Minor components in natural flavors and fragrances

By Louis Peyron, Lautier Aromatiques, Paris, France
Président d'Honneur, Société Technique des Parfumeurs de France

Translated by Richard A Bonneau, Rhone Poulenc, Inc.

In most flavors and fragrances from natural plant or animal origins we find organic compounds in minor if not infinitesimal quantities whose sensory contribution to odor and flavor is fundamental. We are affected constantly by these minor components. As Mr. Jourdain says, they appear

- in our food and drink; this is the broad field of flavors
- in our odoriferous environment; these are perfumes, cosmetics, body odors, pollution, and certainly pheromones

Chemical communication messengers directly or indirectly affect much of the behavior of living beings: feeding, reproduction, defense. These messengers can occur naturally, be voluntarily created when we prepare our foods, or involuntarily created as artifacts when natural products are treated.

A natural flavor is never the effect of a single component, but is always influenced by a diversity of odoriferous components present in minimal concentrations. The quality and the intensity of the aroma is never totally determined by the component present in the highest proportion. Thus among the 150 currently identified types of aromas, 50 to 800 compounds have been identified in each of the 50 aromas investigated in depth.

Although more than 3,000 natural odoriferous products have been cataloged, we estimate the existence of several thousand others that have not yet been identified. Future developments of separative and analytic methods ought to make it possible to characterize them. We invite you to take a panoramic and schematic view of these minor components, which are also called traces, by examining the difficulties in studying them; the media in which they are found; the importance of their contribution; the different structural types to which they can be related; and their origins and formation.

Study of minor components in flavors and fragrances

Until the middle of our century, the isolation of natural components and the elucidation of their structure were only possible by chemical means that often required extensive studies using large quantities of raw materials. Progress was slow, and led only to the characterization of the principal components, for example those described in essential oils.

The spectacular developments of physical separative and analytical methods (chromatography and spectroscopic methods), which made it possible to work on traces, revolutionized the study of natural products and particularly that of flavors, whose components are often present as a few grams per metric ton (ppm). One particular method, the gas chromatography/mass spectroscopy couple, presently makes it possible to detect 10-12 molecules of a single compound in a mixture. These methods are varied and very costly due to the equipment infrastructure they require as well as the auxiliary items necessary to use them validly (competent personnel, data banks, and so forth).

Substances in which minor components are found are of three types: specifically odoriferous, specifically savory (flavor-affecting), or mixed (aromas that have a definite flavor).

Odoriferous media (fragrances) can originate from plants or animals. This is the field of odoriferous plants and of extracts prepared from certain of their organs at precise times of their development. Since certain animal organs have very powerful characteristic odors (pheromones, musk), we have sought to isolate the responsible components in order to use their

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particular properties in perfumery, agriculture, or other fields.

Flavor-affecting media. Although there are a large number of trace molecules among the bitter stimuli, the number is more limited among sweets and is negligible for salty or acidic stimuli.

Mixed media (flavors) have been schematically regrouped in nine classes. These include: fruity—terpene (citrus type) or nonterpene (berries); vegetables; spicy (aromatic, lachrymatory, burning type); beverages (fermented, nonfermented, and mixed); meats; fats; cooked (bouillon, vegetable, fruit); empyreumatical (smoked, fried, roasted, grilled); and smelly (cheese).

Importance of the organoleptic contribution

The importance (for better or worse) of the contribution of the minor components is often considerable. They can bring a personality, a character, a signature, a more natural note, an enormous power whose mastery is often delicate. But how should they be defined? By themselves or as a function of the media in which they play a preponderant role? And what parameters should be used? For example, a more systematic knowledge of the olfactory detection threshold or of the quantity of substance just necessary to be preceived by the human olfactory system ought to likewise make it possible to better situate the minor components among all the odoriferous components. Nevertheless, it is necessary to note that this concept of threshold is subject to interferences related both to the stimuli themselves and to the subjects that perceive them. Molecular detection thresholds (threshold/MW) would likewise be worthwhile. To simplify the matter we shall here consider as minor components those whose detection thresholds are less than or equal to 1 gram per metric ton (ppm). A few examples of odoriferous thresholds expressed in mg per metric ton (ppb) are shown in figure 1.

