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Valuable chemicals by the enzymatic modification of molecules of natural origin: Terpenoids, steroids, phenolics and related compounds

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ABSTRACT

A renewed interest for using natural organic molecules for the production of valuable chemicals is observed in current organic processes. Natural compounds provide the access to natural grade chemicals when submitted to physical treatments or biotechnological processes. Dealing with structurally complex molecules, they can provide complex core structures for hemisynthesis purposes, and in many instances they offer the advantage of providing sustainable processes when using renewable resources. These assets could be synergistic with the assets of biocatalytic processes, to end-up with efficient and sustainable processes in the organic synthesis of valuable products.

In this review, we have gathered a selection of examples on the use of enzymes for the modification of molecules of natural origin being either purified compounds (terpenoids, steroids, phenolics) or mixtures (essential oils, natural extracts) to access fine chemicals or organic polymers.

1. Introduction

Natural products have probably been, and remain, the main source of investigations and discoveries for organic chemists to date. The discipline of organic chemistry is considered to be born in 1828 when Friedrich Wöhler serendipitously achieved the synthesis of urea from cyanic acid and ammonia in an attempt to prepare ammonium cyanate, thereby breaking the dogma of the vital force theory of the influential Swedish Professor Jons J. Berzelius. Following this discovery, natural organic molecules such as glucose, quinine, camphor, indigo, have attracted the interest of renowned chemists for the study of their structure, synthesis and properties. Organic chemistry was born, and the 20th century has witnessed an exponential increase of activity in the field, providing the scientific community with problems to solve and questions to address in the structural elucidation of complex molecules, the description and understanding of their stereochemistry and of the stereoselectivity of their biosynthesis, their mode of interaction with their environment including living organisms, and the total synthesis of these complex molecules. As a reward, many conceptual and practical progresses were made, including biosynthetic pathways elucidation, analytical chemistry at the molecular level, the development of a plethora of efficient synthetic methodologies, including enantioselective catalysis, transition metals-catalysed coupling reactions, in addition to the access to bioactive natural products and relatives. In one and a half century, the status of organic compounds thus shifted from Nature-made to Man-made, and with the ever growing ability of organic chemists to create new molecules, their interests also shifted towards synthetic and/or artificial molecules, in other words molecules with no natural equivalents. This attraction for artificial molecules culminated in the nineties in particular in medicinal chemistry with the development of combinatorial chemistry. For several scientific and cultural reasons, a renewed interest for natural molecules has been observed lately.

This trend has also been observed in applied biocatalysis. While in its infancy, the focus of biochemists and chemists interested in using enzymes in organic synthesis was put on the natural metabolic substrate, a trend largely supported by Emil Fisher's dogma 1 enzyme = 1 substrate = 1 reaction. Many studies of the substrate scopes of enzymes followed, and showed that some enzymes could accommodate various structurally related substrates. The idea of a larger substrate scope was significantly improved with the use of enzyme in organic solvents and the pioneering work of Klibanov (Zaks and Klibanov, 1985), in particular for lipases with the interfacial activation phenomenon (Schmid and Verger, 1998). Synthetic substrates of artificial origin could thus be used in enzymatic reactions performed in biomimetic media or in non-conventional media as well (organic solvents, gas phase, RTILs, supercritical fluids, fluorinated solvents). Today, the novel interest for using natural organic molecules for the production of natural

Abbreviations: API, active pharmaceutical ingredient; CaLB, *Candida antartica* lipase B; CPDMO, cyclopentadecanone monooxygenase; CPO, chloroperoxidase; CrL, *Candida rugosa* lipase; DMF, dimethylformamide; LOX, lipoxygenase; PLE, pig liver esterase; RTIL, room temperature ionic liquid; SEM, scanning electron microscopy; XO, xanthine oxidase.

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grade chemicals, for the hemisynthesis of complex molecules, or for the sustainable use of renewable resources is also observed in biocatalysis in organic synthesis.

On another hand, even if the chemical toolbox seems infinite and virtually allows any kinds of transformations and the synthesis of any kinds of molecules, enzymatic steps have been increasingly implemented in synthetic strategies over the last two decades. Main reasons explaining this evolution are (1) the huge increase of the number, quality and diversity of purified enzymes available from recombinant organisms, (2) the unique ability of enzymes to operate selectively even in complex molecular environments providing chemo-, regio-, and stereoselective reactions (Faber, 2004), of critical importance in the late stages of the synthesis of complex molecules, (3) the need for more sustainable chemical processes, and in the case of active pharmaceutical ingredients (APIs), the need for metal-free products, even at the ppm scale (Galaffu et al., 2007), (4) the development of protocols for the use of enzymes in non-conventional media, useful in medium engineering (Carrea and Riva, 2000).

The progress of biosynthetic pathways elucidation and biotechnology have also contributed greatly to the popularity of enzyme-based strategy since it is possible to identify, isolate, clone, and finally over express the genes involved in the biosynthesis of a natural compound of interest, thereby opening the possibility to use the specific biocatalyst in vitro, and to use site-selective mutation if performance needs to be improved, for instance to change or enhanced the enantioselectivity, or to tailor the enzyme to better accommodate other substrates (Fig. 1).

In this review, we have gathered a selection of examples on the use of enzymes for the modification of molecules of natural origin either being purified compounds (terpenoids, steroids, phenolics) or mixtures (essential oils, natural extracts). Our purpose is not to provide a comprehensive encyclopaedia of all the transformations of that category reported to date, but more likely to expose the results published in the recent literature in a selection of compounds leading to valuable products. We will therefore mainly focus on terpenoids, steroids, natural flavours and odorants, phenolics, and natural extracts production and modification.

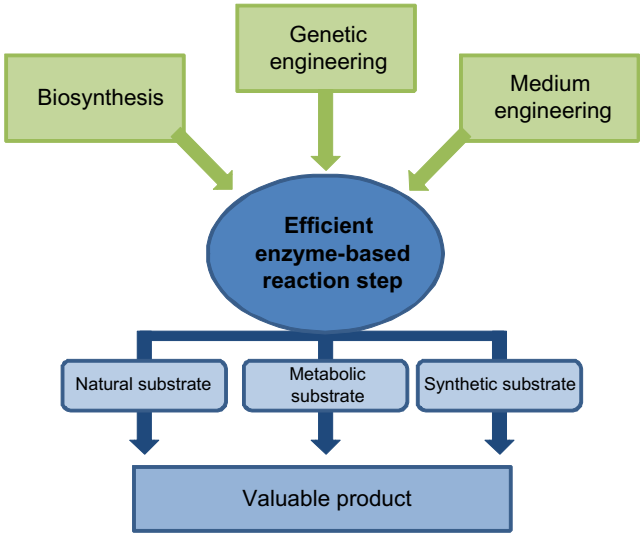

Fig. 1. Rationale of the development of an enzymatic reaction step in organic synthesis. The desired biocatalyst could be identified from biosynthetic studies, isolated and cloned. It could be an existing biocatalyst genetically modified to fit the requirements of the target reaction, and its activity could be tailored by medium engineering. It is used for the transformation of a substrate into a valuable product, the substrate being the natural metabolic substrate of the enzyme, or another substrate, natural or synthetic, structurally related to the metabolic substrate, or not (enzyme promiscuity).

