Chemistry, Sustainability and Naturality of Perfumery Biotech Ingredients

Mathilde Lecourt, Sylvain Antoniotti

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Mathilde Lecourt[a] and Sylvain Antoniotti*[a]

Diversity matters.

Abstract: White biotechnology has emerged in biochemical manufacturing processes to deliver perfumery ingredients satisfying novel interests of the society for natural, eco-responsible and sustainable materials. As a result, an intense R&D activity has taken place on these subjects, resulting in both scientific publications and patent applications reporting combinations of state-of-the-art approaches in biocatalysis, metabolic engineering, synthetic biology, biosynthesis elucidation, gene edition and cloning, and analytical chemistry. In this minireview, we present a smelly selection of novel biotechnological processes and ingredients from a scientific articles and patents survey covering the last 6 years and analysed in terms of chemistry, sustainability and naturality. Classification has been made between metabolic engineering on one side, allowing either biotechnological synthesis of essential oil surrogates or single molecule ingredients, and on another side the optimisation of properties of natural complex substances by specific and selective enzymatic modifications of their chemical composition.

Since 2000, many approaches involving biocatalysis have emerged in order to improve quantitively or qualitatively essential oils and natural extracts used in perfumery. In this regard, pre-treatment of plant material to improve extraction or distillation yields were developed mostly using cellulases, pectinases and glycosidases to facilitate the liberation of volatile molecules from cellular compartments. With a significant paradigm shift, some authors started to work on using biocatalysis directly on natural complexes mixtures such as essential oils and natural extracts in post-treatment procedures, while chemists usually preferred to work on pure substances.[1] The field has however gained in scope and complexity recently. Not only that new enzymes have been used in pre- and post-treatment approaches, but novel biological techniques and processes have been discovered and implemented in industry. This was the birth of novel classes of ingredients such as biotech ingredients obtained by metabolic engineering either as bioproduced essential oils or pure compounds. They are produced in bioreactors by recombinant microorganisms from farnesyl diphosphate or even simple carbon source such as glucose.[2] There are thus highly biosourced (in general up to 100%) and are closely related to their conventional natural counterparts. Following the progresses of molecular biology for the last twenty years, and the sequencing of the genome of numerous species, it is now possible to select genes coding for desired enzymes from their wild natural source (bacteria, fungi, plants, animals, …) and to transfer it into a recombinant organism, typically *E. coli* or *Aspergillus sp.*. If multiple genes coding for different enzymes are transferred, the host metabolism can be modified to produce -relatively- large quantities of a valuable product, whose chemical complexity depends on the number of biosynthetic steps assembled. Metabolic pathways, sometimes from different species, can be mixed or reconstructed in such a way that a biosynthesis, fully or in part, could be performed from simple precursors. Last, the gene can be modified, randomly or selectively, to improve the performance of the bioprocess: yield, volumetric productivity, enantioselectivity, substrate scope, temperature tolerance, …

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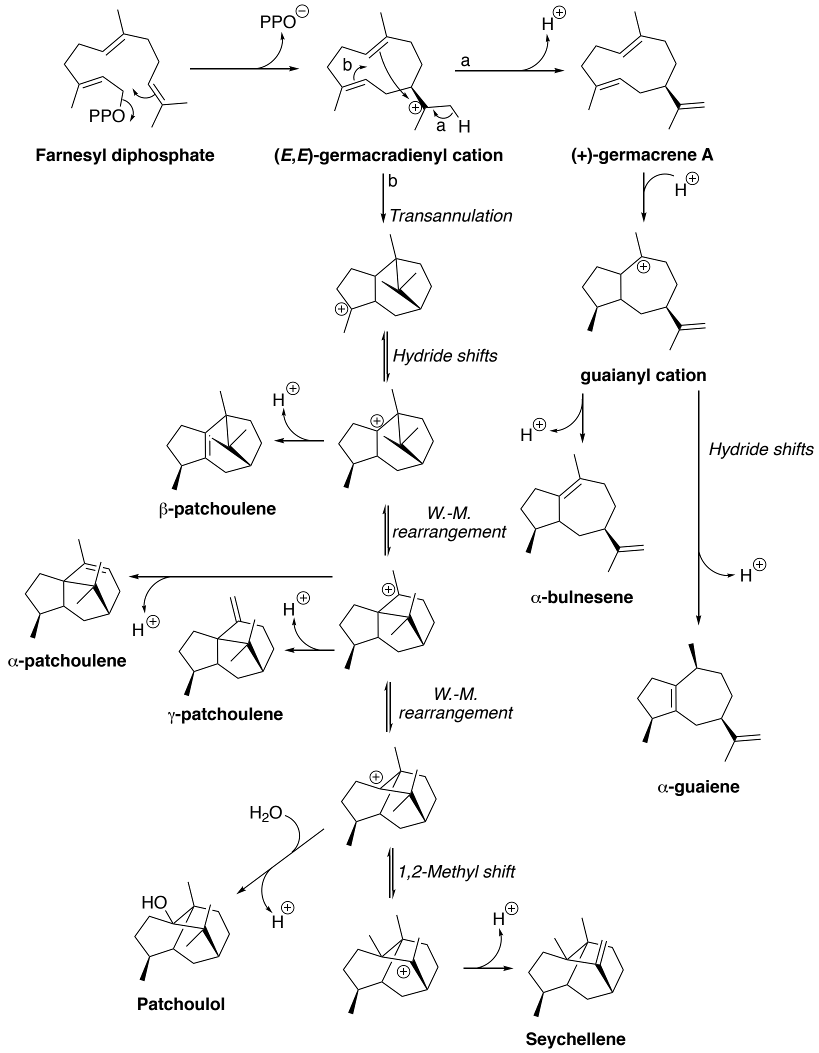
Metabolic engineering and a new class of ingredients