To evaluate the sensory contribution of a defined component with respect to the overall odor of a complex mixture, we have proposed the ratio between the concentration of this component in the medium and the concentration of its detection threshold. This ratio is designated the odor unit. Differences in odor units among the miscellaneous components can constitute an index of odoriferous efficiency. For example, in coffee the odoriferous contribution of methyl mercaptan is about 45,000 times higher than that of acetaldehyde at a comparable concentration. In case of taste-affecting compounds, a bitter or sweet standard is used and the bitter or sweet power of the substance to be tested is compared to it. This means that we are no longer seeking the limits of detectability but the equivalence of effect.

Structural types of minor components

Classifications

Given the large number of diversity of compounds having an odor and a flavor of great power, it was out of the question to list them all here; rather, it was decided to establish a structural chemical classification that made it possible to index them correctly. We shall discuss this classification and illustrate it by a few examples.

In 1977 Boelens pointed out a certain number of natural volatile components in the FEMA/GRAS lists. In 1978 Ohloff limited his investigation to heterocyclic compounds. These investigations are shown in Table I.

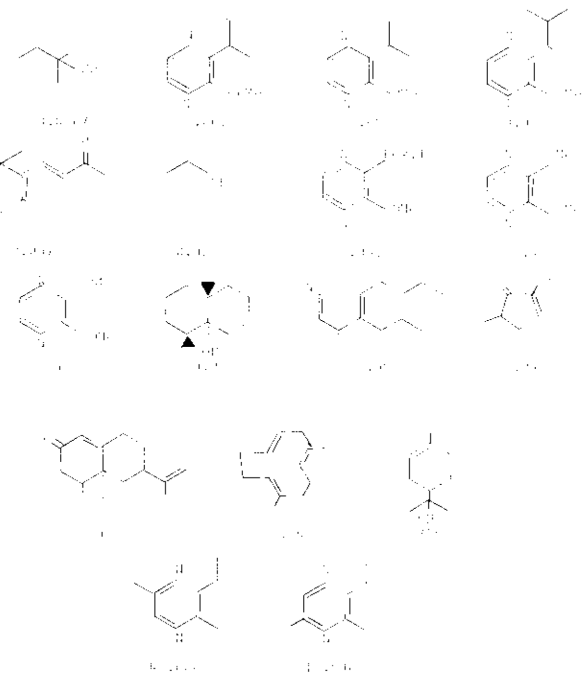


Figure 1

Table I

	Boelens (1977)		Ohloff (1978)	
Hydrocarbons	300			
Hydrocarbons with functional groups				
Oxygenated:	300	alcohols-phenols		
	100	ethers		
	460	carbonylated		
	110	acetals		
	370	acids		
	600	esters		
Sulfurated:	350		22	thiols
			23	monosulfides
			8	disulfides
			5	trisulfides
			1	tetrasulfides
			8	thiol esters
			2	isothiocyanates
Aliphatic nitrogen:	220			
Isoprenoids with functional groups				
monoterpenes				
sesquiterpenes				
polyterpenes				
Heterocyclic compounds				
	160	furans - pyrans	60	furans
	150	lactones	33	pyrazines
	200	pyrazines - imidazoles	13	pyridines
	150	pyridines - pyrroles	7	pyrroles
	90	oxazoles thiazoles	14	thiophenes - thiazoles

The lists of volatile compounds of foods established by TNO also constitute an up-to-date index of components mentioned in the most current natural aromas. The classification of minor components according to their olfactory notes is complex, since each component can exhibit one or several typical notes that each person does not always evaluate in the same way.

Correlations of structures and organoleptic properties

The search for correlations between odor types (or of bitter or sweet odors) and structural types is still a dream. The same note can be found related to miscellaneous structures, while apparently closely-related structures may have very different odors and powers. This is demonstrated in figure 2.

At present, it does not appear possible to define the specific contributions of the skeletons, with or without heteroatoms, with respect to the functional contributions. In summary, are there characteristic increments of skeletons and of functions?