The topic of the enzymatic processing of bioactive glycosides from natural sources has been reviewed in 2010 (Rauter et al., 2010) and will not be covered in the present review. Large-scale synthesis of commodity compounds such as surfactants or processes for the synthesis of biofuels will not covered by this review neither. The general topic of the biocatalytic modification of natural products was reviewed in 2001 (Riva, 2001), and we will not consider older references, excepted for clarity or comparison purposes. In vitro studies of enzymatic reactions involving natural substrates in the elucidation of biosynthetic routes will be excluded from the scope of this review.

2. Terpenoids and related compounds

Terpenoids are an amazing class of natural products found both in the marine and terrestrial realms, with a wide molecular diversity ranging from simple linear non-functionalised to complex polycyclic chiral molecules (Sell, 2003). The wide spectrum of their biological activities has also contributed to their popularity amongst chemists and biologists with compounds with functions of odorants, pheromones, vitamins, and hormones, or properties such as anti-oxidant, anti-inflammatory, anti-bacterial, anti-fungal, anti-tumoral, for example. The high efficiency of terpene cyclases, the enzymes responsible for a significant part of this diversity, has been a source of inspiration for organic chemists for a long while (Wendt et al., 2000) for their regio- and stereoselectivity, and biomimetic catalytic cyclisation reactions of simple polyenes have been proposed only recently (Godeau et al., 2011).

A wide array of terpenoids bearing an alcohol functional group has been used in many instances as substrates in transesterification reactions catalysed by lipases. Lipases are belonging to the class of hydrolases and are known to accommodate a great deal of substrates, particularly when an alcohol is used as nucleophile instead of water, typically in an organic solvent, resulting in a transesterification instead of an hydrolysis (Bornscheuer and Kazlauskas, 2004). They have been the most studied enzymes for applications in organic synthesis to date, and one of their most useful assets is undoubtedly their use in kinetic resolution of racemates, achieved through the enantioselective acylation reactions of alcohols (Ghanem and Aboul-Enein, 2004). In specific applications like fragrance chemistry, the production of enantiomerically enriched material is sometimes required, enantiomers having sometimes different olfactory properties (Abate et al., 2004b). For example, the resolution of racemic lavandulol has been reported by using CaLB and acetic acid as acyl donor to yield at ca. 55% conversion (*S*)-lavandulol in 42% yield and 52% *ee* and (*R*)-lavandulyl acetate in 51% yield and 48% *ee* (Cross et al., 2004). Many other examples of enzymatic resolution for the access to chiral synthetic fragrance materials have been reported with lipases (Abate et al., 2004a).

A parallel protocol has been described to evaluate the substrate scope of hydrolases and notably lipases with various terpenyl alcohols and acyl donors, in a combinatorial fashion (Antoniotti et al., 2008). The reactions were performed in toluene in the presence of 1% (w/w) hydrolases such as CaLB, CrL, PLE, etc. and 1–3 equiv. of vinyl esters as acyl donors. The success of the reaction was greatly influenced by the class of the nucleophilic alcohol used, primary alcohols reacting much better. Tertiary alcohols react indeed much slowly, and efficient enzymatic reactions are preferably based on enzymes isolated from mutant or rationally designed, in particular for resolution purpose (Barsch et al., 2008).

The prenyl side chain is a common feature of terpenes and is sometimes referred to as a hemiterpene (C5) derivative. Prenyl (dimethylallyl) transferases isolated from plants, bacteria or fungi catalyse the transfer of a prenyl moiety from a donor like dimethylallyldiphosphate DMAPP to an acceptor like tryptophan, in-

doles or structurally related compounds (Li, 2009). Several prenyl transferases from fungal strains of *Aspergillus fumigatus* and *Neosartorya fischeri* were cloned and overexpressed as His-tagged proteins from *Escherichia coli* and *Saccharomyces cerevisiae*. They showed broad substrate specificities and valuable regioselectivity in the transfer of prenyl units onto indole derivatives. The prenyl side chain could be transferred either at N1, C2, C3, C4, or C7 on the indole ring, depending on the enzyme used, making this methodology a mild and efficient alternative to Friedel–Crafts type prenyl transfer requiring strong Lewis acid activation, and resulting sometimes in undesired side-reactions. In the synthesis of aszonalenin, a ring closure occurred during the prenylation of the benzodiazepinedione intermediate, and 4 stereoisomers could be obtained, depending on the prenyl transferase used and the absolute configuration of the substrate.

The biomimetic two-phase strategy consisting in a cyclase phase followed by an oxidase phase have been efficiently applied to the total synthesis of complex bioactive terpenes (Ishihara and Baran, 2010). The desired oxidase phase could envisaged by biocatalytic methods, and the topic of the bio-oxidation of terpenes including both biocatalysis and whole cells biotransformations has been reviewed in 2009 (Bicas et al., 2009).

For example, the oxidation of *R*-(+)-limonene by a chloroperoxidase (CPO) from the fungal strain *Caldariomyces fumago* has been reported (Aguila et al., 2008). The reaction was shown to be both regio- and stereoselective with (1*S*,2*S*)-4*R*-limonene-1,2-diol formed with a diastereomeric excess of 99%, however dependant on the absence of chloride ions. The reaction was proposed to proceed through the enzymatic diastereoselective epoxidation of the internal double bond, followed by a S_N2-type hydrolysis to the vicinal diol.

Steroids, derived from triterpenes, are a fascinating class of compounds with well known biological properties. Many enzymes are involved in their biosynthesis to allow such structural diversity, and steroids on the market could be either natural, synthetic, or semi-synthetic. In the nineties, many examples of steroids hydroxyl groups modification by enzymes have been reported, with oxidation and epimerisation by hydroxysteroid dehydrogenases, and regioselective acylation by subtilisin Carlsberg and CaLB (Riva, 2001).

