a key component of SDL. Diacetyl (2,3-butanedione) is a product of citrate metabolism of certain bacteria such as *Lactococcus lactis*. A gene *bud A* encoding alpha-acetolactate when knocked out in strain resulted in the inactivation of diacetyl reductases, thereby eliminating chances of loss of diacetyl owing to its reduction (Zhang et al. 2015). Cofactor engineering has also been applied for enhancing production of diacetyl (Wang et al. 2013); deletion of the gene encoding phosphotransacetylase (*pta*) enhances diacetyl production by about 130% (Wang et al. 2019).

7.2.2 Esters

Ester is mainly produced from an organic or inorganic acid. It contains at least one hydroxyl (-OH) group which is replaced by an alkoxy (-O-alkyl) group. Glycerides are fatty acid esters of glycerol which are the main classes of lipids and generate bulk animal fats and vegetable oils. Low molecular weight esters are used as fragrance that is usually found in pheromones and essential oils.

El Hadi et al. (2013) reported that a number of fruits produce a wide range of volatile compounds which contribute to their flavor. These volatile compounds are composed of esters, aldehydes, lactones, alcohols, apocarotenoids, ketones, and terpenoids. These are produced via biosynthesis initiated from amino acid, lipid, and terpenoid, and once the basic molecules are synthesized, a number of modifications such as acylation, oxidation/reduction, methylation, and cyclic ring closure can be done to allow the compounds to impart proper aroma. Menendez-Bravo et al. (2017) redesigned microbial pathways for the production of routinely used consumer products including waxes, esters, fatty alcohols, and fatty acids. Small-chain aliphatic esters are major elements of flavors and fragrances, while long-chain esters are composed of acids and fatty acids esterified to fatty alcohols. These have been extensively used in paints, coating, lubricant formulas, and cosmetics.

7.2.3 Benzaldehyde

Benzaldehyde is the simplest aromatic aldehyde with a single formyl group. Schade et al. (2001) studied that artificial almond oil is one of the signature vaporized compounds for fragrance of carnation flowers. The production of synthetic and natural benzaldehyde per year equals to 7000 tons and 100 tons, respectively. Usually, 80% of benzaldehyde is produced from cinnamaldehyde by retro aldol reaction (Verma et al. 2017). Benzaldehyde is naturally extracted from cassia, bitter almond oil, cheese, black tea, kernels, fruits, or leaves of apricots, apples, and peaches (Sen 2015). Naturally, benzoic aldehyde is freed from cyanogenic glycoside amygdalin by using β-glucosidase and mandelonitrile benzaldehyde lyase enzymes which gives a cheery and bitter almond taste (Opgrande et al. 2000). Benzenecarbaldehyde is an essential chemical component for the food and chemical industry. This molecule is used for a wide range of value-added chemicals such as production of cinnamic acid, different aniline dyes, aliphatic fragrance, flavoring agents, intermediate for pharmaceutical products, and bee-repellent for harvesting of bees (Wiener et al. 1988).