In this section, we will examine the scientific literature and the many patents dealing with the use of recombinant organisms expressing genes coding for enzymes involved in the biosynthesis of odorant molecules in plants, mostly terpenes and terpenoids, but not only. These modified organisms could then be used to produce essential oils equivalents or single molecules.

New natural complex mixtures

Patchouli (Pogostemon cablin)

Biosynthetic routes to main constituents of patchouli oil have been intensively studied and probably the most important finding was that, in spite of the chemical diversity found in patchouli essential oil (most of it as sesquiterpene hydrocarbons),[3] a limited number of terpene synthase enzymes were actually at work and accounted for a significant part of this diversity.[4] In particular, a single patchoulol synthase cDNA encoding for a multi-functional sesquiterpene synthase was found and could be heterologously expressed from *E. coli*, purified through his-tag technique and used in biocatalytic reactions with farnesyl, geranyl, and neryl diphosphates as substrates. Provided that (*E*,*E*)-farnesyl diphosphate (FPP) is cyclized into (*E*,*E*)-germacradienyl cation and further guaianyl cation,[5] several important sesquiterpenes of patchouli essential oil such as (−)-patchoulol, seychellene, α-, β-, and γ-patchoulene, α-guaiene, and α-bulnesene (δ-guaiene) were formed (Scheme 1).



Schema 1. Chemical diversity of sesquiterpene hydrocarbons obtained from one single starting cyclisation reaction. Hydride shifts could be either 1,2- or 1,3-shifts. W.-M. stands for Wagner-Meerwein.

Thus, using a single natural starting material like (*E*,*E*)-farnesyl diphosphate, a mixture of patchouli essential oil sesquiterpenes and sesquiterpenoids could be produced, thereby giving an entry into a novel class of biotech ingredients sharing chemical and sensorial similarity with the distilled ingredient.

Several patents were submitted by Swiss F&F company Firmenich & Cie and the ingredient Clearwood® was launched in 2014, as a 'clean' equivalent of patchouli essential oil,[6] obtained by a sustainable process with the advantage of the stability of supply.[7] Its natural grade was questioned,[8] not only because of the GMO used in the process to obtain the precious sesquiterpenes mixture, but also for the presence of patchoulol ethyl ether, which is not listed in the 130 published components of patchouli essential oil, and was not previously identified in nature.[9]

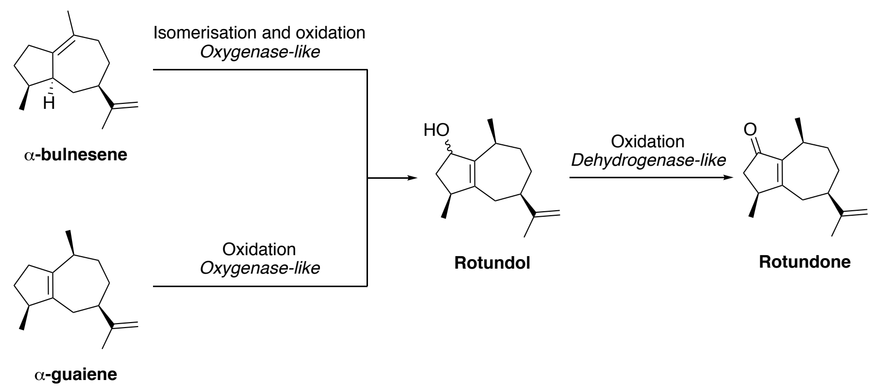
Similarly, a recombinant *Escherichia coli* strain expressing a variant of patchoulol synthase has been successfully used to produce patchoulol and some related sesquiterpenes from (*E*,*E*)-farnesyl diphosphate.[10] Patchouli essential oil compounds were observed, but interestingly, the stereospecificity of the enzymatic reaction was also tested using (2*Z*,6*E*)- and (2*E*,6*Z*)-farnesyl diphosphate. The former furnished mostly α- and γ-gurjunene, and germacrene B, and the latter an unknown sesquiterpene ($C_{15}H_{24}$), and helminthogermacrene. Neither one or the other could be converted into patchoulol, the different stereochemistry of C-C double bonds C2-C3 and C6-C7 leading to other carbenium ion intermediates than the *E*,*E* isomer. For geranyl and neryl diphosphates, a mixture of limonene, α-terpineol, and dephosphorylated terpenyl alcohols was formed.

