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REVIEW

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Biosynthetic pathways and metabolic engineering of spice flavors

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ABSTRACT

Historically, spices have played an important economic role, due to their large applications and unique flavor. The supply and cost of spice materials and their corresponding natural products are often affected by environmental, geopolitical and climatic conditions. Secondary metabolite composition, including certain flavor compounds in spice plants, is recognized and considered closely related to plant classification. Both genes and enzymes involved in the biosynthesis of spice flavors are constantly identified, which provides insight into metabolic engineering of flavor compounds (i.e. aroma and pungent compounds) from spice plants. In this review, a systematic meta-analysis was carried out based on a comprehensive literature survey of the flavor profiles of 36 spice plants from nine families. We also reviewed typical biosynthetic pathways and metabolic engineering of most representative aroma and pungent compounds that may assist in the future study of spice plants as biosynthetic factories facing a new challenge in creating spice products.

GRAPHICAL ABSTRACT
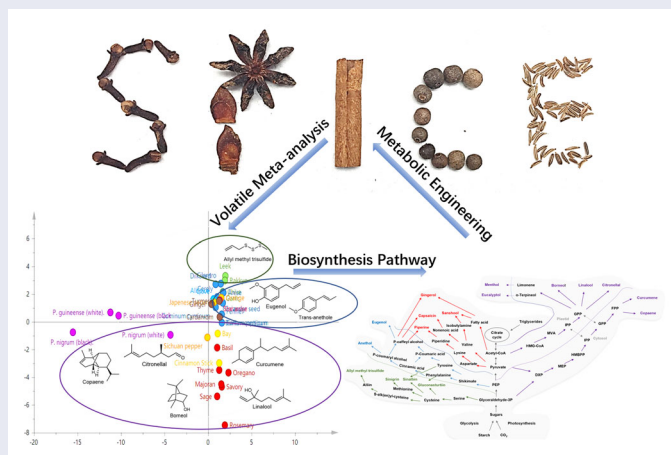
Introduction

Since ancient times, spices, a vast range of plant materials with intensive and distinctive flavors, have been used for a variety of purposes from culinary to medicinal. Spices are derived from numerous plant parts: bark (cinnamon), bulbs (garlic or onion), flowers (clove), fruits (chili), rhizomes (ginger), seeds (sesame) or the entire plants. However, plant leaves used in cooking, such as mint or bay, are commonly denominated as culinary herbs (Viuda-Martos et al. 2011). In this paper, both culinary herbs and spices are considered as a generalized definition of spices. All spices are considered fresh or dried plant organs residing in different phylogenetic categories that correspond to several vegetal families such as *Allioideae* plants, including garlic, chive, leek, onion, or *Apiaceae* plants, including anise, caraway, celery and cumin. Spices are typically known for their flavor profiles, especially those of aromatic properties (aroma or odor) as well as trigeminal stimulation (i.e. pungency), which are the focus here. Spices with a strong smell are considered aromatic spices (cumin, savory, clove) and those with a pungent sensation are considered hot spices (chili, pepper, ginger).

Worldwide, spices are commonly used as seasoning in cuisine due to their ability to enhance flavor; playing an important role in the food industry. Since all spice plant materials have been generally recognized as safe (GRAS, Jessica Elizabeth et al. 2017), they are also a good resource

for other applications, such as indigenous medicines, pharmaceuticals, nutraceuticals, aroma therapy, preservatives, beverages, natural colors, perfumes, cosmetics and botanical pesticides. These applications are based on a diverse array of chemicals synthesized by these spices (Jessica Elizabeth et al. 2017; Nanasombat and Wimuttigosol 2011). These metabolites, including terpenes, phenols, coumarins, flavonoids, and alkaloids, provide the majority of spice related benefits such as flavoring (Lampe 2003), antimicrobial (Solórzano-Santos and Miranda-Novales 2012; Ribeiro-Santos et al. 2017), antioxidant (Embuscado 2015), anticancer (Butt et al. 2013), anti-inflammation (Viljoen et al. 2007), diabetes managements(Bi, Lim, and Henry 2017), wound healing and skin-penetration in medicine (Gupta 2010), insecticidal properties (Çalmaşur, Aslan, and Şahin 2006) in plant disease management, and antibiotic replacement in animal feed (Brenes and Roura 2010).

The importance of secondary metabolite composition, including types of flavor compounds in spice plants, is currently recognized to have a strong relationship with plant systematics. Spice plants were systematically and phylogenetically analyzed using macroscopic and microscopic morphological observation assays. Nowadays, chemotaxonomy combined with molecular biology is applied to study phylogenetics of certain species. For instance, 216 plants were successfully classified based on their known incomplete metabolite content (Liu et al. 2013b). A convenient and reliable method for characterizing chemotaxonomy combining the use of vibrational spectroscopy and hierarchical cluster analysis was developed (Baranska et al. 2005). Additionally, leaf metabolite profiles were applied to taxonomical and phylogenetic studies of the genus, providing support for current taxonomic evidence (Lamine et al. 2018). Although, the distribution of secondary metabolites showing frequent allelochemicals of high structural specificity has been found in unrelated plants families (Trindade 2010), due to plant evolution, aroma compounds of certain spices clearly explain the relationship to their systematic properties. The function of each gene encoding enzyme involved in the biosynthesis pathway can be identified by cloning and expressing the enzyme that catalyzes the corresponding reaction and controls its product. Studies have demonstrated that metabolic engineering on a certain biosynthase gene could enhance the level of corresponding pleasant aroma compounds, such as linalool in tomato (Lewinsohn et al. 2001) and valencene in orange (Shen et al. 2016), or reduce unpleasant ones, such as lachrymatory factors in onion (Oa et al. 2008).

In order to illustrate the relationship between the chemotaxonomical and metabolic properties of flavor compounds amongst traditional spice materials from different families, a systematic literature survey and meta-analysis was implemented based on the flavor profiles of spice plants from different families. We further reviewed typical biosynthetic pathways and metabolic profiling based on the most related aroma and pungent compounds, which may lead to a better understanding of the biosynthesis of the spicy flavors in spice plants.

Meta-analysis of key flavor compounds in spices from different families

Aimed to find key flavor compounds from spice materials commonly used on a daily basis as listed by the European Spice Association (ESA, www.esa-spices.org), a meta-analysis was performed using a systematic literature survey of flavor in particular aroma profiles for 36 spice plants from nine families. As the chemical characterization of aroma compounds requires consistent qualitative and quantitative data for the entire set, the following pre-specified normalization was applied to the data-analysis.