In insects, phenylmethanal serves as chemical defense (harvester ants) and pheromones (noctuid lepidoptera, trigona stingless bees) (Beroza 2012). It is also reported to exhibit antitumor, antibacterial and antifungal activities. In 1922, Match suggested that a minimum benzaldehyde concentration of 0.2% in saline could induce loosening and antispasmodic effect in involuntary non-striated muscles of the urinary bladder, uterus, bronchi, arteries, and stomach from different animal species. The process of forming phenylmethanal is possible by single-step bioconversion and de novo synthesis by utilizing flora, microorganisms, or even isolated enzymes (Krings et al. 1998). The bacteria *P. putida* and different species of white rot fungi such as *Trametes suaveolens*, *Polyporus tuberaster* (Kawabe et al. 1994), *Bjerkandera adusta* (Lapadatescu and Bonnarme 1999), *Phanerochaete chrysosporium* (Kim 2005), and *Pleurotus sapidus* (Lomascolo et al. 1999) naturally synthesize benzenecarbaldehyde through the de novo pathway (Paula Dionísio et al. 2012). The amino acid degradation pathways beginning from lipid oxidation of phenylalanine give benzoic aldehyde at pH 3. The presence of 13-hydroperoxide of linoleic acid (LOOH) or 4-oxononenal (ON), derivatives of phenylalanine (phenylacetaldehyde), metabolizes into benzaldehyde and releases a fruity-like GRAS flavoring molecule that has shown to be present in volatile fractions of almonds, cherry, tea leaves, and other food products (Hidalgo and Zamora 2019). Microbes *Lactobacillus plantarum* (Groot et al. 1998), *Streptococcus thermophilus* (Dan et al. 2018), and *L. helveticus* (Klein et al. 2001) induce production of phenylmethanal via catabolism of amino acids. From the manganese transport system of *L. plantarum*, it has been concluded that phenylalanine gets converted into benzoic aldehyde by pyridoxal 50-phosphate-dependent aminotransferase (Petrovici et al. 2018). In plants, through β-oxidative and non-β-oxidative pathways, phenylalanine is converted to benzaldehyde. Recently scientists have found a clue step of β-oxidative pathways where synthesis of benzoate takes place, which serves as the precursor molecule for benzaldehyde in a metabolic engineered pathway of microbes (Kunjapur and Prather 2015). The microbial biosynthesis of phenylmethanal from phenylmethanol takes place in methylotrophic yeast, *Pichia pastoris*, in single-step bioconversion via involvement of different enzymatic roles such as alcohol oxidase and formaldehyde dehydrogenase. The solid-liquid two-phase system (TPPB) uses an immiscible organic solvent for isolating benzenecarbaldehyde compounds (Jain et al. 2010).

Genetic recombination of *Escherichia coli* K-12 MG1655 strain resulting in deletion of gene encoding aldehyde reductase and recombinant carboxylic acid reductase yielded 350 mg/L production of benzaldehyde from benzoate with only 12% conversion to benzyl alcohol (Carroll et al. 2016). After deletion of cinnamyl alcohol dehydrogenases (*YahK* and *YjgB*) gene, accumulation of benzaldehyde in *E. coli* was observed. In pharmaceutical companies, benzaldehyde is used for the formation of precursor compound L-phenylacetylcarbinol which is involved in the synthesis of ephedrine (Kunjapur et al. 2014).

Benzaldehyde is majorly found in plants such as cucumber. Benzaldehyde synthase is the final enzyme in the bio-formation of benzaldehyde from phenylalanine. Baoxiu Liu and researcher showed that in *Cucumis sativus* L., genes cinnamaldehyde:NADP+oxidoreductase *CsaCCR7* (root cytosol-specific), *CsaCCR9* (peroxisome), and *CsaCCR18* (flower cytosol-specific) are in charge for the production of benzaldehyde from benzoyl-CoA. This was the first time when CCR as in benzaldehyde synthase added a new biochemical role in flora (Liu et al. 2019).

Though in the skin benzoic aldehyde quickly converts into benzoic acid, it shows no unpleasant reaction toward skin irritation or sensitization. Artificial almond oil (benzaldehyde) easily diffuses and is absorbed by the skin and the lungs tissue but does not amass in any particular tissue type. After metabolism of C_6H_5CHO, conjugates are formed with glycine or glucuronic acid and discharged in urine. Benzaldehyde is also confirmed by the US Bureau of Alcohol, Tobacco and Firearms for its usage as a denaturant in different denatured alcohol at 27CFR21.151. Natural benzaldehyde can be used in bath preparation (2%), non-coloring preparation (1%), skin care preparation (1–2%), and fragrance preparation in perfumes and colognes at 0.5% concentration (Andersen 2006).