Numerous authors have attempted to objectively relate certain physicochemical parameters of the molecule to its organoleptic qualities; they have worked with shape, chelating potential, functional groups (type, number, position), molecular weight, partition coefficient between water and lipids, or volatility, vapor pressure, to name some approaches.

The results are modest, but it appears that the apparent shape of the molecule alone is not a preponderant factor in the odor, while factors related to certain physicochemical properties that depend on the shape of the molecule influence the odor to a greater degree.

The enormous differences in sensitivity of our olfactory-gustatory systems with respect to certain organic components are difficult to explain. If we consider the stimulus alone, we can nevertheless assume the presence of a much greater number of odoriferous molecules at the level of the receptors than in the solid or liquid phase where they originate. This is the case of highly volatile molecules. We can also assume the existence of certain highly specific molecular receptors that are better adapted to very active bonds and have powerful responses.

At a higher level, a specific role of the sensory system of integration and interpretation with respect to certain groups of signals transmitted by the receptors cannot be excluded a priori. This could be the case with vital molecules such as the pheromones.

We can also ask the question: How do certain minor components behave when they are no longer used in low quantities but insignificant proportions? Think of the fatty aldehydes in a famous perfume.

We have indexed minor odoriferous, bitter, and sweet components separately according to their chemical systems. A few representative examples of minor components are listed below.

Minor odoriferous components

Hydrocarbons: 1,3,5-undecatriene (galbanum)
Hydrocarbons with functional groups:
Alcohols: 1-octen-3-ol (mushroom) (1 ppb) and cis-3-cis-6-nonadiene-1-ol (watermelon)
Aldehydes: trans-2-hexenal (leaf), cis-3-hexenal

(soybean), cis-4-heptenal (cream) (1 ppb), trans-4-heptenal (green), cis-6-nonenal (musk melon) (0.02 ppb), trans-6-nonenal (milk artifact) (0.7 ppb), trans-2-cis-6-nonadienal (violet leaves), and trans-2-trans-4-decadienal (0.07 ppb)

Ketones: (trans and cis) 5-octen-3-ones (bergamot) and 3,5-octadien-2-one (fruity) (300 ppb)

Acids: lower fatty acids

Thiols and derivatives: 3-methylthio-l-propanol (wine) and thiol methane (2 ppb)

Iso and thiocyanates: allyl isothiocyanate (mustard)

Amines, nitriles....

Isoprenoids with functional groups:

Terpenoids: hydrocarbons: myrcene, alcohols: ipodienol (pheromones), aldehydes: dolichodial (teucrium), ketones: beta ionone (0.007 ppb), and para mentha-8-thiol-3-one (black currant)

Sesquiterpenoids (Among 2000 representative compounds, more than 500 have a flavor of which 20 are very important.)

alpha farnesene (apple)

(−) caryophyllene (64 ppb) (1)

humulene (120 ppb) (2)

geosmine (0.02 ppb) (3)

alpha sinensal (0.05 ppb) (4)

(+) nootkatone

(−) nootkatone (5)

Biodegradation products of higher terpenoids with high molecular weights, odorless or tasteless, such as carotenoids, diterpenes and polyterpenoids—non-isoprenoids from carotenoids

theaspirone (6)

damascone and derivatives: beta-damascenone (oil rose Bulgarian, tobaccos, raspberry, buchu, cooked Brambley apple seeds, Roman chamomile, raisins and wines, tea, coffee, beer) (7), 3-hydroxydamascone (oil rose Bulgarian, tobaccos) (8), beta damascone (oil rose Bulgarian, Burley tobacco) (9), and alpha damascone (tea) (10)

Biodegradation of thumberganoids (macrycyclic diterpenoids) solanone and derivatives (tobaccos) (11)