As indicated in Table S1, information listed, such as taxonomy, sampling methods and column usage, for the aroma compounds from 36 spice materials that belong to nine different families, including *Allioodeae* (Nielsen and Poll 2004; Pino, Fuentes, and Correa 2001; Kim et al. 2011), *Apiaceae* (Potter 1996; Organica et al. 2004; Chemat et al. 2005; G. Singh et al. 2008; Idalgo 2005; Oroojalian et al. 2010; Jirovetz et al. 2003; Bhuiyan, Begum, and Sultana 2009), *Lamiacea* (Politeo, Jukic, and Milos 2007; Bezić et al. 2009; Vera and Chane-Ming 1999; Szumny et al. 2010; Figiel et al. 2010; Bouaziz et al. 2009), *Lauracea* (Abdelwahab et al. 2017; Sellami et al. 2011), *Myrtaceae* (Monteiro et al. 2011; Della Porta et al. 1998), *Piperaceae* (S. Singh et al. 2013; Jirovetz et al. 2002), *Ruteceae* (Högnadóttir and Rouseff 2003; Jiang and Kubota 2004; X. Yang 2008), *Schisandraceae* (Della Porta et al. 1998) and *Zingiberaceae* (Kutti Gounder and Lingamallu 2012; Husain and Ali 2014; Z. Yang et al. 2009). Hydrodistillation or so-called simultaneous distillation-extraction was the traditional method for obtaining essential oil from spice plants. However, this may lead to heat degradation and water solubilization of some aroma constituents and artifact formations. Solvent assisted extracts always contaminate foods and fragrances with residues from the solvent added. The supercritical CO_2 extraction process can be implemented at relatively low pressures, at approximately room temperature, but is costlier. For this method, CO_2 evaporation from the product should be complete once the extraction process is finished (Della Porta et al. 1998). Solid phase microextraction (SPME) concentrates the volatiles into the headspace without solvent use, and has been applied to a broad range of food types.

The aroma composition from spices were collected from references (Table S1), which was profiled using gas chromatography-mass spectrometry (GC-MS). Spices selected were systematically grouped by their corresponding families to evaluate the correlation between their aroma composition and plant families. All the data were then normalized into the percentage of the concentration of each aroma compound from the total aroma profile. Using this data collection and transformation strategy, the normalized aroma profile data was statistically analyzed with PLS-DA (Partial least squares discriminant analysis) model using SIMCA-P software (Umea, Sweden). The confidence of tolerance ellipse in the PLS-DA model was 95% on the basis of Hotelling's T-squared distribution. According to the visualized results of analysis in Figure 1, four parts could be circled among 36 spices from nine families based on their
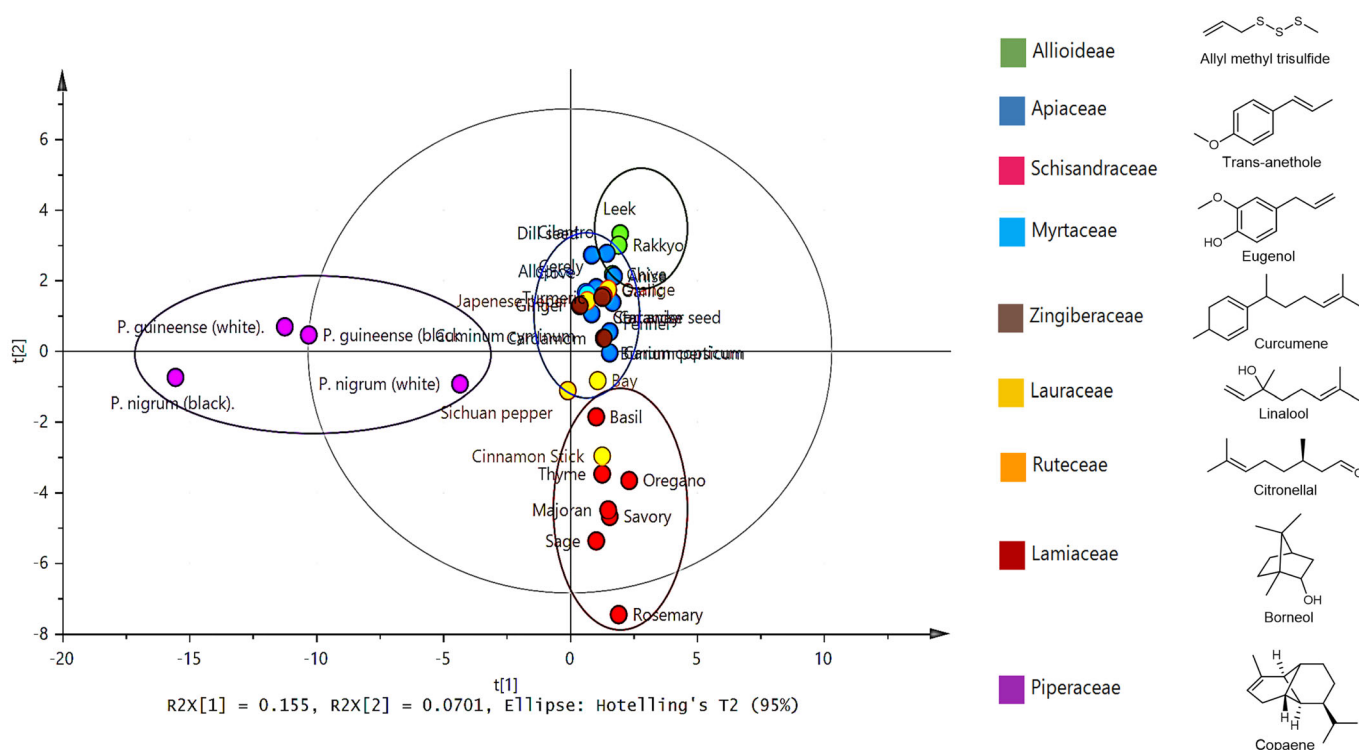
Figure 1. The visual distribution of spice plants grouped by family was tested with a PLS-DA model and the statistically related aroma compounds of spice plants from each family.

aroma composition to identify the variables responsible for the spice plant differentiation. Therefore, the *Lamiaceae* and *Piperaceae* families were significantly separated from the others. The *Alliodeae* or *Allium* family was localized on the upper-most edge, yet close to family *Apiaceae*, and mixed with the families *Mytaceae*, *Schisandraceae*, *Rutaceae* and *Zingiberaceae*. Most spices of one family were closely distributed near each other due to their aroma compound constitutes, revealing a similarity in their aroma component metabolism. Similar methods have also been used to identify and discriminate between commercial spices based on their GC fingerprints with mass spectra collected by HS-SPME (Matsushita et al. 2018).