7.2.4 Alcohols

Excessive alcohol production by yeast continues to be a major concern in the field of distilled liquors. Medium-chain alcohols, among others, have a major effect on the final flavor profile of alcoholic beverages even at low concentrations. It is now widely accepted that the synthesis of chemical compounds in yeasts adversely affects the development of alcohol, in particular carbon metabolites. Conversely, it may not be clear how homeostasis of oxygen and carbohydrates could ultimately impact the alcohol levels in fermented beverages. Consequences between the depletion of oxygen by glucose spike mainly on the accumulation of alcohol in liquid culture and on inclusion of glucose (20 g/L) and leucine (9.8 g/L) as aggregants in alcoholic beverages have been tested using *Saccharomyces cerevisiae* from the industrialized Brazilian cachaça type. The alcoholic fermentation compounds as well as the carbon dioxide balance were analyzed in order to relate the results to biochemical evidence. All findings indicate that the aggregation of isoamyl alcohol by yeast may not be impaired by the presence of oxygen in the atmosphere. Elevated conjugated linoleic acid tests showed a recent and unforeseen accumulation of isobutanol, active amyl alcohol, and 2-phenylethanol that may be linked to gene expression of valine, isoleucine, and phenylalanine biosynthesis. From the emission contexts, findings further demonstrate that the metabolism of isoamyl alcohol, isobutanol, and effective amyl alcohol, even if not 2-phenylethanol, through wild yeast in some kind of stagnant era, indicates the importance of these higher alcohols as both a carbon supply for carrying costs and/or oxidation/reduction of immune function during that period (Espinosa et al. 2015).

Methodologies for improving energy utilization in the fermentation process of ethanol are now based almost exclusively on the development of technology centered on the use of lignocellulose carbohydrate fractions. Some of the so-called "second-generation" innovations require metabolically adjusted production strains, which maintain a high level of catabolic versatility and are homoethanologenic. It may have been suggested that the processing of ethanol at extreme temperatures could promote its production. The expected offspring of *Geobacillus thermoglucosidasius*, *Thermoanaerobacterium saccharolyticum*, and *Thermoanaerobacter mathranii* already constitute the platform technology of many modern biotech companies. All originally proposed achievements in the development of these processing varieties specially focus on the establishment of productive sources of cellular competence, gene removal, or subsequent activation (Taylor et al. 2009).

Renewable energy technology production would gradually be implemented through efforts of societies or industries to fulfill the nation's financial production targets, as well as to overcome the issues of energy production and climate change. Typically, nearly the entire production of bioethanol tends to be from the cultivation of agricultural crops or biomass. There appears to be a great deal of debate over the cost or energy efficiency of this form of biomass-based ethanol production operation. Conversion may have been achieved using a double homologous recombination process that inserts pyruvate decarboxylase (*pdc*) and alcohol dehydrogenase II (*adh*) ethanol-producing genes *Zymomonas mobilis* into the *Synechocystis* PCC 6803 chromosome under the influence of a solid, light-charged psbA II promoter. The PCR-dependent analysis or ethanol performance test is used to test healthy processors. For the analysis of cyanobacterial cultured cells and the production of ethanol, an automated photobioreactor platform for something like analytical preparation and device selection has been developed. The approach described above suggests an improvement in yield of 5.2 mmol OD_{730} unit/L/day with no required antibiotic/selective agent (Dexter and Fu 2009).

7.2.5 Ketones

4-(4-Hydroxyphenyl)-butan-2-one (raspberry ketones) is an important natural flavoring agent (Liu et al. 2020). It shows several activities such as it attenuates cyclophosphamide-induced pulmonary toxicity (Mohamed et al. 2020), controls hyperlipidemia and insulin resistance (Mehanna et al. 2018), de-pigmenting activity (Lin et al. 2011), antimicrobial activity (Gupta et al. 2015), acts as an antioxidant (Mohamed et al. 2018), antiandrogenic activity, etc. (Ogawa et al. 2010). Due to wide usages in soft drink, sweet, and ice cream, it has a high market demand. Plant gene has been incorporated for microbial production (Beekwilder et al. 2007). Microbial production has also been achieved by using other strategies such as catalytic aldolization of precursors (Feron et al. 2007), pathway engineering and synthetic enzyme fusion (Lee et al. 2016), and construction of synthetic pathways in *E. coli* (Wang et al. 2019).