Akigalawood® is another of these novel biotech ingredients launched recently with a filiation with patchouli oil. This one is currently a captive ingredient at Givaudan.[11] It is clearly mentioned in a patent as a *rotundone-containing material*.[12] Rotundone is an odorant sesquiterpenoid occurring in several natural products, and notably in red wines, featuring a spicy-woody olfactory note.[13] The biosynthesis of rotundone from α-guaiene in *Vitis vinifera* has been elucidated and the genes identified.[14]

Production of α-guaiene and rotundone from farnesyl diphosphate by recombinant microorganisms expressing sesquiterpene synthases from *Vitis vinifera* and *Pogostemon cablin* and an oxidase from *Aquilaria sp* (agarwood) is mentioned in a recent patent application also from Givaudan.[15] The patent mentioned that farnesyl diphosphate could be produced in vivo from sugar by the microorganism, but this specific point was not listed in the claims.

In another series of patents, an enzymatic process to access rotundol from α-guaiene and α-bulnesene is proposed.[16] It is based on the use of laccases, in the presence or not of a chemical mediator (e.g. 1-hydroxybenzotriazole), and indicates specifically that the starting sesquiterpenes could be replaced by light fractions of patchouli essential oil, which are typically containing varying ratios of α-, β-, and δ-guaienes, seychellene, and β-, δ-patchoulenes. Considering the known reactivity of laccases, upon rotundol formation by the direct allylic oxidation of α-guaiene, it is very likely that rotundone will also be obtained among other oxidation products (Scheme 2).[17]

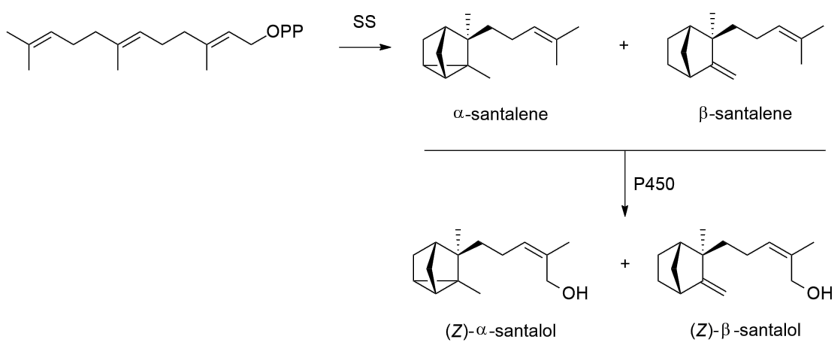
A recent patent application submitted by Ginkgo Bioworks describes the formation of sesquiterpenes and sesquiterpenoids, including guaienes and derivatives, by using chimeric terpene synthases built up by the assembly of nucleic acid sequences from rare or extinct plants such as *Hibiscadelphus wilderianus*, *Leucadendron grandiflorum*, *Macostylis villosa*, *Orbexilum stipulatum*, *Shorea cuspidate*, and *Wendlandia angustifolia*.[18]



Scheme 2. Conversion of α-guaiene and α-bulnesene into rotundol and rotundone.

Sandalwood (Santalum sp.)
Sandalwood oil is another important woody natural ingredient obtained by steam distillation of the heart of *Santalum sp.* wood. The east Indian quality is obtained from *Santalum album* L. typically found in Indonesia and Sri Lanka, and recently in Northern Western Australia plantations.[19] Sandalwood oil is mostly composed of sesquiterpene alcohols, among which (*Z*)-α-santalol and (*Z*)-β-santalol account for 80-90%. It is accepted that these santalols are also the main contributors to the sandalwood odor, attributed to (+)-(*Z*)-α-santalol and (–)-(*Z*)-β-santalol enantiomers. Biosynthetic studies recently unveiled a common sesquiterpene synthase at work in *Santalum sp*. to transform farnesyl diphosphate into α- and β-santalene, *trans*-β-bergamotene, epi-β-santalene and (*Z*)-β-farnesene.[20]

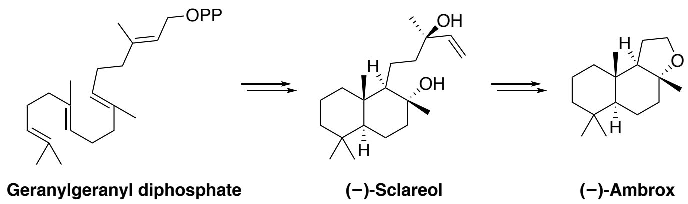
Upon a chemoselective hydroxylation of the pro-(*Z*) methyl group of α- and β-santalene by a P450 monooxygenase, the fragrant sesquiterpene alcohols of sandalwood are formed.[21] By cloning these genes in recombinant cells, fermentation processes could be implemented industrially to deliver the ingredient Dreamwood® launched in May 2020 (Scheme 3).[22]



Scheme 3. Bioconversion of farnesyl diphosphate into a mixture of α- and β-santalene by a sesquiterpene synthase (SS) from *Santalum sp.* followed by a chemoselective hydroxylation by a P450 monooxygenase.