The most distinctive compound in each family was selected according to the PLS-DA analysis and is shown in Figure1. Focusing on the eight compounds that were the most closely related to the regarding classification, three compound types were classified based on the structure of their carbon framework, which were found to represent the aroma source of their corresponding spice plants. Allyl methyl trisulfide, belonging to the sulfocompounds, always give rise to the characteristic onion- or garlic-smell in the *Alliaceae* family or *Allioideae* subfamily in plants (Kubec et al. 2013). Trans-anethole and eugenol are the representative aroma active compounds in family *Apiaceae* and *Myrtaceae,* respectively, as is the main natural phenylpropanoids synthesized from the amino acid phenylalanine via conversion to cinnamic acid as well (Cássia et al. 2014). Borneol from *Lamiaceae*, linalool from *Lauraceae* and citronellal from *Rutaceae* are all monoterpenes, one of the main groups of plant aroma compounds. Another main aroma

compound found in plants is sesquiterpenes, such as copaene from *Piperaceae* (B. Singh and Sharma 2015). Although, the statistically related compounds here may not be the most conclusive representative from each species, their biosynthetic pathways and metabolic systems are expected to be organized, which has been attempted in current investigations of some spice families such as *Allium* (Böttcher et al. 2018), *Apiaceae* (Borg-Karlson, Valterová, and Nilsson 1993), *Lamiaceae* (Zielińska and Matkowski 2014), *Zingiberaceae* (Theanphong 2016) and *Cupressaceae* (Lin et al. 2011). Limonene is one of the important cyclic monoterpenes found in the plant resource of essential oils, including *Laminaceae* (mints), *Pinaceae* (conifers), *Rutaceae* (citrus), and in *Angelica archangelica* from the *Apiaceae* family. Limonene accounts for more than 95% of essential oil in citrus plants, but may not be the major aroma active compound that affects overall-liking of citrus juice products (Rodríguez et al. 2017), providing evidence that our meta-analysis was able to select the major aroma active compounds from the overall aroma or volatile profile.

Biosynthetic pathways and metabolic systems of flavor compounds in spice plants

Spicy flavor including aroma and pungency is attributed to the production of a wide range of flavor compounds resulting from several biochemical pathways. According to the meta-analysis and statistical data, plus referencing the KEGG database (http://www.kegg.jp/), Figure 2 indicates a spice aroma schematic diagram, along with the
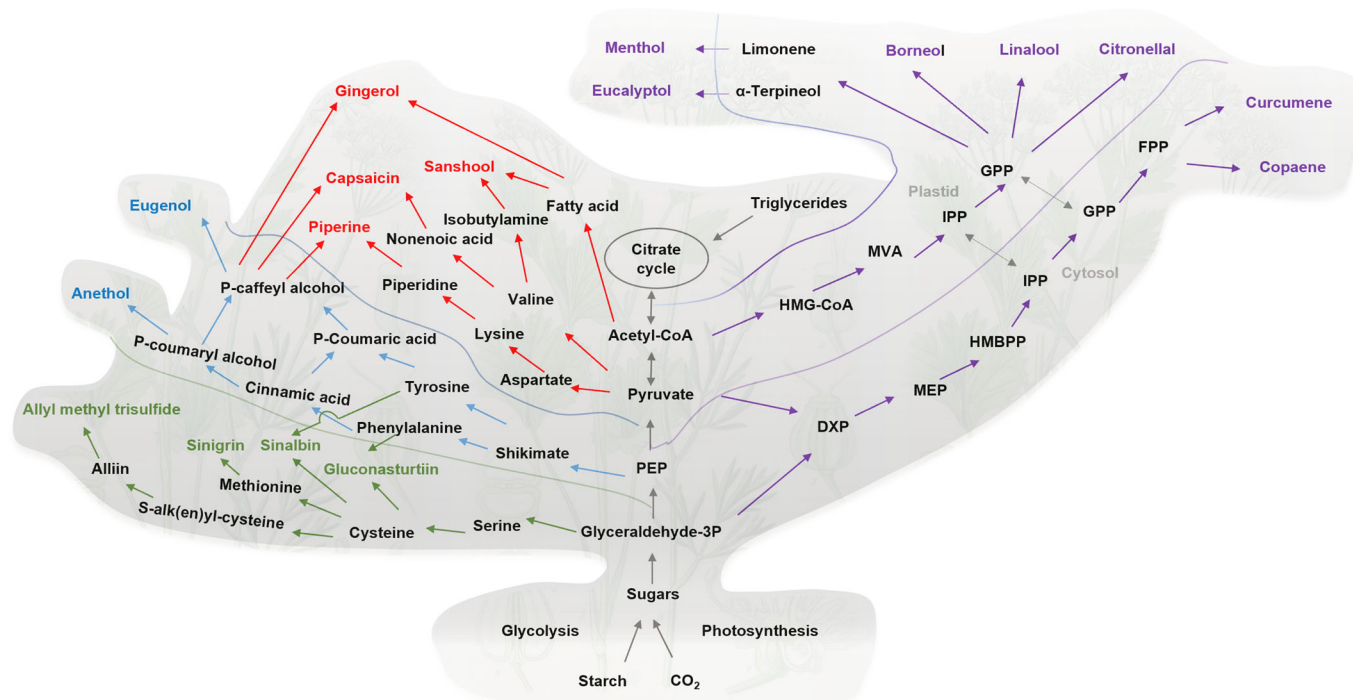
Figure 2. Spicy flavors biosynthetic tree. Spice aroma and pungent compounds are produced by different biochemical pathways. The methyl-erythritol phosphate (MEP) pathway gives rise to the formation of monoterpenes and diterpenes. Sesquiterpenoids are generated by farnesyl pyrophosphate (FPP), derive from the cytosolic mevalonate (MVA) pathway (purple arrow). Aroma compounds, such as eugenol, derive from phenylpropanoids via shikimate pathway (blue arrow). Sulfocompounds, such as allicin, are catalyzed from alliin, via the cysteine pathway, and isothiocyanates are derived from glucosinolates biosynthesis pathway (green arrow). Pungent compounds combine the pathways of phenylpropanoids, some amino acids and fatty acids (red arrow).

representative molecules involved in each branching pathway. Three main biosynthetic pathways result in the production of terpenoids, phenylpropanoids and sulfocompounds respectively, which correspond to the aroma of spice plants or products. Some pungent compounds like capsaicin, sanshool, and menthol are also organized to show their metabolic interrelationships.