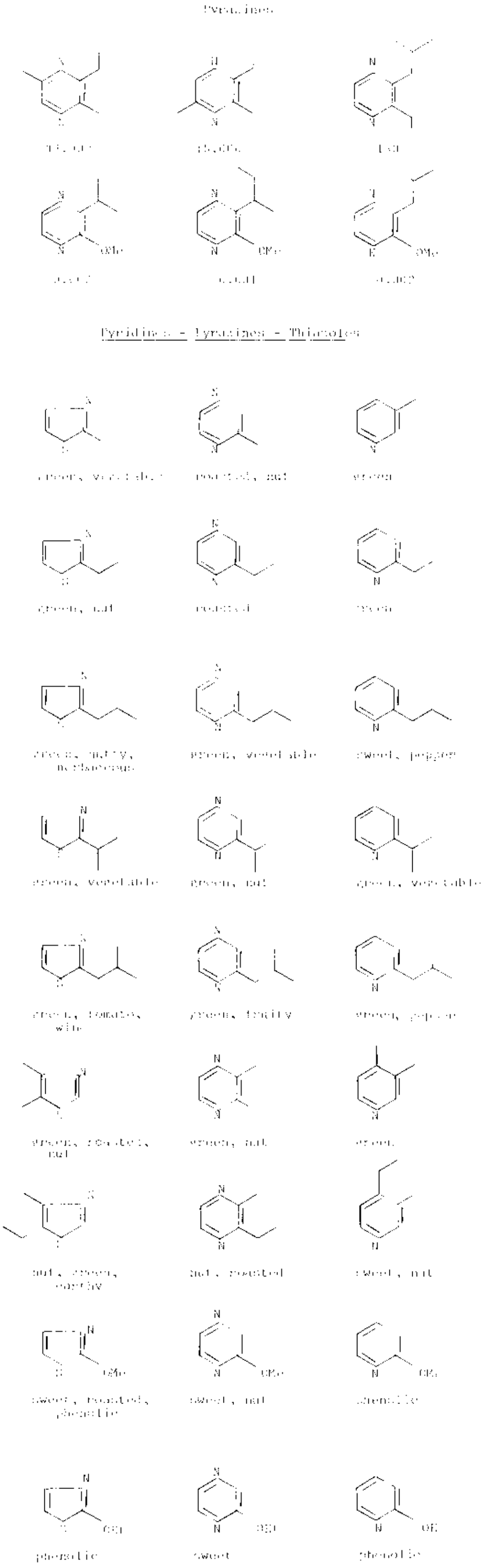
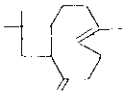


Figure 2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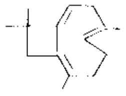
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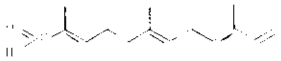
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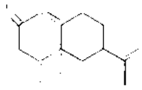
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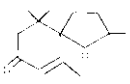
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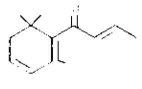
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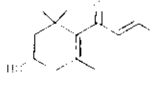
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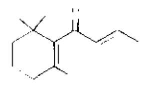
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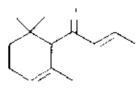

(10)


(11)

Oxygenated heterocyclic compounds

Furans: Di and tetrahydrofurans (12): rose oxide, perillene, furan rose, methofuran, and 2-furanones (or gamma lactones) (fig. 3), 4-hydroxy-2,5-dimethyl-3(2H)—furanone (burnt pineapple), 4-hydroxy-5-methyl-3(2H)-furanone (roasted chicory), and bis (2-methyl) furyl tetrasulfide (10 ppb) (braised beef)

Pyrans: Di and tetrahydropyrans (13): Maltol (14), 2-ethyl-3-hydroxy-6-methyl pyran-4-one (4H) (soybean sauce) (15), 2-pyranones (or delta lactones), and tuberolactone

Macrycyclic compounds: Ambrettolide (musk) (16)

Heterocyclic sulfides

Thiophenes and derivatives (17): numerous thiphene derivatives in coffee, 3-2(2-thienyl) propenal (meat), and 3,4-dimethyl-2(5H)-thiophenone (onion)

Thiopyrans and derivatives: tetrahydrothiopyrans and homologs (roasted peanut) and mint sulfide (Mentha piperita) (18)