Monoacylated derivatives of 2,3- and 3,4-vicinal diols of steroids could be prepared by regioselective lipase-catalysed acylation reactions (Cruz Silva et al., 2005). Lipases from various source were assayed and afforded selective reactions with their ability to discriminate vicinal hydroxyl groups located on the A-ring of the steroid, without requiring activating/protecting groups.

Besides acylation reactions, galactosylation reactions have also been studied with ginsenoside Rg1, isolated from *Panax ginseng* C.A. Meyer and belonging to the protopanaxatriol family, galactosylated by β-(1,4)-galactosyltransferase (GalT) from bovine colostrum and using UDP-galactose as a sugar donor yielding a mixture of mono- and digalactosylated derivatives (Danieli et al., 2000).

The Baeyer–Villiger oxidation of 3 and 17-ketosteroids by a recombinant cyclopentadecanone monooxygenase from *Pseudomonas* sp. was reported to yield the corresponding lactones with a full control of the regiochemistry, albeit with moderate yields (Beneventi et al., 2009). NADPH was used as cofactor and recycled in situ by D-glucose-6P oxidation to D-gluconate-6P by glucose 6-phosphate deshydrogenase.

3. Flavour precursors

Natural flavours are a class of food ingredients extracted from foodstuffs or from plants by physical methods or produced by biocatalytic reactions or fermentation of natural precursors (Serra et al., 2005). Biocatalytic routes also provide greener industrial processes and secure a high level of purity for compounds used in food and edible products, compared to metal-based processes, where trace amounts of metal could remain in the final ingredient. Industrial processes based on fermentation and whole cells for the production of natural flavours such as vanillin, γ-decalactone, C6 aldehydes, esters, and some alcohols (2-phenylethanol, (*Z*)-hex-3-en-1-ol) have been reviewed in 2004 (Schrader et al., 2004) and a book chapter published in 2010 covers the broad biotechnological production of flavours either by micro-organisms, plant catalysts or isolated enzymes, including genetically engineered biocatalysts (Berger et al., 2010).

β-Carotene is a water-insoluble natural hydrocarbon which can be produced from algae and cyanobacteria (Johannisbauer et al., 2006), although most of β-carotene used in dietary supplements is of synthetic origin. In vivo, β-carotene oxidative cleavage yields small volatile molecules with pleasant odours and tastes (Kaiser, 2002; Ravichandran, 2002; Silva et al., 2008). Dioxygenases such as lipoxygenase (LOX) or monooxygenases such as xanthine oxidase (XO) have been used to generate reactive oxygen species involved in the subsequent oxidation of β-carotene to β-ionone, a precious fragrance ingredient with woody, fruity, berry-like odor and taste (Waché et al., 2006). Enzymatically generated radicals being inactive in organic solvents, β-carotene had to be dispersed in an aqueous medium containing surfactants such as Span (20, 40, 80) or Tween (40, 80, 85) to form reverse micelles. Surprisingly, a microbial peroxidase able to oxidatively cleave β-carotene was identified from *Lepista irina* (Zorn et al., 2003).

Biocatalytic oxidative C–C double bond cleavage is a very interesting alternative to ozonolysis. Another example was given with vegetal oils, converted into valuable C6- and C9-aldehydes by the combined action of soybean lipoxygenase (LOX) and recombinant hydroperoxide lyase (HPL) (Noordermeer et al., 2002). Natural hexanal, (*E*)-hex-2-enal, and (*Z*)-hex-3-enal, commonly used for their green notes, could be obtained by this method from hydrolysed safflower oil (73% linoleic acid) and hydrolysed linseed oil (44% linolenic acid/20% linoleic acid).

(+)-Nootkatone is a naturally occurring sesquiterpene possessing an appreciated grapefruit-like flavour with a low odour threshold (1 μg/L in water) for which many synthetic routes including biocatalytic processes have been devised and reviewed in 2009 (Fraatz et al., 2009). (+)-Valencene is a natural and inexpensive sesquiterpene isolated from Valencia oranges (*Citrus sinensis*) essential oil which possesses the bicyclic skeleton and the requisite absolute stereochemistry to serve as starting material for (+)-nootkatone synthesis. Whole cells processes have been the preferred systems to achieve this synthesis, due to the need for 2 oxidation steps, namely an allylic oxidation typical of monooxygenase activity, and the oxidation of a secondary alcohol to a carbonyl group, typical of a deshydrogenase activity, requiring the presence of co-factors (Furusawa et al., 2005). However, solutions with isolated enzymes such as microsomial enzymes (de Kraker et al., 2003), lipoxygenases (Muller et al., 1997), or CYP450 have been reported (Sowden et al., 2005).

It is worth mentioning that such selectivity between the two allylic positions would have been hardly obtained by conventional methods such as Sharpless oxidation with SeO_2.

4. Phenolics

Phenolics are an ubiquitous and wide class of natural products with a large structural diversity, their biosynthesis being derived from the phenyl propanoid pathway or from malonyl CoA in polyketide-like pathways (Genta-Jouve et al., 2011). Being both

substrates and products of many metabolic pathways, they have been used in several synthesis involving enzymatic reactions.

Flavonoids are a well-known subclass of phenolic compounds exhibiting a biological activity mainly based on their anti-oxidant properties. Bergenin is a trihydroxybenzoic acid glycoside derived from 4-methoxy gallic acid which presents anti-arthritic effect in addition to the anti-oxidant character (Nazir et al., 2007). A two-step enzymatic modification of solid-supported bergenin followed by cleavage from the support by the action of a lipase has been reported (Akbar et al., 2009). A first step of bromination by a chloroperoxidase in the presence of NaBr was followed by an acylation step with vinyl butyrate in the presence of subtilisine Carlsberg. The modified bergenin was then released from the controlled pore glass by lipase-catalysed cleavage of the ester bond connecting the product with the support.

Phenolics are substrates of peroxidases (EC number 1.11.1.x), a class of enzymes that uses a hydroperoxide, in most instances H_2O_2, and an organic substrate as electron donor, to achieve an electron transfer (Poulos, 2010). When the electron donor is a phenol or a phenolic derivative, phenoxyl radicals are formed and dimerisation occurs in the solution phase. If the reaction is carried out in water, the dimmers, trimmers, or oligomers rapidly reach their saturation concentration and precipitate out. By adding small volumes of an organic solvent miscible with water (MeOH, EtOH, DMF, etc.) or surfactants, it is possible to maintain the oligomer in the solution phase and to grow up to a thousand kDa molecular weight, in a biomimetic fashion resembling lignin biosynthesis.