Terpenoids are synthesized de novo by an enzymatic cascade reaction, combining acetyl CoA with pyruvate in plastids and the cytoplasm (Figure 3) (Schwab, Davidovich-Rikanati, and Lewinsohn 2008). Volatile terpenoids including hemiterpenes, monoterpenes and sesquiterpenes are major constituents of the plant volatilome (Tholl et al. 2006). Generally, spice plants synthesize isopentenyl diphosphate (IPP), a key precursor for the production of monoterpenes and sesquiterpenes via plastidial methyl-erythritol-4-phosphate (MEP) and cytosolic acetate-mevalonate (MVA) pathways (Parvin et al. 2014). Primary metabolism by conversion of IPP and dimethylallyl diphosphate form monoterpenes through synthesis of intermediate, geranyl diphosphate (GPP) by action of the plastidial prenyltransferase geranyl diphosphate synthase. For instance, GPP undergoes subsequent cyclization by limonene synthase to cyclic monoterpene limonene (Ringer et al. 2003). It is the key precursor of most essential oil components in mint (family *lamiaceae*) such as menthol combined with a chain of biosynthases like cytochrome P450 (-)-limonene-3-hydroxylase, (-)-*trans*-isopiperitenol dehydrogenase, (-)-isopiperitenone reductase, (+)-*cis*-isopulegone isomerase, (+)-pulegone reductase and finally (-)-menthol reductase (Ringer et al. 2003; Ringer, Davis, and Croteau 2005).

Moreover, primary cyclic monoterpene synthases in sage (family *lamiaceae*) were investigated at the gene expression level, in which 1,8-cineole (eucalyptol) synthase was responsible for 1,8-cineole, (+)-sabinene synthase leading to the initial step on the way to thujone, and (+)-bornyl diphosphate synthase forming borneol and camphor (Schmiderer et al. 2010). Acyclic monoterpenoids, linalool and citronellal, are synthesized by linalool synthase and geraniol synthase respectively where the catalytic substrate shares an identical compound and function within an important branch of the pathway (Masumoto, Korin, and Ito 2010). In the cytosol, the condensation of one dimethylallyl pyrophosphate (DMAPP) molecule with two IPP molecules forms farnesyl pyrophosphate (FPP), the linear 15-carbon isoprene pyrophosphate substrate catalyzed by terpene synthases into sesquiterpenes such as curcumene and copaene (Pichersky, Noel, and Dudareva 2006). Four cDNAs encoding sesquiterpene synthases including γ-curcumene synthase were isolated from patchouli (family *Lamiaceae*), and revealed that FPP was catalyzed into different corresponding sesquiterpene products (Deguerry et al. 2006). The sesquiterpene synthases gene from *Ricinus communis* (family *Euphorbiaceae*) was also cloned and expressed in either *E. coli* or *Saccharomyces cerevisiae* strains and found to produce (-)-α-copaene, (+)-δ-cadinene and (E,E)-α-farnesene (Xie, Kirby, and Keasling 2012). Meanwhile, a model system plant such as Arabidopsis, with a genome comprised of over 30 genes thought to encode terpene synthases, has been used to study the evolution, structural properties and biological functions of plant terpene secondary metabolism (Chen et al. 2004).
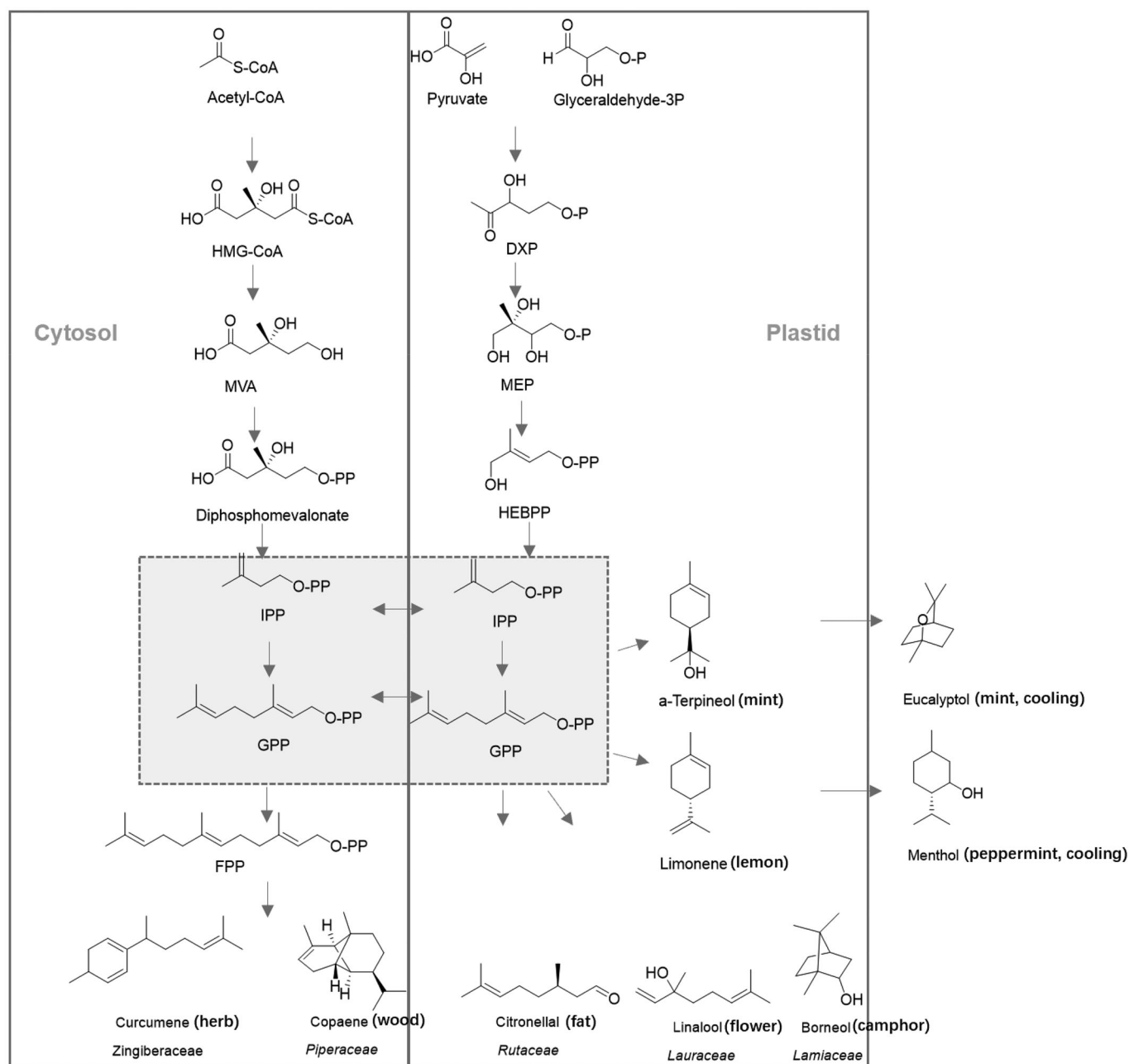
Figure 3. Plastidial methyl-erthritol-4-phosphate (MEP) and cytosolic acetate-mevalonate (MVA) pathways to form isopentenyl diphosphate (IPP) and geranyl diphosphate (GPP) which act as key precursors for the productions of monoterpene and sesquiterpenes. Menthol and eucalyptol, which taste cool, are then further modified from monoterpene. Single arrow may show a multistep reaction. Flavor quality of each compound are presented in parentheses.