Pure molecules produced by recombinant organisms

Ambrox
Ambrox is a sesquiterpenoid with many tradenames (Ambroxide®, Ambroxan®, Ambrofix®, Ambermox®, Ambermor®, Ambrox® super). It is the odorant principle of the animal raw material ambergris, a pathological secretion of sperm whale (*Physeter macrocephalus*).
Viable synthetic routes were developed to avoid ethical, economical and supply issues. Hemisynthesis from the diterpenediol sclareol was established, this compound being a co-product in the manufacture of clary sage essential oil (*Salvia sclarea*). However, due to the stable and moderate consumption of clary sage essential oil and the massive and increasing use of ambrox, such coupled manufacturing process was not satisfying the demand in sclareol. Upon cloning the two enzymes involved in its biosynthesis from geranylgeranyl diphosphate in *Salvia sclarea*, engineered *E. coli* strains co-expressing geranylgeranyl diphosphate synthase from *Pantoea agglomerans* and both diterpene synthases could be produced (Scheme 4).[23] As a result, sclareol was formed in concentrations up to 1.5 g/L, together with 15% of 13-episclareol, starting with a feed of 30 g/L of glycerol as the carbon source. Further optimisation studies including metabolic engineering followed.[24]

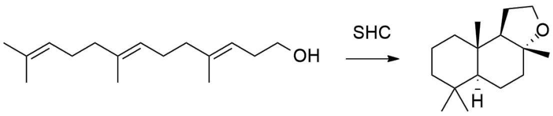


Scheme 4. Sclareol and ambrox from geranylgeranyl diphosphate.

Sclareol could also be similarly produced from recombinant microorganisms expressing labdenediol synthase and sclareol synthase from tobacco (*Nicotiana glutinosa*), and be further transformed into ambrox by various known reactions.[25]

Ambrofix® via (E)-β-farnesene
Biobased (*E*)-β-farnesene has become a commodity chemical after the successful development of a biotechnological manufacturing process with modified yeasts by the biotech company Amyris. In *Saccharomyces cerevisiae*, (*E*)-β-farnesene is produced from FPP via heterologous expression of the *Artemisia annua* farnesene synthase. Acceptable productivity requires both heterologous expression of farnesene synthase and over-expression of endogenous mevalonate pathway enzymes to continuously supply the yeast with FPP. High concentrations of (*E*)-β-farnesene, which separates from culture broth, are tolerated by *S. cerevisiae* allowing good productivity (up to 23.8 g (*E*)-β-farnesene for 100 g of glucose).[26] (*E*)-β-farnesene can thus be chemically converted to (*E*,*E*)-homofarnesol in two steps by chemoselective and stereospecific cyclopropanation of the terminal double bond,[27] followed by a Bronsted acid-catalysed

ring-opening in the presence of water.[28] (*E*,*E*)-homofarnesol is then cyclised to (–)-ambrofix® by a squalene hopene cyclase (SHC) enzyme, either isolated or heterologously expressed by a microorganism (Scheme 5).[29] SHC from *Alicyclobacillus acidocaldarius* could be optimized by random mutagenesis and *E. coli* strains expressing SHC variant allowed the conversion of up to 188 g/L (*E*,*E*)-homofarnesol in less than 72 hours in the presence of SDS as surfactant.[30]

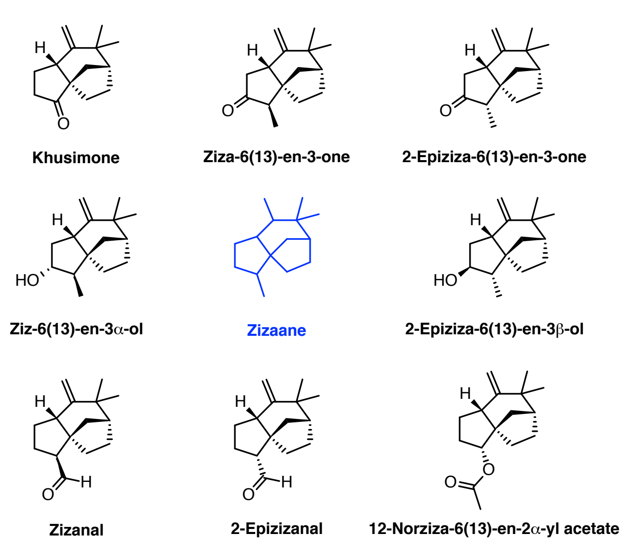


Scheme 5. Cyclisation of (*E*,*E*)-homofarnesol into (–)-ambrox by squalene hopene cyclase (SHC).

Similarly, a BASF patent mentions a homofarnesol ambroxan cyclase which could lead to (–)-ambrox® from citral.[31]
The case of amber oxepin (aka amberketal), known under the trademark Z11®,[32] is kind of unique in the portfolio of biotech fragrance ingredients since it is originally an artificial ingredient discovered in the 50's when a huge synthetic work was done on amber odorants. Amber oxepin could be obtained from sclareol, manool or sclareol oxide upon fermentation followed by clean oxidation and acetal formation. It is thus biobased, the process is sustainable but could not be considered natural.