Horseradish peroxidase (HRP) and soybean peroxidase (SBP) have been used in this regard for the oligomerisation of natural substituted *ortho*-methoxyphenols (Antoniotti et al., 2004). Starting from natural product apocynin, the oligomers obtained upon *ortho* C–C couplings were characterised, and shown to inhibit the migration of breast cancer cells at the micromolar level as a result of intracellular signalling break down (Klees et al., 2006).

Enzymatic polymerisation of phenols appeared as a safe alternative to the phenol–formaldehyde process which was questionable in terms of both health and environment impact, and the topic of the polymerisation of phenolics by peroxidases from the polymerist viewpoint has been reviewed in 2006 (Reihmann and Ritter, 2006). The general topic of biocatalysis in polymer chemistry has been addressed in a book published in 2010 (Loos, 2010). Many studies have been directed towards the control of the final size and dispersity of the polymer. The HRP-catalysed oxidative polymerization of phenol in the presence of PEG-derived triblock copolymers has been reported to yield ultrahigh molecular weights (Kim et al., 2008). The formation of a micellar aggregate of the phenol growing polymer and the PEG derivative through hydrogen bonding served as template and maintained the polymer in the aqueous phase which allow to reach a molecular weight up to 10^6 Da. Polyphenol-graft-polyethylene glycol copolymer could also be obtained from HRP-catalysed phenol polymerisation combined with living anionic polymerisation of ethylene oxide, yielding polymers of 2000–8000 Da (Cui et al., 2010). The resulting copolymer showed improved solubility in solvents such as water, ethanol, and dichloromethane.

Since the critical issue of enzymatic polymerisation of phenolics is solubility, the SBP-catalysed polymerisation of phenols in ionic liquids (RTILs)/water was studied (Eker et al., 2009). Commercially available RTILs such as 1-butyl-3-methylimidazolium tetrafluoroborate, [BMIM][BF_4], and 1-butyl-4-methylpyridinium tetrafluoroborate, [BMPy][BF_4], were used in 50% (v/v) mixture with water. The molecular weight of the polymer obtained with *para*-cresol as substrate was increased of ca. 80% (up to 1300 Da), compared to the polymer obtained without added RTILs. In 90% (v/v) [BMPy][BF_4]/H_2O, a DMF-insoluble polymeric material was obtained, and its molecular weight determined by MALDI-TOF mass spectrometry analysis as being of 1750 Da.

Some attempts to confer biological properties to a polymeric material by an appropriate choice of the phenolic monomers used were done. In that regard, the antibiotic and antioxidant properties of anacardic acid attracted attention. Anacardic acid is the trivial name which was given to the major constituent of cashew nut (*Anacardium occidentale*) shell extract, and which remained even after it was discovered that it was actually a mixture of salicylic acid derivatives, bearing a 15 carbon atoms chain at carbon atom 6, featuring 1, 2 or 3 double bonds.

This natural compound appeared as an attractive monomer for the synthesis of coatings for antifouling applications. The SBP-catalysed synthesis of polyanacardic acid in water/propan-2-ol or water/MeOH was thus reported to yield polymers of 3900 and 5000 Da molecular weight, respectively (Chelikani et al., 2009). FT-IR and ^{1}H NMR analysis suggested the formation of a cross-linked polymer with C–C and C–O couplings, involving the reaction of stabilised phenoxyl radicals. Some decarboxylation also occurred, but the unsaturated side chain remained unchanged. The antibiofouling properties were assayed by SEM time-course analysis of biofilms formation of *Leuconostoc mesenteroides* (Gram-positive) and *Pseudomonas aeruginosa* (Gram-negative) onto coated and non-coated polypropylene slides, showing that polyanacardic acid coating was effective against both bacteria.

Nanocomposites based on polymerised phenol and cellulose have been prepared by SBP-catalysed polymerisation coupled with TEMPO-oxidation of cellulose (Li et al., 2010). SEM imaging of fracture surface indicated that the phenol polymer consisted of a spherical cluster of ca. 0.1 μm diameter. Physical and chemical interactions between the fibre and the matrix were characterised by FT-IR spectroscopy, and the material exhibited enhanced thermal stability, compared to its components taken separately.

5. Vegetal materials and enzyme-assisted extraction

Plants constitute an important renewable source of small molecules easily harvested by extraction from roots, barks, stems, leaves, or flowers. Even if natural extracts such as essential oils, concretes or absolutes are typically mixtures of dozen of compounds, a fair number of extracts are rich in one compound in particular, making them an attractive source of building blocks for fine chemical synthesis. For example, the essential oil of *Mentha pulegium* contains up to 83% of (+)-pulegone (Agnihotri et al., 2005), (+)-limonene is industrially produced from citrus fruit (Martin-Luengo et al., 2011), and 10-deacetylbaccatin could be obtained in large quantities from *Taxus baccata* needles and serve as an advanced starting material for paclitaxel and docetaxel synthesis, both compounds being the APIs of anti-cancer drugs on the market (Gueritte-Voegelein et al., 1986).

In general, the yields of extraction from vegetal sources are low, typically below 5% (w/w), and enzymatic strategies to assist the extraction process have been developed. One strategy is to weaken the cells to facilitate their disruption and therefore increase the release of metabolites in the extracting phase. Such coarse strategy involves the use of cellulases. Another strategy concerns the cleavage of metabolites existing as conjugates with sugars and is based on the use of glucosidases. When the aglycon is an active compound (carotenoids, capsaicinoids, flavonoids, pro-vitamin A, etc.), the enzyme-assisted extraction results in improved chemical compositions of the extracts in terms of concentration of active compounds.

With the purpose of enhancing the yield of steam distillation of spices, compared to the yield of hydrodistillation, the effect of hydrolytic enzymes on the extraction of the volatile oil of cumin (*Cuminum cyminum* L.) has been reported (Sowbhagya et al., 2011). The oil yield could be improved in many instances when

pre-treatment of cumin seeds with cellulase, pectinase, protease and Viscozyme, was performed. The yield improvement was of ca. 20%, while no significant change in the chemical composition was observed, with the content of cuminaldehyde, the main flavouring constituent of the extract, remaining similar.