The large diversity of phenylpropanoids is derived from efficient modification and amplification of a very limited set of core structures via the shikimate pathway leading from shikimate to phenylalanine and consequently to secondary volatile molecules (Figure 4) (Vogt 2010). Phenylalanine and tyrosine are the main aromatic amino acids that form aromatic compounds. Phenylalanine is deaminated by phenyl-alanine ammonia lyase into cinnamic acid followed by conversion to p-coumaric acid, which could be also derived from tyrosine as shown in Figure 4. The resulting p-cou-maroyl-CoA is successively reduced by cinnamoyl-CoA reductase and cinnamyl alcohol dehydrogenase to produce coumaric alcohol. Along with caffeoyl alcohol, this alcohol acts as the precursor of the phenylpropene biosynthetic

pathway, producing volatile phenylpropanoids like trans-anethol and eugenol. Eugenol and isoeugenol are synthe-sized through the reduction of coniferyl alcohol ester by an NADPH-dependent reductase belonging to structural family. This is similar to sweet basil (family Lamiaceae) glandular trichomes and Petunia (model system plant) flowers (Takao Koeduka et al. 2006). Both anise cDNA encoding trans-anol/ isoeugenol synthase 1 (AIS1) and O-methyltransferase 1 (AIMT1), which convert trans-anol or isoeugenol into trans-anethol or methylisoeugenol, were identified and character-ized, indicating that unlike the situation in basil, the phenyl-propenes synthesized within the anise plant (family Apiaceae) are not stored in glands (T. Koeduka et al. 2009). Also, the Arabidopsis genome database that encodes a
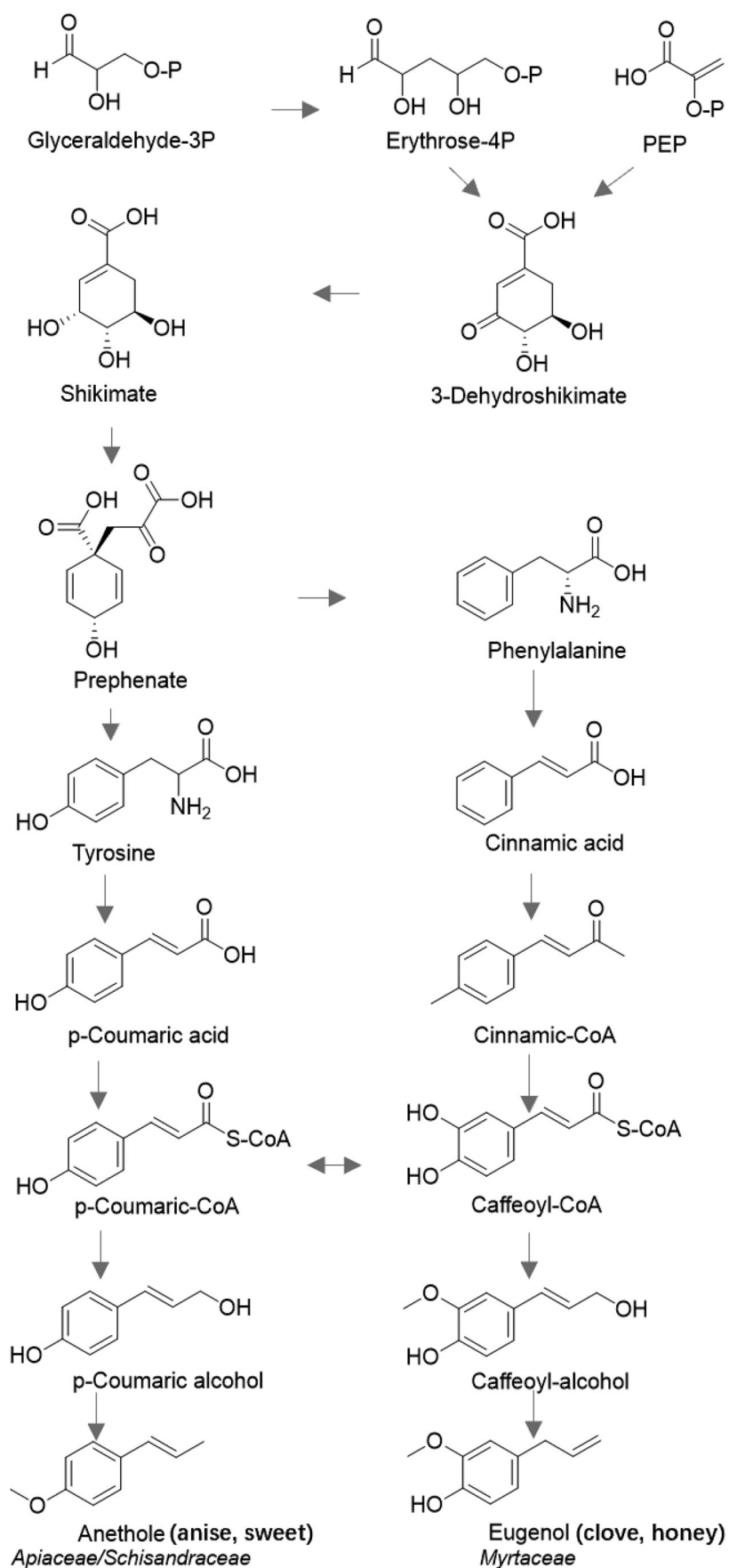
Figure 4. Phenylpropanoids such as anethol or eugenol biosynthesized through shikimate pathway from shikimate to phenylalanine and then to an array of primary and secondary nonvolatile compounds. Single arrow may show a multistep reaction. Flavor quality of each compound are presented in parentheses.

potential synthesase involved in phenylpropanoid biosynthesis was used to study gene function and the cascade reaction of the phenylpropanoid metabolic networks (Costa et al. 2003).