Polysulfides: 1,2 (3H)—dithiole (cooked asparagus) (19), 1,2,4-trithiolane (cheese) (20), and lenthionine: 1,2,3,4,6-penta thiepane (mushroom) (21)

Mixed oxygenated and sulfurated compounds: 2-methyl-4-propyl-1,3-oxathiane (passion fruit) (22) and 6-methyl-2,3-dihydro thieno (2,3-c) (coffee)

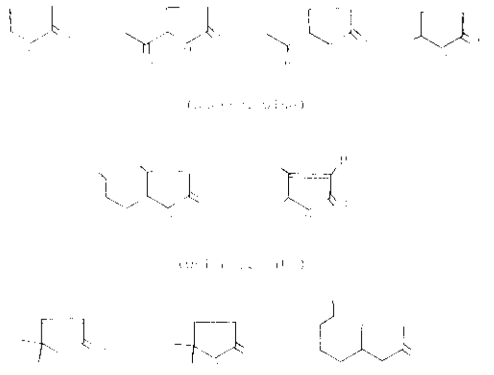
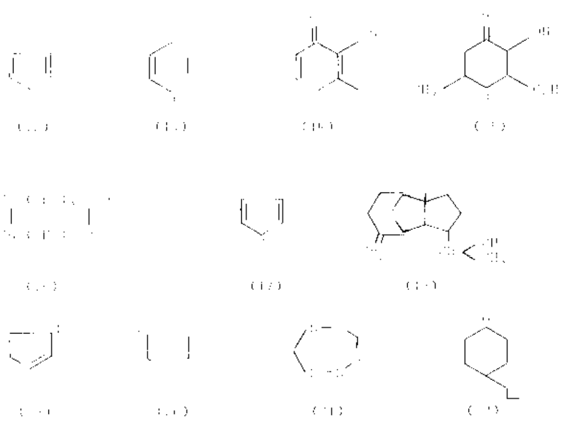


Figure 3



Nitrogenated heterocyclic compounds:

Pyrroles and derivatives (23): Numerous pyrroles are found in roasted products, coffee in particular.

pyrrolidine (German cheeses, caviar)

2-acetyl-1-methyl pyrrolidine (bread) (24)

1-ethyl-2-pyrrolidone (cooked pork) (25)

Pyridines and derivatives (26): There are numerous pyridines in roasted products.

1,4,5,6-tetrahydro-2-acetyl pyridine (bread) (27)

Pyrazines/pyrimidines (28,29): There are numerous alkylated and methoxylated pyrazines that are particularly powerful.

2-secbutyl-3-methoxy pyrazine (galbanum),

2-isobutyl-3-methoxypyrazine (green pepper) (30)

alkyl-pyrrolo (1,2-c) pyrazines (roasted meat) (31)

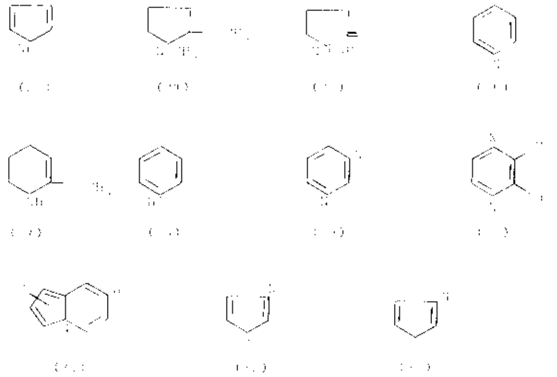
Oxazoles and derivatives (32): 5-acetyl-2-methyl oxazole (coffee) and oxazolines (meat)

Thiazoles and derivatives (33): There are numerous thiazoles in broiled meat.

2,3-dimethyl thiazole (0.05 ppm) (braised meat)

1-n butyl-2-isobutyl-3-methoxy thiazole (2 x 10^{-4} ppm) (vegetable soup)

2-acetyl-2-thiazoline (beef)



Minor, bitter components

The extreme diffusion of the bitter flavor in all the conventional groups makes difficult a search for correlation between structural characteristics and bitterness.