The enzyme-assisted selective extraction of capsaicinoids and carotenoids from chili guajillo "puya" (*Capsicum annuum* L.) flour has been studied (Santamaria et al., 2000). When the flour was pretreated with enzyme preprations exhibiting pectolytic, cellulolytic, and carbohydrase activities, extraction yield increases were observed for carotenoids and capsaicinoids. Sequential extraction after enzymatic treatment afforded up to 60% of the initial capsaicinoids content and 83% of carotenoids content present in the flour. The method was improved with the use of enzymatic extracts from *Rhizopus nigricans* which exhibited high cellulase and pectinase activities and allowed the recovery of 85% of carotenoids content and 96% of capsaicinoids content (Salgado-Roman et al., 2008).

The effects of various enzymes on the extraction of volatile oil of celery (*Apium graveolens* L.) has been reported (Sowbhagya et al., 2010). The oil yields of steam distillation could be improved by ca. 25% when cellulase, pectinase, protease or viscozyme pretreatment was performed. Physicochemical and sensory properties of the essential oils obtained were not modified. Similarly, the use of cellulase, pectinase, protease and viscozyme pretreatment resulted in improved yields of steam distillation as well as hydrodistillation (Sowbhagya et al., 2009). The oil yield of steam distillation was increased of 40–80%, of 45–84% for hydrodistillation. The best improvements were observed when pectinases and proteases were used, and the enzymatic pre-treatment had virtually no impact on the olfactory quality of the product.

Pectinase 690 L (endogalacturonase, cellulase from *Aspergillus* sp. and *Trichoderma* sp.), pectinase 62 L (endogalacturonase from *Aspergillus* sp.), and cellulase C013P (from *Trichoderma* sp.) has been reported to be useful for the release of flavonoids and oligosaccharides from bergamot (*Citrus bergamia* Risso) peel (Mandalari et al., 2006). Up to 80–90% of the flavonoids were recovered after deglycosylation, thanks to some β-glucosidase and α-rhamnosidase activities exhibited by the enzyme preparations. This process allowed recovering valuable compounds such as flavonoids having healthcare applications from bergamot peel, a by-product of the essential oil industry.

When used in aqueous environment during the extraction process, enzymatic cellulolytic pre-treatment is expected to assisted cell walls destruction and to catalyse the hydrolysis of glycosylated conjugates. When extracts are obtained by cold pressing, the preferred technique for certain types of vegetal materials, enzymatic pre-treatment could also be used to improve yields. It was reported that enzyme mixtures with pectinase, cellulase and hemicellulase activities could be used prior to the cold-pressing of rose-hips to allow more than 50% yield improvements (Concha et al., 2004). Although rose-hip oil contains significant amounts of *E*-retinoic acid (pro-vitamin A), the effect of enzymatic treatment on the quantitative composition was not described. Similar results, e.g. yield improvement without modification of the properties, have been reported for lemongrass and lemon eucalyptus (Dudai et al., 2001), and mustard seeds (Szakács-Dobozi et al., 1988).

A β-glucosidase-assisted hydrolysis approach was applied to *Osmanthus fragrans* Lour. flowers extraction (Yao et al., 2010). Concrete and refined absolute could be produced with increased yields of 147% and 178%, respectively, as compared with the procedure without β-glucosidase hydrolysis. Detailed chemical analyses showed a significant increase of the amount of high-impact aroma components such as 6,10,14-trimethyl-2-pentadecan-2-one, nonanal, dihydro-β-ionol and (*E*)-β-ionone, resulting in an overall stronger fragrance.

Similarly, the use of β-glucosidases was applied to the vapour extraction of rose essential oil, one of the most precious natural material used in fine perfumery (Zhiping et al., 2006). Interestingly, a particular emphasis was put on the chemical composition of the headspace, analysed by GC/MS. It was shown that the chemical composition was qualitatively and quantitatively modified, with 13 compounds varying in their concentration, and with the apparition of 20 new compounds, terpenoids and low molecular weight alcohols. Sensory evaluation by a panel of 12 persons was performed to show that a difference between the hydrolysed sample and the control could be detected.

A study of the essential oil composition of various parts of *Psoralea bituminosa* has been reported (Bertoli et al., 2004). It was shown that when aqueous residues of hydrodistillation were treated by β-glucosidases and hydrodistilled again, significant amounts of compounds such as 3-(*E*)-hexen-1-ol, α-pinene, 1-octen-3-ol, β-myrcene, caryophyllene, β-farnesene, and germacrene D could be extracted. The cases of 3-(*E*)-hexen-1-ol and 1-octen-3-ol were remarkable since none of these compounds were detected in the leaves extracts, while they accounted for 36.7% and 27.1%, respectively in the hydrolysed leaves extracts, which undoubtedly confirmed that these compounds are glycosylated in the plant.

Terpenoids (farnesol and nerolidol) eugenol, isoeugenol, and aliphatic alcohols from dried clove buds and fresh green clove leaves of *Syzygium aromaticum* (L.) merr. et perry (Myrtaceae) could also be recovered using both β-glucosidase and α-amyloglucosidase hydrolysis (Menon and Narayanan, 1992). Similar results were described for essential oils from different parts of hyssop (*Hyssopus officinalis* L.) (Schulz and Stahl-Biskup, 1991).

6. Natural extracts enzymatic post-treatment

Natural extracts are typically mixtures of organic compounds with close physico-chemical properties but sometimes very divergent biological properties. Rose essential oil for example contains delicate odorant molecules such as β-damascone, β-damascenone and rose oxide, rather inert hydrocarbons such as eicosene, and carcinogen methyleugenol. The final properties of the natural extract, either desired (a pleasant odour), or deleterious (toxicity) are determined by the concentration of active compounds, and could therefore be modified by post-treatment, and in particular by enzymatic treatment.

The recent years have witnessed a significant evolution of the manufacturing practices and the regulations of the western countries in regards of the use of natural extracts in the cosmetic and perfume industry. In addition to the regulation REACH (Registration, Evaluation, Authorisation and Restriction of Chemical substances), an important number of chemical compounds found in natural extracts and with suspected or demonstrated toxicity such as putative carcinogens like methyleugenol (Johnson and Abdo, 2005), safrole (Tsujimura et al., 2006), skin sensitizers such as atranol derivatives, contained by tree moss extracts (Johansen et al., 2006), or hepatotoxic suspected agents such as estragole (Smith et al., 2002) are also under close surveillance and their concentration in final products should not reach certain levels.

The removal of allergenic eugenol from rose essential oils by the use of a combination of peroxidase and catalase has been studied (Bouhlel et al., unpublished results). The purpose of the work was to set up a protocol for the selective removal of the target compound without altering the overall appreciated quality of the mixture. The chemistry used herein was based on the HRP-mediated oligomerisation of eugenol to unsoluble oligomeric species, allowing their removal by simple filtration.