Two biosynthetic pathways have been proposed for S-alkyl cysteine sulphoxides such as alliin. One of which is obtained from the alkylation of glutathione via γ-glutamyl peptides, while the other one is from thioalkylation of serine, then oxidized to the sulphoxide as shown in Figure 5 (Collin, Trueman, and Cosstick 2007). These pathways were investigated by checking the products of undifferentiated callus cultures exposed to potential pathway intermediates in garlic and onion (Hughes et al. 2005). The glutathione route involves allylation of the cysteine in glutathione, typically by the glutathione-S-transferase family, followed by transpeptidation to cut the glycyl group, oxidation of cysteine into a sulphoxide and disjunction of the glutamyl group to form alliin. The other route allows the active site to evolve the capacity to insert substrates instead of sulfide into O-acetyl-serine through thioalk(en)ylation or alk(en)ylation of O-acetyl-serine or cysteine by cysteine synthase (Collin, Trueman, and Cosstick 2007). Notably, once the Allium plant tissue is damaged, the onion-like flavor precursors are converted enzymatically by alliinase to form an array of volatile sulfur compounds followed by further vapor phase chemical transformations (Yu-Yan and Lijuau 2001).

In addition to the aroma property of spice materials, the pungent sensation is a presentative property as well, which is not actually a typical taste. Instead it is considered a taste modulator or causes a trigeminal effect, such as hot or cool, tingling-sensation in the mouth (Starkenmann, Cayeux, and Birkbeck 2011). Isothiocyanates, the main pungent component of Cruciferae family plants such as wasabi, horseradish, and white mustard, are liberated by myrosinase from their precursors, glucosinolates, such as sinalbin and sinigrin, when tissues of these plants are damaged (Figure 5) (Terada, Masuda, and Watanabe 2015). Menthol and eucalyptol are the main cooling flavor compounds from spice plants (Plevkova et al. 2013). Both of them are derivative of monocyclic monoterpenes such as limonene and α-terpineol as mentioned in the terpenoids pathway (Figure 3) (Liu et al. 2013a). The biosynthesis pathway of hot or tingling compounds is organized and presented in Figure 6. Capsaicinoids primary lead to the spicy taste of chili pepper fruit, and their biosynthesis is defined by two routes: phenylpropanoid for the phenolic part and fatty acid metabolism for the fatty acids part (De Lourdes Reyes-Escogido, Gonzalez-Mondragon, and Vazquez-Tzompantzi 2011; Aza-González, Núñez-Palenius, and Ochoa-Alejo 2011). Piperine, a pungent alkaloid from black peppers, is of basic unit of phenolic from the phenylpropanoid pathway, and piperidine, derived from L-Lysine metabolites (Okwute and Egharevba 2013). Sanshool, the tingling paresthesia ingredient in plants of the prickly ash plant family, is combined with dodeca-2,6,8,10-tetraenoic acid form the fatty acid pathway and the valine metabolic product, isobutylamide (Lennertz et al. 2010; Greger 2016). The compounds mentioned above are all amides with strong pungency. However, gingerol, the pungent active compound in ginger is not amide, though combines with the phenylpropanoid and fatty acid pathways as well (Ramirez-Ahumada, Timmermann, and Gang 2006; Walstab et al. 2013).

Metabolic engineering in biosynthesis of spice flavor compounds and its applications

Research on flavor compounds of spice plants is focused on their chemical characterization, along with biosynthesis mechanisms to identify certain genes or enzymes involved in the biosynthesis pathway, or to modulate the aroma property to meet different practical demands of plant derived products in the food, perfume or medicine industry. Plant biochemists have been focusing on identifying the genes and enzymes regarding secondary metabolites such as aroma compounds, and to genetically modify their biosynthesis using metabolic engineering approaches. Recent metabolic engineering technology has enabled customized stimulation of metabolic products connecting primary and secondary metabolism using foreign microbial genes expressed under the control of plant tissue-specific promoters (Aharoni and Galili 2011). It appears that plant aroma profiles have large variability and not only result from differential gene expression, but also frequent changes in the enzymatic functions along with plant development (Takao Koeduka 2018). Plants generate several different aroma compounds via varying the reaction mechanisms that involve each biosynthetic enzyme (Takao Koeduka 2018). Some representative enzymes or genes involved in the metabolic engineering biosynthesis of spice flavor compounds were summarized in Table S2.

Terpene synthases, defined as catalyzing the formation of terpenoids including hemi-, mono-, and sesquiterpene from DMAPP, GPP and FPP respectively (Pichersky, Noel, and Dudareva 2006), have been characterized in various plants. Rapid functional identification of numerous terpene synthases is facilitated by advanced experimental improvements, such as surrogate splicing based on genomic-sequence information (Tholl 2006). Functionalized terpenoids can be produced from carbon sources of simple sugar in E. coli by plant P450s obtained from the relative native biosynthetic pathway, which is more economical compared to feeding strategies based on large amounts of expensive sources (Chang et al. 2007). The function of CitAP2.10 encoding (+)-valencene biosynthease was identified with the Arabidopsis homolog (AtWRI1) combined with transient over-expression of CitAP2.10 in sweet orange fruit (Shen et al. 2016). S-linalool and 8-hydroxylinalool were synthesized and accumulated in ripening tomato fruits after a transgenic strategy with a heterologous S-linalool synthase gene, controlled by the tomato late-ripening-specific E8 promoter (Lewinsohn et al. 2001). Three linalool synthase genes from Citrus unshiu Marc. were characterized and identified as having linalool-mediated resistance against Xanthomonas citri subsp. and Penicilium italicum in the leaves and fruits (Shimada et al. 2014). A novel sesquiterpene synthase gene (cDNA), ZoTps1 was cloned and characterized from ginger (Z. officinale) and revealed to encode
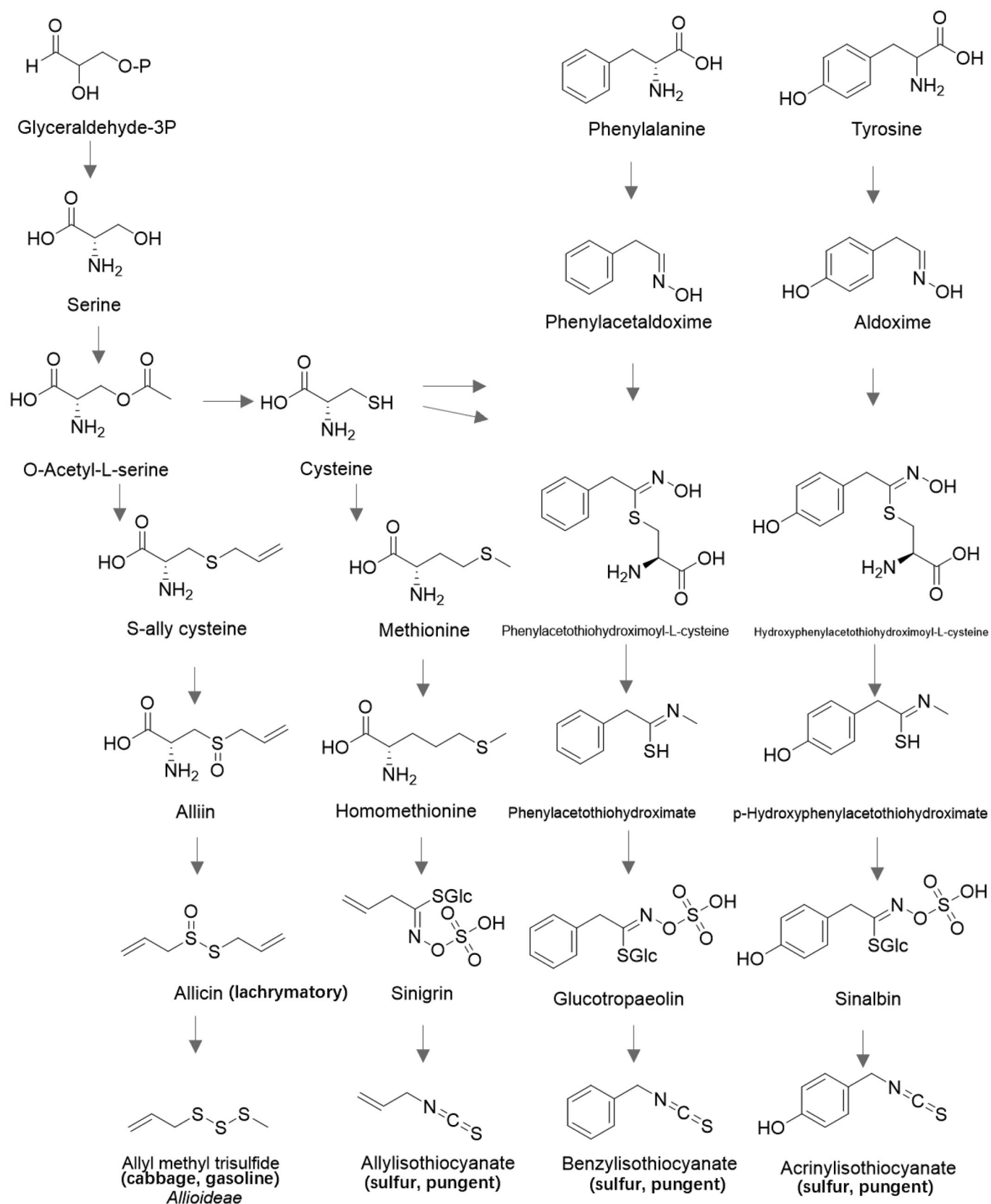
Figure 5. The biosynthetic pathway involving alkylation of the cysteine in glutathione, followed by cleavage and oxidation to form the sulfoxides or (thio)alk(en)yla-tion of cysteine or *O*-acetyl serine. Isothiocyanates are derived from glucosinolates biosynthesis pathway connected with cysteine mediated pathway. Single arrow may show a multistep reaction. Flavor quality of each compound are presented in parentheses.