Hydrocarbons with functional groups: Humulon

Isoprenoids with Functional Groups:

Monoterpenoids: amarogentin (70 ppb) and aucubin

Sesquiterpenoids: lactucin (2000 ppb)

Diterpenoids: columbin (10,000 ppb) (34)

Triterpenoids: quassin (35) and ailantin (200 ppb)

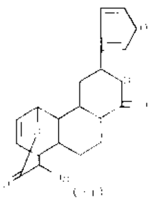
Steroids: sarcostin

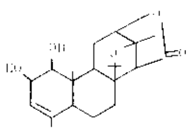
Oxygenated Heterocyclic Compounds: Compounds with a lactone function whose importance is very great among bitters have been classified according to their basic structure and not as derivatives of oxygenated heterocyclic compounds.

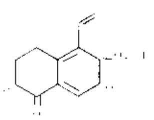
n-guaione (36)

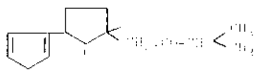
gentiopicroside (100,000 ppb) (37)

Nitrogenated and Sulfurated Heterocyclic Compounds: amines, aminoacids, peptides, proteins, heterocyclic compounds, peptides from the hy-









drolysis of casein: brucine (200 ppb) (HCl, 80 ppb) and quinine, HCl (6600 ppb)

Minor sweet components

Sweetening power, SP, evaluated with respect to sucrose.

Monellin (Protein MW 11,000)
SP: 2500 (or 8.4 x 10^4 on a molecular basis)
Neohesperidin dihydrochalcone
SP: 2000 (3500 on a molecular basis)
Thaumatin (Protein MW 20,400 to 21,000)
SP: 1600 (1 x 10^5 on a molecular basis)
Naringin dihydrochalcone
SP: 300 (500 on a molecular basis)
Stevioside (steviol disaccharide: aglycone diterpenoid)
SP: 280
Glycyrrhizin (glycyrrhetinic acid glucoside: triperpene)
SP: 100
Phyllodulcin and analogs
(Substituted isocoumarin)
2-(3-hydroxy-4-methoxy phenyl) benzo-1,3-dioxane
Osladin (bis glucoside saponin steroid)
n-Propane thiol
SP: 50 to 70

Minor components typed by origins

The knowledge of precursors and of the formation mechanism of odoriferous and taste-affecting compounds, whether or not they are minor, in animal or plant organisms or starting from them is still partial in spite of considerable work by many investigators. Nevertheless, it is necessary to distinguish the natural flavors and fragrances that can be considered as direct metabolites manufactured by organisms, for example food flavors and fragrances, produced principally by extracellular biogenetic routes from those produced by sequences of chemical reactions that are more or less complex.

In the first case, the organoleptic quality depends on genetic factors, maturation, and preservation. In the second case, the odoriferous products result from the degradation of high molecular weight precursors with the fragment formed contributing directly to the flavor or fragrance, or contributing indirectly to these items after intramolecular or intermolecular conversion with other biochemical processes. In certain cases the precursors themselves can react with their fragments or with external agents, then forming more particularly odoriferous products.

Minor components can be grouped in four classes according to whether they are naturally present; they have been created from natural media; they are synthetic, that is identical to those present in nature; or they are artificial, that is, still not found in nature. We shall not deal here with the last category, but for the three others we shall mention a few examples of minor components present in miscellaneous media.

Naturally present minor components

Knowledge of odoriferous molecules in living animals or plants is far from being complete. Are they metabolites that are indispensable for life, or are they catabolites that are consequences of life? Their biogenesis has given rise to numerous and important studies. Conversion schematics have been suggested, starting from simple elements such as CO_2 or from more complex elements such as storage products (fats, starches, proteins). The cell and enzyme systems capable of such conversions are varied, but it is necessary to recall that the formation of such products can depend on genotypes, physiological conditions, the state of development, and the location in the plant.

Let us note with respect to the biogenesis of essential oils, or more exactly their components, that essential oils are not biological products. Only certain components and sometimes part of these components are biological in nature. The minor components for jasmin, rose, pear, and tomato are discussed below.