7.2.6 Lactones

Lactones are defined as cyclic carboxylic esters and are ubiquitous in nature such as saturated and unsaturated γ-lactones (plants) and δ-lactones (animal products). The organoleptic property of aliphatic lactones is used for components of food. The rising demand of natural flavors and fragrance makes biosynthesis of lactone production more enticing. Flavored lactones are formed via chemical synthesis or microbial and enzymatic conversion (Gatfield et al. 1997). Lactone has various tastes and aroma say oily, creamy, coconut-like, fruity, milky, honey, peachy, and others. Different species of fungi can produce lactone from triolein, sebum, oleic acids, lecithin, and Tween 80 as substrates (Shaaban et al. 2016).

Derivatives of lactone serve as a building block in the environment such as enzyme lactonase, epinepetalactone, and food additive glucono delta-lactone. Majorly δ-lactones and γ-butyrolactone serve as structural scaffolds for different active natural compounds (Andrushko et al. 2013). Lactone derivatives are remarkably used as pheromones and flavoring compounds derived from many flowers and fruits (Mori 1984). Lactone of D-gluconic acid (gluconolactone) is used as a food additive, an acidifier, or leavening agent (Feiner 2016). Spironolactone is a steroid lactone that plays a role in diuretic, antihypertensive, and antiandrogen. Macrolides consist of macrocyclic lactone rings that have antifungal and antibiotic properties against *S. pneumoniae*, *Bordetella pertussis*, and *Haemophilus influenzae*. Sesquiterpene lactone (vernolepin) and epothilones are used as anticancer and antitumor drugs. The 16 macrocyclic lactone (avermectin) is used as a drug and pesticide (Heravi et al. 2019). The most important bacterial signaling lactone molecule (*N-acyl homoserine* lactone) is involved in bioluminescence, production of biofilm, expression of virulence factors, and formation of antibiotics (Yajima 2016; Gohil et al. 2018). Even polycaprolactone is extensively used in scaffolding and tissue engineering (Ducheyne et al. 2015).

Fungus *Trichoderma viride* EMCC-107 produced 6-amyl-α-pyrone through solid-state fermentation of sugarcane bagasse and imparted strong coconut fragrance (Fadel et al. 2015). Other coconut lactone flavors can be produced by *Tyromyces sambuceus* and *Cladosporium suaveolens* from ricinoleic acid of castor oil (-γ-decalactone) and linoleic acid (δ-dodecalactone) (Sharma et al. 2020). Lactones used in dairy and milk products have milky, buttery, and coconut flavors produced by microbiological pathways. Lactone also tends to give out stale flavor when used in excess quantities of heated milk (Gupta et al. 2015). *Candida tropicalis*, *Yarrowia lipolytica*, and *Sporidiobolus salmonicolor* yeasts amass δ-dodecalactone by β-oxidation of ricinoleic acid that gives fruity, oily aroma which is used to impart apricot, strawberry, and peach flavors (Schrader et al. 2004; Longo et al. 2006). The production of γ-decalactone by using *Y. lipolytica* CCMA 02042 and *Lindnera saturnus* CCMA 0243 yeasts through β-oxidation of ricinoleic acid or crude glycerol yields 214.8 mg/L and 512.5 mg/L, respectively (de Andrade et al. 2017). Waché et al. (2001) stated that decreasing the activity of acyl-CoA oxidase helps in collecting a higher concentration of γ-decalactone. In 2011, strain *Y. lipolytica* TA1 was studied to investigate about the enzyme AOX3 encoded by the *POX3* gene which is responsible for a low yield due to breakdown of lactone. By introducing copper resistance gene *CRF1* to *Y. lipolytica locus* of *POX3* gene, it was demonstrated to accumulate 2.9 times (0.531 g/L at 63 h) higher yield of lactone than 0.194 g/L at 57 h in *Y. lipolytica* AS2.1045. That enlightened the troubleshooting for the fragrance producing industry (Guo et al. 2011). Biotransformation using microorganisms such as *C. boidinii*, *Mucor circinelloides*, and *S. ruinenii* produced γ-decalactone yielding 40.9 g/L, 10.5 g/L, and 5.5 g/L from ricinoleic acid, ethyl decanoate, and methyl ricinoleate, respectively (Kourist and Hilterhaus 2015). The production of lactone in cytosol before import of protein into peroxisomes takes place in the presence of *SmOHYp* gene in strain ST8822. The engineered strain ST7584 (Δpox1–6 LaLHY YlPOX2), by deletion of FAA1, exhibited 0.17 ± 0.03 mg/L production of δ-decalactone. The enhancement of δ-decalactone by copying cytosolic *LaLHY* gene titer 1.74 ± 0.3 mg/L suggested that low levels of hydratase activity influence the flux of lactone (Marella et al. 2020). Microbial production of lactones has become advanced in the field of biorenewable molecules (triacetic acid lactone), fragrance and natural flavors, and pharmaceutical intermediate sector.