To avoid the side-reactions involving H_2O_2 and unsaturated terpenes, a sequential procedure was developed, including the

action of a second enzyme, catalase, after HRP activation by H_2O_2 and before the introduction of essential oil in the reaction medium to oxidise residual H_2O_2. GC/MS analysis of the modified essential oil showed no remaining eugenol, and only slight modifications of the concentration in citronellol. Sensory evaluation by triangular testing was performed and showed that the modified essential oil was not significantly different from the non modified essential oil from a sensory viewpoint. This result supported the relevance of using enzymes for the selective removal of toxic compounds from sensitive natural extracts.

7. Conclusion

The interests of organic chemists, either in academia or industrial settings, for biocatalytic processes is increasing everyday, for example with the recent upgrade of the manufacturing process of sitagliptin, with a greener and chemically more efficient process, in terms of yield and enantioselectivity (Desai, 2011). Natural organic compounds grant access to natural grade chemicals when submitted to biocatalytic processes, with still unmet goals like the synthesis of natural frambinone, an important flavouring ingredient. Natural products can provide complex core structures for hemisynthesis, and in most instances, they provide sustainable processes when coupled with biocatalysis.

The feasibility of a given reaction in a biocatalytic version is also increasing by the ability to tailor enzymes function by genetic engineering and/or medium engineering, and by enzyme promiscuity, the use of an enzyme to catalyse alternate reactions (Bornscheuer and Kazlauskas, 2004).

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References

Abate, A., Brenna, E., Fuganti, C., Gatti, F.G., Serra, S., 2004a. Odor and (bio)diversity: single enantiomers of chiral fragrant substances. Chem. Biodivers. 1, 1888–1898.

Abate, A., Brenna, E., Fuganti, C., Gatti, F.G., Serra, S., 2004b. Lipase-catalysed preparation of enantiomerically enriched odorants. J. Mol. Catal. B: Enzym. 32, 33–51.

Agnihotri, V.K., Agarwal, S.G., Dhar, P.L., Thappa, R.K., Baleshwar, Kapahi, B.K., Saxena, R.K., Qazi, G.N., 2005. Essential oil composition of *Mentha pulegium* L. growing wild in the north-western Himalayas India. Flavour Frag. J. 20, 607–610.

Aguila, S., Vazquez-Duhalt, R., Tinoco, R., Rivera, M., Pecchi, G., Alderete, J.B., 2008. Stereoselective oxidation of *R*-(+)-limonene by chloroperoxidase from *Caldariomyces fumago*. Green Chem. 10, 647–653.

Akbar, U., Shin, D.-S., Schneider, E., Dordick, J.S., Clark, D.S., 2009. Two-step enzymatic modification of solid-supported bergenin in aqueous and organic media. Tetrahedron Lett. 51, 1220–1225.

Antoniotti, S., Santhanam, L., Ahuja, D., Hogg Michael, G., Dordick, J.S., 2004. Structural diversity of peroxidase-catalyzed oxidation products of o-methoxyphenols. Org. Lett. 6, 1975–1978.

Antoniotti, S., Fernandez, X., Duñach, E., 2008. Reaction design for evaluation of the substrate range of hydrolases. Biocatal. Biotransform. 26, 228–234.

Barsch, S., Kourist, R., Bornscheuer, U.T., 2008. Complete inversion of enantioselectivity towards acetylated tertiary alcohols by a double mutant of a *Bacillus subtilis* esterase. Angew. Chem., Int. Ed. 47, 1508–1511.

Beneventi, E., Ottolina, G., Carrea, G., Panzeri, W., Fronza, G., Lau, P.C.K., 2009. Enzymatic Baeyer–Villiger oxidation of steroids with cyclopentadecanone monooxygenase. J. Mol. Catal. B: Enzym. 58, 164–168.

Berger, R.G., Krings, U., Zorn, H., 2010. Biotechnological flavour generation. In: Taylor, A.J.L., Robert, S.T. (Eds.), Food Flavour Technology. Wiley-Blackwell, Chichester, pp. 89–126.

Bertoli, A., Menichini, F., Noccioli, C., Morelli, I., Pistelli, L., 2004. Volatile constituents of different organs of *Psoralea bituminosa* L.. Flavour Frag. J. 19, 166–171.

Bicas, J.L., Dionísio, A.P., Pastore, G.M., 2009. Bio-oxidation of terpenes: an approach for the flavor industry. Chem. Rev. 109, 4518–4531.

Bornscheuer, U.T., Kazlauskas, R.J., 2004. Catalytic promiscuity in biocatalysis: using old enzymes to form new bonds and follow new pathways. Angew. Chem., Int. Ed. 43, 6032–6040.

Bouhlel, C., Dolhem, G.A., Antoniotti, S., unpublished results.

Carrea, G., Riva, S., 2000. Properties and synthetic applications of enzymes in organic solvents. Angew. Chem., Int. Ed. 39, 2226–2254.

Chelikani, R., Kim, Y., Yoon, D.-Y., Kim, D.-S., 2009. Enzymatic polymerization of natural anacardic acid and antibiofouling effects of polyanacardic acid coatings. Appl. Biochem. Biotechnol. 157, 263–277.

Concha, J., Soto, C., Chamy, R., Zúñiga, M., 2004. Enzymatic pretreatment on rose-hip oil extraction: hydrolysis and pressing conditions. J. Am. Oil Chem. Soc. 81, 549–552.

Cross, H., Marriott, R., Grogan, G., 2004. Enzymatic esterification of lavandulol – a partial kinetic resolution of (*S*)-lavandulol and preparation of optically enriched (*R*)-lavandulyl acetate. Biotechnol. Lett. 26, 457–460.

Cruz Silva, M.M., Riva, S., Sá e Melo, M.L., 2005. Regioselective enzymatic acylation of vicinal diols of steroids. Tetrahedron 61, 3065–3073.

Cui, Y., Hu, A., Lu, Z., Zhang, L., Luo, X., Du, K., 2010. Synthesis of water-soluble polyphenol-graft-poly(ethylene oxide) copolymers via enzymatic polymerization and anionic polymerization. Polym. Int. 59, 676–679.