The minor components of the jasmin flower belong to two groups: the sasmonoids and the nitrogenated compounds to which must be added a few light constituents of the aldehyde or ester types (fig. 4). The following are nitrogenated components:

3-ethyl pyridine
3-vinyl pyridine
3-ethyl-4-methyl pyridine
3-vinyl-4-methyl pyridine
methyl pyridine-3-carboxylate
ethyl pyridine-3-carboxylate
methyl-4-methyl pyridine-3-carboxylate
methyl-5-ethylpyridine-3-carboxylate
ethyl-5-ethylpyridine-3-carboxylate
methyl-5-vinyl pyridine-3-carboxylate
ethyl-5-vinyl pyridine-3-carboxylate
methyl-5-ethyl-4-methylpyridine-3-carboxylate
methyl-5-vinyl-4-methylpyridine-3-carboxylate
ethyl-5-ethyl-4-methylpyridine-3-carboxylate
2-phenylacetonitrile
2-phenylnitroethane
methyl anthranilate
dimethyl anthranilate
indole

The single-terpene components of rose flowers (Damascena) illustrated in figure 5 represent less than 1% of the essential oils.

According to the species, the nature and number of the components in the flavor of the pear vary. Some are cited below.

2-hexenal
2,4-decadienoic acid (trans-cis)
methyl-2-octenoate (trans)
ethyl-2-octenoate (trans)
methyl and ethyl-3-hydroxyoctanoate
methyl-2-decenoate (trans)
ethyl-2-decenoate (cis)
methyl-2,4-decadienoate (trans-cis and trans-trans)
ethyl-2,4-decadienoate (trans-cis and trans-trans)

The minor components of the tomato (fresh and concentrate) are:

Alcohols: miscellaneous alkenes
Carbonylated compounds:
trans-2-pentenal
3-pentenal
trans-3-hexenal
2-methyl-2-butenal
aliphatic aldehydes of the types octenal and dienal
2,4-hexadienal (trans-trans)
2,4-heptadienal (trans-cis)
2,4-nonadienal (trans-trans)
2,4-decadienal (trans-trans)
miscellaneous aliphatic ketones:
  6-methyl-5-hepten-2-one
Esters: Branched aliphatic compounds
Heteroatomic compounds:
methylthioalkanols
thiophenes:
  2-formyl
  3-formyl
  2-formyl-5-methyl
  2-acetyl
thiazoles:
  2-isobutyl (threshold: 1.3 ppb in fresh and concentrate tomatoes)
  2-propyl
  2-sec.butyl
  benzothiophene

For the sake of information figure 6 shows the formation of certain minor volatile components in the tomato from the conjugated reaction of lipoxygenase and dehydrogenase alcohol.

Minor components created from complex natural media

The odor and flavor of different odoriferous and food products can undergo great changes during treatment, transportation, and storage. Primary aromatic compounds can be lost and other secondary compounds formed, for example during aging, sometimes to the benefit of the final product. But occasionally such changes correspond to the loss of interesting sensory properties. Minor components here play a fundamental role.

These modifications of odor or of flavor can include unintentional modifications, for example, from the ac-