7.2.7 Vanillin

Vanillin is an important aromatic flavoring compound that is widely used in beverages, foods, perfumes, and medicines (Priefert et al. 2001). Currently, more than 10 tons per year of this compound is produced through chemical synthesis worldwide. Bio-based production of vanillin is achieved through the conversion of lignin, isoeugenol, aromatic amino acids, phenolic stilbenes, and eugenol using bacteria, fungi, plant cells, and engineered microorganisms (Priefert et al. 2001). Shimoni et al. (2000) had stated that natural aroma compounds are major attention to the fragrance and flavor industry. A number of microorganisms have the natural ability to produce these aromatic aldehydes as intermediates in phenylpropanoid degradation pathway. In a study, researchers isolated *Bacillus subtilis* and used that for bioconversion of eugenol into vanillin. In the presence of eugenol, *B. subtilis* produced 0.61 g/L vanillin (molar yield 12.4%) in growth culture, while in cell-free extract, it was observed 0.9 g/L vanillin (molar yield 14%). Similarly, Walton et al. (2000) isolated *Pseudomonas* strains that were able to produce 6 g/L vanillin from ferulic acid. They characterized biosynthetic pathways at the enzyme and molecular-level for the production of vanillin from eugenol via ferulic acid. This study suggests that it can be further expanded toward higher vanillin production at industrial scale.

Krings and Berger (1998) reported that natural aroma compound (vanillin, benzaldehyde (bitter almond, cherry) and 4-(R)-decanolide (fruity-fatty)) demands have been significantly increasing which demands its scale-up to several thousand tons per year. A single-step biotransformation, de novo synthesis, and bioconversion have been attempted for the production of these compounds using microorganisms and plant cells.

7.2.8 Terpenes

Compounds grouped as terpenes have five carbon isoprene units (2-methyl-1,3-butadiene) which are further classified into groups based on the number of isoprene units, such as two (monoterpenes), three (sesquiterpenes), four (diterpenes), six (triterpenes), eight (tetraterpenes), or more (polyterpenes). Terpenes are potential biofuel (Mewalal et al. 2017) and antioxidants (Ng et al. 2000). These can be converted into various important compounds which hold importance in pharmaceutical, agrochemical, and flavor and fragrance industries (Monteiro and Veloso 2004). Apart from that, terpenes are also part of essential oils (Noriega 2020), resin ducts (Rahman et al. 2008), and rubber (Hattori et al. 2008) (Table 7.1).

Limonene is widely used in the food and cosmetic industry. Several strategies have been tested for microbial production such as employing heterologous mevalonate pathway in *E. coli* by incorporation of single plasmid, i.e., pMevT and pMBI (a pMBIS variant) for production (Alonso-Gutierrez et al. 2013). Expression of MmMK with ScPMK, ScPMD, and ScIDI under FAB80 promoter resulted in an efficient midstream module to produce 181.73 mg/L of limonene (Wu et al. 2019).

7.2.8.1 Valencene

Valencene is a biosynthetic product of farnesyl pyrophosphate (FPP) that has a characteristic juicy, orange, sweet, and woody fragrance, and it is also an aroma component of citrus fruits. It contributes to the production of nootkatone which is the most significant and expensive aromatic component of grapefruit (Bomgardner 2012). Since it helps in decreasing somatic fat ratio, it has a higher demand in cosmetics and fiber sectors. The skin of Valencia oranges has a significant amount of valencene. Valencene has significance in the fragrance and flavoring industry and finds application in fruit-flavored beverages, perfumes, chewing gum, personal care, and cleaning products (Waltz 2015; Gupta et al. 2015).