bisabolene synthase by expression combined with genes involved in the mevalonate pathway in *E. coli* cells (Fujisawa et al. 2010).

The phenylpropanoids branching pathway synthesizes monolignols such as *p*-coumaryl alcohol or coniferyl alcohol, followed by acetylating these monolignols into *p*-coumaryl
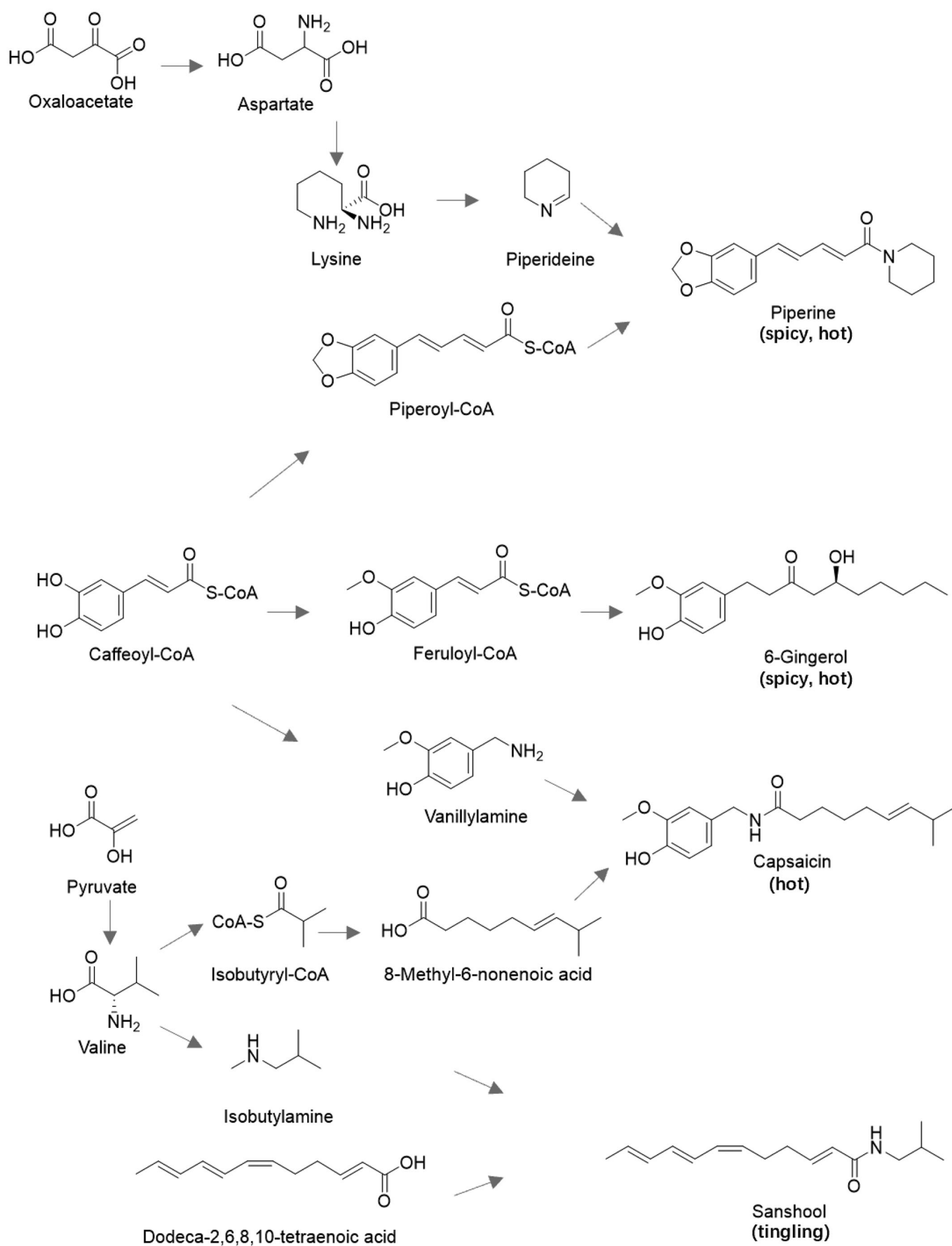
Figure 6. The biosynthesis pathway of hot or tingling compounds such as capsaicin, sanshool, gingerol and piperine, mediated by some related amino acids and fatty acids. Single arrow may show a multistep reaction. Flavor quality of each compound are presented in parentheses.