Zizaene

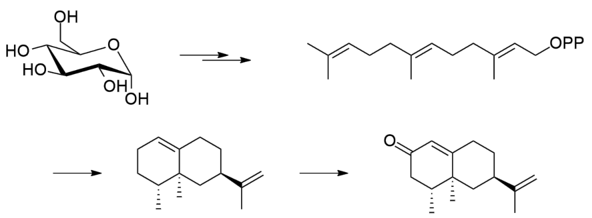
Vetiver essential oil obtained by hydrodistillation of roots of *Chrysopogon zizanioides* in Haiti, Indonesia, Brazil, Ile de la Réunion, or Sri-Lanka is a precious natural ingredient with woody-earthy-ambery facets. It is considered by analytical chemists one of the most complex natural substances used in perfumery.[33] Several odour-impact components of vetiver essential oil share a common zizaane skeleton as in khusimone, khusimol, zizaen-3α-ol, ziza-6(13)-en-3-one, and 2-epiziza-6(13)-en-3-one, for example (Scheme 6).[34]
Terpene synthase involved in (+)-zizaene biosynthesis in *Chrysopogon zizanioides* has been transferred in recombinant *Escherichia coli* together with genes involved in the mevalonate pathway.[35] As a result, engineered whole-cell biotransformation of glucose directly into (+)-zizaene could be proposed. Fermentation of modified *E. coli* strain was performed in a biphasic DNB broth/isooctane and delivered under optimised conditions up to 25 mg/L of (+)-zizaene, formed with 10% of β-acoradiene, for an initial feed of glucose of 5 g/L.
A patent for an access to premnaspirodiene, a spiradiene sesquiterpene, by fermentation further leading by conventional organic synthesis to 5-*epi*-β-vetivone and related odorant compounds has been recently granted.[36] β-vetivone is a constituent featuring a spirodecane skeleton and a contributor to the odour of vetiver essential oil with fruity-woody notes.



Scheme 6. Odour-impact molecules featuring a zizaane skeleton found in vetiver essential oil from ref. [34]

Nootkatone

Oxygenated sesquiterpene nootkatone is a constituent of vetiver and citrus essential oils, and plays an important role in the grapefruit facet typically associated with vetiveryl acetate.[34] Nootkatone could be obtained by chemoselective oxidation of sesquiterpene hydrocarbon valencene, found in Valencia orange (*Citrus* x *sinensis* Valencia) essential oil. A gene coding for valencene synthase could be expressed heterologously in recombinant bacterial cell and allow for the production of valencene from a carbon source such as glucose via farnesol diphosphate at concentration up to 356 g/L.[37] (Bio)chemical oxidation could then convert valencene into nootkatone, enzymatic reactions being known to deliver selectively the desired isomer (Scheme 7).[38]
Monoterpene hydrocarbons such as myrcene also attracted the interest of biotech companies recently.[39] A myrcene synthase from *Ocimum basilicum* and a geranyl diphosphate synthase from *Quercus ilex*, *Abies grandis*, *Antirrhinum majus*, *Arabidopsis thaliana*, *Aegilops squarrosa*, *Alstroemeria peruviana* or *Picea abies* were cloned and expressed in a microbial recombinant organism to produce myrcene, with other terpenes such as α- and γ-terpinene, from a carbon source such as a monosaccharide (e.g. glucose), a disaccharide (e.g. sucrose), or a non-fermentable carbon source (glycerol). Although myrcene is not olfactorily interesting as itself, biobased myrcene can be converted into various valuable products thereby becoming themselves partly or completely biobased.



Scheme 7. Biocatalytic route from glucose to nootkatone.

In another patent application, recombinant cells transformed with genes coding for the entire mevalonate pathway, and selected terpene synthase were claimed to convert AcetylCoA to citronellal and citronellol.[40]

Muscone

Besides terpenes, macrocycles such as muscone, the odorant principle of animal raw material musk, civetone and normuscone have been prepared using recombinant organisms.[41] L-isoleucine was used as the precursor, providing the correct absolute configuration for the methyl-substituted asymmetric carbon atom. Upon the action of a transaminase, L-isoleucine is converted to (*S*)-3-methyl-2-oxopentanoate.

Transketolase activity delivers (*S*)-2-methylbutanal, further oxidized to (*S*)-2-methylbutanoic acid, thioesterified to (*S*)-2-methylbutanoic acid CoA.

The latter is then incorporated in (S)-anteiso fatty acid biosynthesis by fatty acid synthases leading either to 14, 16, or 18 carbon atoms chain. Oxidation of the terminal position yields the corresponding diacid, further converted into its diacid CoA and cyclized into muscone, presumably by cyclocondensation/hydrolysis/decarboxylation (Scheme 8).

(Z)-hex-3-en-1-ol

The typical odorant considered the reference of the green grassy note is (*Z*)-hex-3-en-1-ol. A biotechnological approach from linolenic acid (from soybean flour) involving a 13-lipoxygenase (LOX) and a 13-hydroperoxide lyase (HPL) was developed to obtain (*Z*)-hex-3-enal, further reduced to (*Z*)-hex-3-en-1-ol. The performance of a 13-HPL isolated from guava (*Psidium guajava*) was improved by directed evolution. After four cycles of gene shuffling and random mutagenesis, a 15-fold increase of the desired product yield was obtained.[42]