Valencene has a high value in industry, but its extraction is very expensive, and therefore several researches have been conducted for achieving its high yield. In 2014, Beekwilder et al. (2014) engineered the valencene synthase gene (*CnVS*) in *Rhodobacter sphaeroides* strains and also included the expression of mevalonate operon from *Paracoccus zeaxanthinifaciens*. Recombinant *Corynebacterium glutamicum* was constructed having valencene synthase from *Nootka cypress* and genes *erg20* and *ispA* were overexpressed which gave positive results with about 2.41 mg/L valencene yield (Frohwitter et al. 2014). Microorganism such as *S. cerevisiae* is used as gene expression host because it is safe and comes with

Table 7.1 Important terpenes and their plant sources and activities

Sr. No.	Terpene compound	Sources	Activity	References
1	α-Bisabolol	*Plinia cerrocampanensis*	Antimicrobial	Vila et al. (2010)
		Nectandra amazonum	Anti-*trichomonas vaginalis*	Farias et al. (2019)
		Salvia species	–	Sandasi et al. (2012)
		Laserpitium zernyi	Antimicrobial	Popović et al. (2010)
		Eremanthus erythropappus		Santos et al. (2019)
2	β-Caryophyllene	*Psidium guajava*	–	Arain et al. (2019)
		Zingiber nimmonii	Antimicrobial	Sabulal et al. (2006)
		Plectranthus barbatus	Mosquito larvicidal activities	Govindarajan et al. (2016)
		Aquilaria crassna	Antimicrobial	Dahham et al. (2015)
		Murraya paniculata	Antioxidant activity	Selestino et al. (2017)
3	α-Humulene	*Cheilocostus speciosus*	Insecticidal activity	Benelli et al. (2018)
		Syzygium zeylanicum	Larvicidal activity	Govindarajan and Benelli (2016)
4	(+)-limonene	Citrus lemon, *Mentha spicata*	Antibacterial, insecticidal, repellent	Lopresto et al. (2014), Aggarwal et al. (2002), Ibrahim et al. (2001)
5	Linalool	*Croton cajucara*, *Litsea glaucescens*	Antimicrobial, antioxidant activity, anticonvulsant activity, antidepressant	Alviano et al. (2005), Seol et al. (2016)
6	Myrcene	Lemon grass, *Citrus aurantium*	Analgesic activity	Lorenzetti et al. (1991), Bonamin et al. (2014)
7	α-Pinene	*Syzygium cumini*	Anti-*Leishmania* activity	Rodrigues et al. (2015)
8	β-Pinene		Anti-inflammatory and chondroprotective activity	Rufino et al. (2014)

highly recommended physiological properties (Chen et al. 2019; Ozaydin et al. 2013; Asadollahi et al. 2008).

7.2.8.2 Nootkatone

Sesquiterpenoids are highly value-added aroma compounds found in plants such as strawberry (Hampel et al. 2006). (+)-Nootkatone was first reported from *Cupressus* *nootkatensis* and is found in grapefruits in trace amount (MacLeod et al. 1964). It is also isolated from *Alpiniae oxyphyllae* (He et al. 2018) and *Cyperus rotundus* L. (Jaiswal et al. 2014).

Multifaceted activities such as anti-inflammatory (Khan et al. 2011; Tsoyi et al. 2011), neuroprotective (He et al. 2018), antiplatelet (Seo et al. 2011), antibacterial (Yamaguchi 2019), and anticancer (Yoo et al. 2020) are exhibited by (+)-nootkatone. Studies also report that (+)-nootkatone acts as repellent to termites (Maistrello et al. 2003), and maize and rice weevil (Mao et al. 2010), shows toxicity against arthropods (Anderson and Coats 2012) and anti-biofilm efficacy against *Staphylococcus aureus* (Farha et al. 2020). Majorly it is used in food (Shaw et al. 1981; Del et al. 1992), cosmetics, and pharmaceuticals (Leonhardt and Berger 2014).