acetate and coniferyl acetate, and then being reduced by NADPH-dependent reductases including eugenol synthase or isoeugenol synthase (Ferrer et al. 2008). Chavicol and eugenol in sweet basil leaves could be enzymatically O-methylated by O-methyltransferase leading to methyl chavicol and methyl eugenol, respectively (Lewinsohn et al. 2000). Additionally, enzymes catalyzing the formation of eugenol or isoeugenol were characterized from basil, one of which was a closely related enzyme from petunia (Takao Koeduka et al. 2006). Biosynthases from different plant sources should be recombined to make various molecules. For example, several biosynthetic genes from either plants or bacteria were applied to construct a set of biosynthetic pathways in *E. coli* to generate valuable target compounds (Wang et al. 2015). Metabolic engineering is also applied to illustrate the detailed enzymatic reaction based on the understanding of the biosynthetic route. A TtgR gene cloned from *Pseudomonas putida* was expressed in *E. coli* and the TtgR protein that responds to resveratrol used to identify 4CL variants with enhanced activity in *in vivo* directed evolution as an efficient resveratrol biosensor (Xiong et al. 2017).

Cysteine synthase was found primary in bundle sheath and phloem cells using antibodies against enzyme proteins, whereas alliinase was detected almost exclusively in bundle sheath cells in three Allium species tested (Yamazaki, Sugiyama, and Saito 2002). Cysteine synthase was associated with sulphur metabolism which occurs primarily in leaves. Once produced in leaves, cysteine synthase can be transported to the bulb (Mitrová et al. 2018). The cDNA clones for two cysteine synthase and serine acetyltransferases involved in the biosynthesis of sulfur-containing constituents were isolated and investigated for their biochemical properties and expression (Urano et al. 2000). An isoform of onion γ-Glutamyl transpeptidase was purified from sprouting onion bulbs, and its partial cDNA and genomic sequences were cloned with Arabidopsis (Shaw, Pither-joyce, and Mccallum 2005). Among major Allium plants, alliin is the representative cysteine sulfoxide in garlic, while isoalliin is characteristic for onion and other related species (Jan and Michael 2010). Allicin, which was found in disrupted garlic, but not found in disrupted onion, is enzymatically catalyzed by alliinase from alliin, and attributed to garlic health benefits (Thomson et al. 2013). Wounded onions produced significantly smaller amounts of tear-inducing lachrymatory factor, which leads lachrymatory synthase activity up to 1,544-fold, after using RNA interference silencing (Oa et al. 2008). In addition, onions grown with repressed lachrymatory factor synthase activity causes these onions to produce increased amounts of allicin, which enable tearless modified onions that may also contribute to weight control (Tuzlukov 2013).

For the phenylpropanoids pathway related pungent compounds, phenolic parts are generated through the shikimate pathway, in which caffeoyl-CoA plays a vital role. Enzyme activities like polyketide synthases, cytochrome p450 hydroxylases, and S-adenosyl-L-methionine-dependent O-methyltransferases, were identified in both ginger and turmeric tissues providing direct evidence for enzymatic involvement of the phenylpropanoid pathway in these plants (Ramirez-Ahumada, Timmermann, and Gang 2006). The gene involved in capsaicin biosynthesis is unequivocally revealed to support the suggestion of a pungency gene (Prasad et al. 2006). Discovery of special taste or trigeminal compounds and their corresponding sensory analysis have been performed to enhance overall taste whilst reducing the sugar or salt content of foods (McNamara, Randall, and Gunthorpe 2005; Terada, Masuda, and Watanabe 2015). Starkenmann et al outlined some of the natural products screened, such as pungent alkaloids and isothiocyanates, to integrate all parameters of pungency to help flavorists design their flavor innovatively (Starkenmann, Cayeux, and Birkbeck 2011). Moreover, not only flavor modulation, but also the bioactivity or medical application of some pungent compounds such as capsaicin has attracted increased interest into their biosynthesis mechanism (De Lourdes Reyes-Escogido, Gonzalez-Mondragon, and Vazquez-Tzompantzi 2011).

Conclusion

Spice plants produce a large number of flavor compounds that attribute to their aroma property. Due to their antimicrobial activities and pleasant smell, essential oils are typically severed as preservatives and flavorings (Takao Koeduka 2018). Spice plants are closely correlated to high industrial demand, which leads to the search for alternatives such as synthetic products. However, a great number of consumers still prefer natural products due to safety concerns or cultural reasons. The search for elucidation of flavor compound biosynthesis pathways in spice plants regarding the enzymes and genes involved and the molecular reaction mechanisms have only been reported in recent decades. As discussed in this study, the major aroma compounds vary greatly among the various spice plant families. To the best of our knowledge, spice plants from the family *Lamiaceae*, such as basil and sage, seem to be the greatest resource chosen to identify the biosynthesis mechanism of major aroma compounds from either terpenoids or phenylpropanoids. Future research and application of spice flavor compounds could be directed to the combination of chemotaxonomical and metabolic properties, since compositional differences were found in the essential oils or aroma compounds isolated from species (Dogaru et al. 2015).

Aroma from spice plants may contain many individual chemicals, some of which have overlapping components, and others are specific, even to closely related species. Here, a literature survey and meta-analysis on aroma or pungent compounds of spice plant and their production is presented to enable a better understanding of the biosynthetic pathways and metabolic engineering. Recent advances in the biochemical and molecular analysis of flavor compounds have improved our understanding of the genes and enzymes involved in the biosynthesis of spicy flavor compounds. This may also provide us with tools for metabolic engineering in an effort to meet the increasing demand of spice flavor using screening techniques to design novel flavor compounds or excavate the biosynthases involved. In addition,

motivated by the current reality and high demand to reduce the salt in the diet, food researchers and industry have been searching to find novel pungent compounds that help enhance the taste, while, at the same time reduce the use of salt and monosodium glutamate.

ABBREVIATIONS

DMAPP	dimethylallyl pyrophosphate
ESA	European spice association
FPP	farnesyl pyrophosphate
GC-MS	gas chromatography-mass spectrometry
GPP	geranyl diphosphate
HS-SPME	head space-solid phase microextraction
IPP	, isopentenyl diphosphate
MEP	Methyl-erthritol-4-phosphate
MVA	mevalonate;
PLS-DA	partial least squares discriminant analysis

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