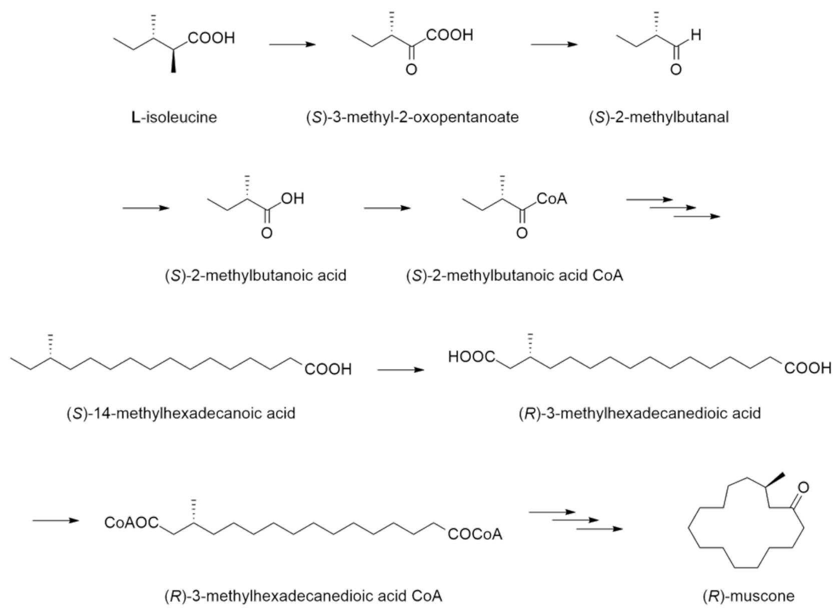
Santalol

Following studies on santalene synthase (vide supra), a renewable biotech santalol was made available to the market by BASF in July 2020 after its acquisition of Isobionics.[43]

Flavours

Flavoring molecules with the natural grade produced by biotechnology are present on the market for longer than perfumery ingredients.

Thus, natural vanillin produced by biotechnology, either in whole-cell fermentation processes or with isolated enzymes, remain a hot target considering the market price of natural vanillin compared with its synthetic counterpart and the supply instability.[44] Several routes were developed from natural and available starting materials such as ferulic acid,[45] eugenol,[46] and curcumin.[47] Recombinant microorganisms engineered with several enzymes are now able to convert glucose into vanillin and vanillin β-D-glucoside, the latter being less toxic for microorganisms.[48] Other natural flavors with fruity notes such as furaneol[49] (strawberry) and frambinone[50] (raspberry), and massoia lactone[51] (coconut), have been the focus of recent research to develop viable biotechnological routes.



Scheme 8. Multistep biocatalytic synthesis of (*R*)-muscone.

Optimisation of natural complex substances by isolated enzymes

If whole-cell processes are particularly efficient for multistep synthesis and obviously *de novo* synthesis, when only one reaction is required to convert a readily available starting material, isolated enzymes might be a better choice. Immobilisation techniques are numerous and enzymes can be attached to virtually any type of support (organic, mineral, hydrophobic, hydrophilic, natural, synthetic, magnetic …), allowing either their recovery and recycling or their use in continuous flow processes.[52] On the side of that, considering the substrate specificity of enzymes, they have been used to react with a single compound or a limited number of compounds from a common chemical class within a natural complex substance (NCS). As a result, optimisation of the properties of NCS could be performed by a controlled, specific and selective enzymatic modification of the chemical composition.

Vetiver essential oil

Vetiver essential oil (VEO) is an important natural raw material for fine fragrances (vide supra). Several origins can be found on the market, as well as several specialties, like fractions from molecular distillation featuring some particular olfactory aspects, acetylated derivatives, or combination of both. Crude or fractionated vetiver essential oil, rich in sesquiterpene alcohols, can indeed be submitted to an acetylation reaction to deliver various qualities of vetiveryl acetate, an ingredient still belonging to the woody family but featuring amber and grapefruit notes, less earthy.

Vetiveryl acetate is typically obtained by acetylation of VEO or light fractions of VEO with acetic anhydride in the presence or not of a catalyst, in a solvent and upon heating and classified as a synthetic ingredient.

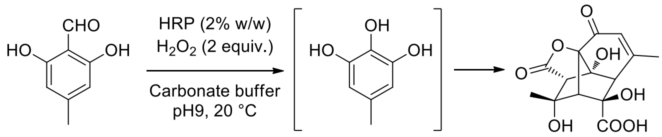
Taking advantage of the large substrate promiscuity of lipase B from *Candida antartica* (CaLB),[53] vetiveryl acetate has been prepared by enzymatic acetylation using natural EtOAc both as the solvent and the acyl donor.[54] In this process, a very high sustainability profile was attained since the enzyme used was immobilized on an acrylic resin and could be recycled more than 12 times with very little decrease of the activity, biobased EtOAc was used and recycled upon evaporation, and the reaction was performed at room temperature within a few hours. As a result, a +95% natural vetiveryl acetate was produced for the first time in quantitative yield and up to the kilogram scale. Interestingly, it was shown by detailed comprehensive GCxGC-MS that the reaction occurred chemoselectively with primary sesquiterpene alcohols, leaving mostly unchanged secondary and tertiary alcohols.