Danieli, B., Falcone, L., Monti, D., Riva, S., Gebhardt, S., Schubert-Zsilavecz, M., 2000. Regioselective enzymatic glycosylation of natural polyhydroxylated compounds: galactosylation and glucosylation of protopanaxatriol ginsenosides. J. Org. Chem. 66, 262–269.

de Kraker, J.-W., Schurink, M., Franssen, M.C.R., König, W.A., de Groot, A., Bouwmeester, H.J., 2003. Hydroxylation of sesquiterpenes by enzymes from chicory (*Cichorium intybus* L.) roots. Tetrahedron 59, 409–418.

Desai, A.A., 2011. Sitagliptin manufacture: a compelling tale of green chemistry, process intensification, and industrial asymmetric catalysis. Angew. Chem., Int. Ed. 50, 1974–1976.

Dudai, N., Weinberg, Z.G., Larkov, O., Ravid, U., Ashbell, G., Putievsky, E., 2001. Changes in essential oil during enzyme-assisted ensiling of lemongrass (*Cymbopogon citratus* Stapf.) and lemon eucalyptus (*Eucalyptus citriodora* Hook). J. Agric. Food Chem. 49, 2262–2266.

Eker, B., Zagorevski, D., Zhu, G., Linhardt, R.J., Dordick, J.S., 2009. Enzymatic polymerization of phenols in room-temperature ionic liquids. J. Mol. Catal. B: Enzym. 59, 177–184.

Faber, K., 2004. Biotransformations in Organic Chemistry, fifth ed. Springer-Verlag, Berlin.

Fraatz, M., Berger, R., Zorn, H., 2009. Nootkatone—a biotechnological challenge. Appl. Microbiol. Biotechnol. 83, 35–41.

Furusawa, M., Hashimoto, T., Noma, Y., Asakawa, Y., 2005. Highly efficient production of nootkatone, the grapefruit aroma from valencene, by biotransformation. Chem. Pharm. Bull. 53, 1513–1514.

Galaffu, N., Man, S.P., Wilkes, R.D., Wilson, J.R.H., 2007. Highly functionalised sulfur-based silica scavengers for the efficient removal of palladium species from active pharmaceutical ingredients. Org. Process Res. Dev. 11, 406–413.

Genta-Jouve, G., Antoniotti, S., Thomas., O.P., 2011. Polyketide assembly mimics and biomimetic access to aromatic rings. In: Poupon, E., Nay, B. (Eds.), Biomimetic Organic Synthesis. Wiley-VCH Verlag, Weinheim, p. 471.

Ghanem, A., Aboul-Enein, H.Y., 2004. Lipase-mediated chiral resolution of racemates in organic solvents. Tetrahedron: Asymmetry 15, 3331–3351.

Godeau, J., Olivero, S., Antoniotti, S., Duñach, E., 2011. Biomimetic cationic polyannulation reaction catalyzed by $Bi(OTf)_3$: cyclization of 1,6-dienes, 1,6,10-trienes, and aryl polyenes. Org. Lett. 13, 3320–3323.

Gueritte-Voegelein, F., Senilh, V., David, B., Guenard, D., Potier, P., 1986. Chemical studies of 10-deacetyl baccatin III. Hemisynthesis of taxol derivatives. Tetrahedron 42, 4451–4460.

Ishihara, Y., Baran, P.S., 2010. Two-phase terpene total synthesis: historical perspective and application to the taxol® problem. Synlett, 1733–1745.

Johannisbauer, W., Mahnke, E.U., Sander, A., Weiss, A., Pulz, O., Franke, H., 2006. Improved process for obtaining carotenoids from algae or cyanobacteria. EP1681060A1, Cognis Ip Management G.m.b.H., Germany.

Johansen, J.D., Bernard, G., Gimenez-Arnau, E., Lepoittevin, J.-P., Bruze, M., Andersen, K.E., 2006. Comparison of elicitation potential of chloroatranol and atranol – 2 allergens in oak moss absolute. Contact Dermatitis 54, 192–195.

Johnson, J.D., Abdo, K.M., 2005. Methyleugenol in the diet: toxic and pathological aspects. Rev. Food Nutr. Toxicol. 3, 1–60.

Kaiser, R., 2002. Carotenoid-derived aroma compounds in flower scents. ACS Symp. Ser. 802, 160–182.

Kim, Y.-J., Shibata, K., Uyama, H., Kobayashi, S., 2008. Synthesis of ultrahigh molecular weight phenolic polymers by enzymatic polymerization in the presence of amphiphilic triblock copolymer in water. Polymer 49, 4791–4795.

Klees, R.F., De Marco, P.C., Salasznyk, R.M., Ahuja, D., Hogg, M., Antoniotti, S., Kamath, L., Dordick, J.S., Plopper, G.E., 2006. Apocynin derivatives interrupt intracellular signaling resulting in decreased migration in breast cancer cells. J. Biomed. Biotechnol. 1–10.

Li, S.-M., 2009. Applications of dimethylallyltryptophan synthases and other indole prenyltransferases for structural modification of natural products. Appl. Microbiol. Biotechnol. 84, 631–639.

Li, Z., Renneckar, S., Barone, J., 2010. Nanocomposites prepared by in situ enzymatic polymerization of phenol with TEMPO-oxidized nanocellulose. Cellulose 17, 57–68.

Loos, K., 2010. Biocatalysis in Polymer Chemistry. Wiley-VCH, Weinhem.

Mandalari, G., Bennett, R.N., Kirby, A.R., Lo Curto, R.B., Bisignano, G., Waldron, K.W., Faulds, C.B., 2006. Enzymatic hydrolysis of flavonoids and pectic