Due to limited biological production of (+)-nootkatone, it is currently produced through chemical synthesis to fulfil market demand. Due to lesser availability of its natural form in the market, it is alternatively being produced in bacteria, fungi, and plant cells. Cell extracts and purified enzymes are used for the conversion of allylic oxidation of (+)-valencene that allows natural production of (+)-nootkatone in high yields (Fraatz et al. 2009). Terpenoid compounds have always played an important role in the aroma industry. Microbial production leads to the production of desired products. Engineered organisms can be utilized for the production of such terpenes at low cost. Various strategies have been implemented to enhance production such as increase in supply of carbon source (Schindler 1982), high flux toward the central C5 prenyl diphosphate precursors (Schempp et al. 2018), expression of a synthetic amorpha-4,11-diene synthase gene, and the mevalonate isoprenoid pathway from *S. cerevisiae* into *E. coli* (Martin et al. 2003).

7.2.8.3 Patchoulol

A frequently used sesquiterpene alcohol in the chemical industry is patchoulol ($C_{15}H_{26}O$). It can be extracted using steam distillation from the leaves of patchouli plant. It is a major component of patchouli oil, and its odor is known for its characteristic pleasant and long-lasting woody, earthy, camphoraceous odor. Therefore, it is a widely used fragrance ingredient in products like soaps, cosmetics, and perfumes (Bauer et al. 1997). The responsible factor of the typical fragrance of patchouli is (−)-optical isomer. Patchoulol shows anti-inflammatory (Jeong et al. 2013; Xian et al. 2011; Su et al. 2016), anti-viral, anti-ulcer, anti-aging (Zheng et al. 2014; Feng 2014), antidepressant (Sah et al. 2011), antitumorigenic, and anti-influenza activities (Jeong et al. 2013; Lee et al. 2020).

Earlier, the predominant commercial source of patchoulol was patchouli plants (Näf 1981). But now, with advancements in research, there are other promising approaches that present cost-effective synthetic routes for producing enantiomeric pure patchoulol (Martin et al. 2003; Besumbes 2004). Also, steam distillation process is energy- and resource-intensive, and therefore for minimizing its environmental footprint and improving production yield, fermentation process for production of patchoulol is used. By converting FPP to patchoulol using patchoulol synthase enzyme (*Pc*PS; PTS, uniprot: Q49SP3) (Deguerry et al. 2006; Munck et al. 1990) and using different microbial hosts, say *S. cerevisiae* (Gruchattka et al. 2013), the moss *Physcomitrella patens* (Zhan et al. 2014), and the green microalga *Chlamydomonas reinhardtii* (Lauersen et al. 2016), the heterogeneous expression of patchoulol has been achieved. Bacteria such as *E. coli* and *C. glutamicum* also tend to give better results through this approach (Henke et al. 2018).

7.2.8.4 Sclareol

A fragrant amber-colored chemical named sclareol found in *Salvia sclarea* is a type of diterpene alcohol (Lawrence 1986). It has a sweet and balsamic fragrance, alike *Cistus creticus* (Cistaceae) (Demetzos et al. 1999), *Nicotiana glutinosa* (Solanaceae) (Guo and Wagner 1995), and *Cleome spinosa* (Brassicaceae) (McNeil et al. 2010). It not only has its importance in the perfume industry but also possesses properties like antibacterial, antifungal, and growth regulating activities (Demetzos et al. 1999; Ulubelen et al. 1994). Sclareol is the basic component for synthesizing Ambrox (Schmiderer et al. 2008) that ultimately contributes as a better substitute for ambergris (Barrero et al. 1993). Ambergris is secreted by sperm whales which has a musky, sweet earthy odor, a heavily demanded product in the perfume industry. As it originates from an endangered species, its use is controversial.

The annual production of sclareol from leaves and flowers of clary sage (*S. sclarea*) is greatly affected due to several environmental factors; therefore, many researches were conducted to find alternative cost-efficient and scalable enzymatic production platforms for improving it on high yield. *E. coli* and *S. cerevisiae* have been used as alternatives by elucidating and reconstructing sclareol biosynthetic pathways (Schalk et al. 2012; Ulubelen et al. 1994; Cutler et al. 1977). Genetic manipulation in yeast and moss *P. patens* also enhances sclareol production (Trikka et al. 2015; Pan et al. 2015).