Oakmoss absolute

Also possessing earthy notes, oakmoss absolute, the solvent extract of the lichen *Evernia prunastri*, is a natural raw material used in fine fragrances for almost a century in the chypre and fougère accord.[55] Mostly composed of monoaromatic compounds and depsides, oakmoss absolute contains small amounts of contact allergens atranol and chloroatranol. The European Union (EU) Commission Regulation 2017/1410 of 2 August 2017 has set that cosmetic products containing these substances shall not be made available on the Union market from 23 August 2021.[11]

Following our previous work on eugenol,[56] a peroxidase-based strategy has been developed to specifically remove atranol and chloroatranol from oakmoss absolute.[57] The reaction consisted in a tandem process were both aromatic aldehydes were submitted to a Dakin oxidation by H_2O_2 at pH9, followed in the same pot by oxidative coupling of the resulting pyrogallol derivatives catalyzed by horseradish peroxidase, HRP (Scheme 9). Under these oxidative conditions, a cascade starting by the enzymatic oxidation was proposed to account for the complexity of the product formed.[58] The product being hydrosoluble, it could be simply removed from the absolute by liquid-liquid extraction.

The resulting modified oakmoss absolute showed only ppm levels of atranol and chloroatranol. Sensory analysis was performed by triangular testing on a panel of 56 persons and revealed that the atranol-free absolute was not significantly different from an olfactory viewpoint from the genuine absolute at the 99% level of confidence.



Scheme 9. Oxidation of atranol to 5-methylpyrogallol leading to a densely fused dimeric product via an oxidative cascade triggered by HRP.

Sustainability & Naturality

Biocatalysis is 'naturally' sustainable.[59] Not only because reactions are performed in water (and anyway solvent is sometimes necessary to collect products in downstream processing) and at temperatures close to room temperature, but for many other points. Considering the 4 main items of green and sustainable chemistry, to which the famous 12 principles refer to,[60] one can list the following assets of applied biocatalysis towards sustainability:

Waste prevention

- High selectivity, in the ability to deliver only the desired product (few byproducts);
- High enantioselectivity, in the ability to deliver only the active stereoisomer, including cases where enantiomery matters. This is particularly important in fragrance chemistry where chirality matters and may impact both qualitatively and quantitatively the properties of odorant molecules;[61]
- Frugality, in the limited requirements of biocatalytic processes in terms of additives, activators, protecting groups, and so on, mostly thanks to the often high specifity of enzymes;
- Biodegradability of nature-identical molecules for which degradation biochemical cascades already exist or can be readily activated by living organisms.

Resources

- Enzymes, which are stable renewable biological catalysts, are used rather than chemical reagents of petrochemical and therefore depleting resources with sometimes limited shelf-lives;
- Glucose can be used as the carbon source, when it is estimated that 50% of biomass is consisting of carbohydrates;
- Commonly starting from natural substrates or biobased substrates otherwise.

Safety

- Water as a non-flammable and non-toxic solvent excludes the risk upon scale-up. Industrial wastewaters however require treatment before discharge,[62] mostly to remove microbial pathogens and heavy metals.
- Extrinsic safety is rarely required;
- Besides cases of allergenicity, enzymes are not considered harmful material during handling or upon exposure;
- No high pressure/high temperatures, pyrophoric or energetic material requiring special safety processes. Limited surveillance;

- Removing specifically regulated compounds flagged for health concerns using biotech approaches improves the safety of products put on the market.

Energetic sobriety

- Room temperature processes, meaning no heating or cooling which are costly operations industrially.

Metrics

Measuring all these factors and impacts with a single metric is not an easy task. In general, it is more convenient and insightful to consider homogeneous groups of impacts, and to compare ingredients or processes on a relative rather than an absolute approach.[63] Several initiatives have flourished in industry to implement and measure sustainability in their activities, more or less focusing on concepts directly related to green chemistry.

The French company Mane et fils has developed the green motion metric in the early 2010's.[64] It is based on the assessment of health, safety and environmental impacts of manufactured ingredients on a 0-100 scale.

In France again, the innovation cluster PASS, now Innov'Alliance, has set up the Eco-Responsible Ingredient label (ERI-360) in 2018 with 12 companies of the Flavour and Fragrance sector.[65] The label can be attributed with several degree of eco-responsibility upon the analysis of agricultural and manufacturing processes, together with societal aspects.

In 2018, Symrise has developed the Product Sustainability Scorecard to evaluate the sustainability of their solutions[66] and IFF the "Toward a circular future" program.[67]

In 2019, Givaudan has launched the FiveCarbon Path™ as a part of "A sense of tomorrow" program, aimed at *increasing the use of renewable carbon, increasing carbon efficiency in synthesis, maximising biodegradable carbon, increasing the 'odour per carbon ratio' with high impact material, and using upcycled carbon from side streams*.[68]

Following EcoScent Compass in 2018, Firmenich has announced the launch of the EcoIngredient Compass in June 2020, a new proprietary tool for the immediate assessment of fragrance molecules with respect for renewable carbon, biodegradability and green chemistry.[69]

Society

Societal impacts are not really addressed by the twelve principles of green chemistry, besides health issues addressed globally as safety concerns. Biodiversity can be endangered by the massive consumption of a given vegetal or animal resource. If ingredients of animal origins are of scarce use nowadays for ethical reasons, some precious odorant woods, heavily exploited for the last decades, have been now placed under the protection of international conventions such as CITES to avoid their extinction. Consumers may be sensitive to these types of impacts of their way of life.