oligosaccharides from bergamot (*Citrus bergamia* Risso) peel. J. Agric. Food Chem. 54, 8307–8313.
Martin-Luengo, M.A., Yates, M., Diaz, M., Rojo, E.S., Gil, L.G., 2011. Renewable fine chemicals from rice and citric subproducts: ecomaterials. Appl. Catal. B 106, 488–493.
Menon, A.N., Narayanan, C.S., 1992. Glycosidically bound volatiles of clove *Syzygium aromaticum* (L.) merr. et perry (Myrtaceae). Flavour Frag. J. 7, 155–157.
Muller, B., Dean, C., Schmidt, C., Kuhn, J.-C. 1997. Method for preparing nootkatone. WO 9722575 (A1), Firmenich & cie, CH.
Nazir, N., Koul, S., Qurishi, M.A., Taneja, S.C., Ahmad, S.F., Bani, S., Qazi, G.N., 2007. Immunomodulatory effect of bergenin and norbergenin against adjuvant-induced arthritis – a flow cytometric study. J. Ethnopharmacol. 112, 401–405.
Noordermeer, M.A., van der Goot, W., van Kooij, A.J., Veldsink, J.W., Veldink, G.A., Vliegenthart, J.F.G., 2002. Development of a biocatalytic process for the production of c6-aldehydes from vegetable oils by soybean lipoxygenase and recombinant hydroperoxide lyase. J. Agric. Food Chem. 50, 4270–4274.
Poulos, T.L., 2010. Thirty years of heme peroxidase structural biology. Arch. Biochem. Biophys. 500, 3–12.
Rauter, A.P., Vogel, P., Queneau, Y., Weignerová, L., Křen, V., 2010. Enzymatic processing of bioactive glycosides from natural sources. In: Carbohydrates in Sustainable Development II. Springer, Berlin/Heidelberg, pp. 121–146.
Ravichandran, R., 2002. Carotenoid composition, distribution and degradation to flavor volatiles during black tea manufacture and the effect of carotenoid supplementation on tea quality and aroma. Food Chem. 78, 23–28.
Reihmann, M., Ritter, H., 2006. Enzyme-catalyzed synthesis of polymers. In: Kobayashi, S., Ritter, H., Kaplan, D. (Eds.), Advances in Polymer Science. Springer, Berlin/Heidelberg, pp. 1–49.
Riva, S., 2001. Biocatalytic modification of natural products. Curr. Opin. Chem. Biol. 5, 106–111.
Salgado-Roman, M., Botello-Àlvarez, E., Rico-Martìnez, R., Jiménez-Islas, H., Cardenas-Manrìquez, M., Navarrete-Bolaños, J.L., 2008. Enzymatic treatment to improve extraction of capsaicinoids and carotenoids from chili (*Capsicum annuum*) fruits. J. Agric. Food Chem. 56, 10012–10018.
Santamaria, R.I., Reyes-Duarte, M.D., Barzana, E., Fernando, D., Gama, F.M., Mota, M., Lopez-Munguia, A., 2000. Selective enzyme-mediated extraction of capsaicinoids and carotenoids from chili guajillo puya (*Capsicum annuum* l.) using ethanol as solvent. J. Agric. Food Chem. 48, 3063–3067.
Schmid, R.D., Verger, R., 1998. Lipases: interfacial enzymes with attractive applications. Angew. Chem., Int. Ed. 37, 1608–1633.
Schrader, J., Etschmann, M.M.W., Sell, D., Hilmer, J.M., Rabenhorst, J., 2004. Applied biocatalysis for the synthesis of natural flavour compounds – current industrial processes and future prospects. Biotechnol. Lett. 26, 463–472.
Schulz, G., Stahl-Biskup, E., 1991. Essential oils and glycosidic bound volatiles from leaves, stems, flowers and roots of *Hyssopus officinalis* L. (lamiaceae). Flavour Frag. J. 6, 69–73.
Sell, C.S., 2003. A Fragrant Introduction to Terpenoid Chemistry. The Royal Society of Chemistry, Cambridge.
Serra, S., Fuganti, C., Brenna, E., 2005. Biocatalytic preparation of natural flavours and fragrances. Trends Biotechnol. 23, 193–198.
Silva, F.A.C., Monteiro, J., Oliveira, C., Guedes, d.P.P., 2008. Study of major aromatic compounds in port wines from carotenoid degradation. Food Chem. 110, 83–87.
Smith, R.L., Adams, T.B., Doull, J., Feron, V.J., Goodman, J.I., Marnett, L.J., Portoghese, P.S., Waddell, W.J., Wagner, B.M., Rogers, A.E., Caldwell, J., Sipes, I.G., 2002. Safety assessment of allylalkoxybenzene derivatives used as flavouring substances – methyl eugenol and estragole. Food Chem. Toxicol. 40, 851–870.
Sowbhagya, H.B., Purnima, K.T., Florence, S.P., Appu Rao, A.G., Srinivas, P., 2009. Evaluation of enzyme-assisted extraction on quality of garlic volatile oil. Food Chem. 113, 1234–1238.
Sowbhagya, H.B., Srinivas, P., Krishnamurthy, N., 2010. Effect of enzymes on extraction of volatiles from celery seeds. Food Chem. 120, 230–234.
Sowbhagya, H.B., Srinivas, P., Purnima, K.T., Krishnamurthy, N., 2011. Enzyme-assisted extraction of volatiles from cumin (*Cuminum cyminum* L.) seeds. Food Chem. 127, 1856–1861.
Sowden, R.J., Yasmin, S., Rees, N.H., Bell, S.G., Wong, L.-L., 2005. Biotransformation of the sesquiterpene (+)-valencene by cytochrome P450cam and P450BM-3. Org. Biomol. Chem. 3, 57–64.
Szakács-Dobozi, M., Halász, A., Kozma-Kovács, E., Szakács, G., 1988. Enhancement of mustard oil yield by cellulolytic pretreatment. Appl. Microbiol. Biotechnol. 29, 39–43.
Tsujimura, K., Asamoto, M., Suzuki, S., Hokaiwado, N., Ogawa, K., Shirai, T., 2006. Prediction of carcinogenic potential by a toxicogenomic approach using rat hepatoma cells. Cancer Sci. 97, 1002–1010.
Waché, Y., Bosser-De Ratuld, A., Belin, J.-M., 2006. Dispersion of b-carotene in processes of production of β-ionone by cooxidation using enzyme-generated reactive oxygen species. Process Biochem. 41, 2337–2341.
Wendt, K.U., Schulz, G.E., Corey, E.J., Liu, D.R., 2000. Enzyme mechanisms for polycyclic triterpene formation. Angew. Chem., Int. Ed. 39, 2812–2833.
Yao, W.-R., Zhang, Y.-Z., Chen, Y., Yang, Z.-P., 2010. Aroma enhancement and characterization of the absolute *Osmanthus fragrans* Lour.. J. Essent. Oil Res. 22, 97–102.
Zaks, A., Klibanov, A.M., 1985. Enzyme-catalyzed processes in organic-solvents. Proc. Natl. Acad. Sci. USA 82, 3192–3196.
Zhiping, Y., Weirong, Y., He, Q., 2006. Studies on vapor phase extraction of rose oil enhanced by β-glucosidase. Flavour Frag. J. 21, 776–782.
Zorn, H., Langhoff, S., Scheibner, M., Nimtz, M., Berger, R.G., 2003. A peroxidase from *Lepista irina* cleaves β-carotene to flavor compounds. Biol. Chem. 384, 1049–1056.