7.2.8.5 Steviol

Stevia rebaudiana (sugarleaf) is a naturally produced sweetener like stevioside and rebaudioside (Dan et al. 2018). The leaves consist of 9.1% and 3.8% glycoside diterpenes stevioside and rebaudioside A, respectively (Goyal et al. 2010). Steviol glycoside has zero calorie. Steviol has a methylene bond between C16 and C17, including diterpenoid steviol as a structural compound with three glucose moieties at C 13-hydroxyl/C 19-carboxylic acid group. The methylene bond gives a sweet taste and serves as a pharmacophore for industries (Upreti et al. 2012). Artificial sweetener stevia is used to enhance palatability in the food products, beverages, dietary supplements, and diabetic sugar as it also serves as a heat and acidity stabilizer. It is also used in fermentation, hardening, absorption of soy sauce, pickles, bread, and biscuits. Stevia is also non-cariogenic (Mitchell 2008).

The leaves of stevia when exposed to gamma radiation showed double production of *Reb A* and by treatment of ethyl methanesulfonate resulted in 1.5–2.0-fold increment of genes *Stev* and *Reb A* indifference to control plants. By increasing the gene expression of UGT74G1 (Stev biosynthesis) and UGT76G1 (Reb biosynthesis), it was found that improvement of steviol glycosides take place in EMS and gamma ray-treated plants (Khan et al. 2016). The chameleon enzyme, glucosyltransferase UGT76G1, aims at the metabolism of rebaudioside D (RebD) and rebaudioside M (Reb M). Reb D and Reb M enhance the sweet profile and decrease the bitter taste compared to Reb A and stevioside (Olsson et al. 2016).

The reduction of steviol glycoside has been observed by downregulating the transcription genes involved in steviol metabolism: *ent-KS1* (*ent*- kaurene synthase 1), *ent-KO* (*ent*-kaurene oxidase), *ent-KAH* (ent-kaurenoic acid 13-hydroxylase), *UGT85C2*, *UGT74G1*, and *UGT76G1* in polyethylene glycol-treated stevia plants (Hajihashemi et al. 2016). Constructing steviol metabolic pathways in *E. coli* increases the production of steviol. Isoprenoid serves as a precursor molecule for steviol biosynthesis in engineered *E. coli* strain. Moon and colleagues concluded that by expressing the UtrCYP714A2-AtCPR2 fusion protein obtained from *Arabidopsis thaliana*, 5′-untranslated region engineering of KS (*ent*-kaurene synthase) and modification of N-terminal KO (*ent*-kaurene oxidase) sequence, steviol production enhanced up to 38.4 ± 1.7 mg/L in batch fermentation (Moon et al. 2020). Stevioside protects against food-borne bacteria such as *Bacillus cereus*, *Klebsiella pneumoniae*, and *P. aeruginosa* (Puri et al. 2011). Steviol glycoside is also used to decrease blood pressure, to maintain blood glucose level in diabetes mellitus, and also to avoid atherosclerosis (Hajihashemi et al. 2013).

7.3 Conclusion and Future Remarks

The flavor and fragrance market is exponentially growing, and its global demand is quiet high. In order to fulfill the current demand, microbial production is an alternative source met through modifying or extending natural pathways in heterologous microorganisms for overproduction of these molecules. Currently, a number of stronger and well-characterized genetic parts including promoter, ribosome binding site, degradation tag, and transcription terminator are being employed for replacing native weak parts with strong part for boosting production of flavor and fragrance. Several flavors and fragrance are already available in the market, and many will be available soon for commercial applications. In the near future, it is anticipated that it will increase and improve the production of molecules to meet various industrial, pharmaceutical, and biotechnological applications.

Acknowledgments N.G. acknowledges the Indian Council of Medical Research, Government of India, for financial assistance as Senior Research Fellowship (File No. 5/3/8/63/ITR-F/2020). The financial support from Gujarat State Biotechnology Mission (GSBTM) (Project ID: 5LY45F), Gujarat, India, to G.B. and V.S. is duly acknowledged.

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