The consequences of new technologies such as metabolic engineering and synthetic biology on the wealth of developing countries in Africa or in other places are a matter of concern for some NGO.[70] For example, vanilla cultivation and exportation is an important economical asset of Madagascar and worth 62% of the total vanilla market worldwide in 2018. This point has been taken into account in sustainability programs from most big players of the flavor and fragrance industry to improve revenues of farmers and agricultural practices at the same time. During summer 2020, IFRA and IOFI have launched the IFRA-IOFI sustainability charter.[71]

Economic viability

The question of economic viability of white biotechnology processes is legitimate, particularly when product concentration and productivity are low, compared with conventional processes, and has always been crucial in evaluating their industrial implementation.[72]

For commodity chemicals, the selling price is dependent on crude oil prices variations,[73] and for fine chemicals, including fragrance ingredients, on issues of shortage on key intermediates.[74]

If we consider general aspects of the viability of industrial biotech, we can roughly identify 3 categories of chemicals in decreasing order of valorisation: APIs, fine chemicals, and intermediates and commodities. Industry has increasingly implemented biotech processes these last two decades, culminating with highly valuable chiral APIs such as sitagliptin[75] (in the optimized final process, a 200 g/L concentration of substrate was tolerated by the enzyme) with an increase in the overall yield (13 %) and productivity (53 %) as compared to the chemical process (transition metal-based asymmetric catalysis). The minimum selling price (MSP) of biobased commodities was studied and it was determined that titers of ≥45 g/L result in lower production cost, energy consumption, and GHG emissions, when compared to conventional petrochemical production processes.[76]

In the particular case of fragrance ingredients, it is ingredient- and strategy- dependent, when an ingredient can remain captive (not commercially available and only used in perfume compositions from the fragrance company owning it and used as signature). In some specific cases, it is also dependent on natural ingredients prices variation.

Naturality

From Mendel's manual plant hybridization[77] to metabolic engineering and gene selection and edition by molecular tools[78] how should we define naturality based on scientific inputs? These fields are expanding quickly, empowered by implementation of omics, in silico simulation, and deep learning algorithms to fasten the selection, assembly, and coordination of biological components to achieve the biological synthesis of valuable chemicals from cheap elemental sources. On the other side, essential oils are perceived as highly natural material by consumers although heating terpenes at 100 °C in wet conditions is a suitable manner to perform hydration, dehydration, and isomerization reactions and so on. Easier is the definition of artificial: never seen in nature and thus designed by the human mind. However, the artificial status of a compound could change if the compound happens to be identified in nature afterwards. Nature-identical molecules should deserve a special status because their presence in the metabolome has an evolutionary justification and because nature knows how to deal with such molecules, and eventually dispose of those.

On open question remains on the status of molecules that would be unknown in nature but produced during a fermentation process involving a GMO. Although genetically modified, a GMO remains a living organism and any of its metabolites should be considered a natural product. Ingredients such as Clearwood® and Akigalawood® are good examples of borderline substances when it comes to qualify their naturality. The former is made by a genetically modified living organism but producing a metabolite never seen elsewhere in nature, the latter is a human-made ingredient prepared from a natural starting material by using an enzyme, which although natural should hardly be considered a living organism. With a virtually infinite capacity to modify the metabolism of bacterial host, that would eventually mean that any molecule should be "naturally" made by white biotechnology and therefore the term natural molecule would lose its sense. Squeezing a fruit belonging to a citrus species and smelling its scent is probably the surest way to smell genuinely natural odorants.

Perspectives

The implementation of biotech processes in fragrance industry and the success of recent ingredients launches certainly indicate that the field will continue to grow in the future. Not only that such approaches positively balance issues of supply for raw materials produced in remote locations of the world sometimes submitted to external factors (climate change, natural disasters, political changes, shortage ...), but these processes also comply with the increasing demand for sustainable ingredients and processes. If these high-tech scientific manipulations of genes seem to be reserved to large firms, popularisation of genes editing tools and kits for transfer, expression and purification will surely in a near future allow for a larger use in the sector. Small companies specialised in these types of activities also enter in ventures with F&F companies in this regard.

In this above-mentioned patent submitted by Ginkgo Bioworks, chimeric terpene synthases were built by selecting portions of genes in the DNA of rare or extinct plants.[18] This approach could lead to unprecedented combinations of biosynthetic steps leading either to novel sesquiterpene structures or original mixtures of sesquiterpene derivatives.

The question of GMOs however remains sensitive for certain businesses and products, some end-consumers being reluctant to be exposed to GMOs or to products obtained by using GMOs, sometimes with a lack of knowledge on what is really occurring. Besides that, GMOs that would be accidentally liberated in the environment could have an uncontrolled impact on the ecosystem. This question will definitely impact the future of metabolic engineering and synthetic biology on the field. An alternate solution is to use wild enzymes, even if R&D and development phases could still be operated with enzymes produced by heterologous expression, and to use external approaches instead of genetic manipulations to reach the targeted reactivity: substrate promiscuity, catalytic promiscuity, and medium promiscuity, already studied in research laboratories for years.[53, 79]

Biotechnology could be used together with a better understanding of the human olfactory system to reconstruct natural odor signatures by stimulation of the ca. 400 human olfactory receptors.[80]

The impact of regulatory bodies should not be underestimated in this field considering the difficult definition of naturality which could be in the future limited to non-processed substances.

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