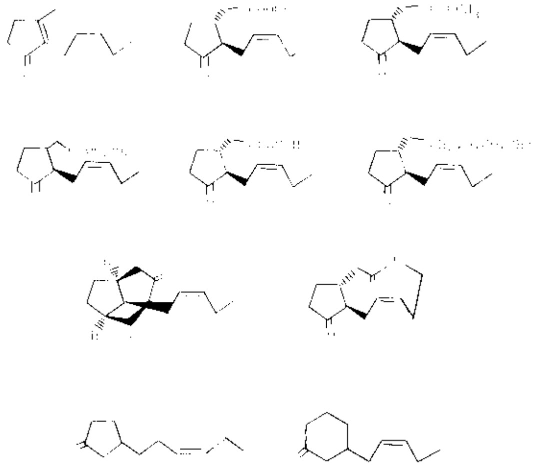


Figure 4

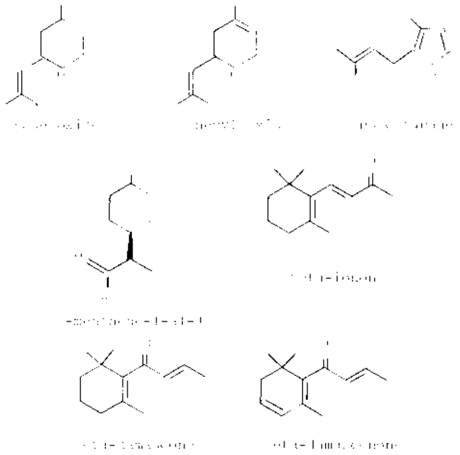


Figure 5

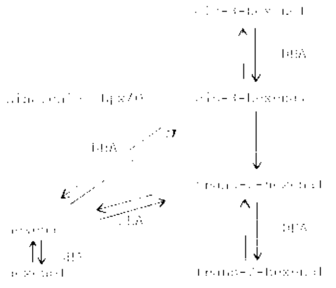


Figure 6

tion of the environment or of faulty techniques. They may also be intentional, for various reasons, such as the regeneration of a lost flavor; the upgrading of residual extraction materials; the neutralization of an unpleasing flavor; the production of a new sensory effect by means of physical chemical or biological agents.

During the last few years, important efforts in interpreting the formation of odors and flavors have made it possible in certain circumstances to explain or predict such formations, by means of mechanical concepts currently used to interpret organic chemistry reactions or by means of well-known enzyme schematics. Unfortunately, these interesting mechanisms cannot be discussed here. We shall only cite a few examples of modifications related to processes used for the most common types of minor components present and for certain modes of formation. These include enzymatic (alcohol-containing beverages, cheeses, tea); thermal and mixed (thermal-enzymatic) (coffee, cocoa, meats); and chemical (hydrolysis, oxidation).

Enzyme processes

Enzyme processes, which are the basis of the manufacturing of traditional flavors in food and beverages provide a fundamental contribution to the formation of minor components. Examples include cheeses and fermented (alcohol-containing) beverages.

Minor components of cheeses are provided by the entirety of enzyme reactions related to the metabolism of lactic acid: lipolysis, oxidation, proteolysis; but also by reactions between the products formed during these different processes. According to the type of cheese, certain constituents are highly specific and are related to the particular microflora of each. A few conventional minor components of cheese flavor are listed below.

methanethiol
ethanethiol
dimethyl sulfide
ethylene sulfide
ethyl methyl sulfide
1-propane-3-thiol
2-propane thiol
2-butane thiol
1-propane-2-methyl thiol
methylthioformate
diethyl sulfide
thiophene
methyl propyl sulfide
butane thiol
methyl thioacetate
2-methyl thiophene
3-methyl thiophene
methyl thiopropanoate
2,3-dithiopentane
dipropyl sulfide
2,5-dimethyl thiophene
methyl thiobutyrate
2,4-dithiopentane
2,4-dithiohexane
2-methyl methyl thiobutyrate
3-methyl methyl thiobutyrate
methional
2,3,4-trithiopentane
dibutyl sulfide
2-methyl-thiophen-3-one
2,5-dimethyl trithiolane (cis)
3,5-dimethyl trithiolane (trans)

Yeasts and fermentation conditions determine the most important aromatic constituents of fermented beverages. Approximately the same components appear in distilled beverages whatever the starting materials may be. Changes take place during maturing, since new components can be formed and others destroyed. Figure 7 shows the fermentation process and minor components resulting in whiskey.

The regeneration of flavors lost during treatment, a very important theme in research since the 1950s, consists of reintroducing an enzyme preparation containing the specific enzymes of the flavor into the impoverished food that still contains usable precursors. The introduction of components that may be converted by the enzymes present in minor components that are characteristic of the flavor would likewise be used. The upgrading of inexpensive residual materials that have served for obtaining aromatic products but still contain usable precursors would have some success. The creation of flavor by seeding natural culture media or by enriching them with suitable bacteria strains is used for cheese aromas. Among the numerous minor components formed are the specific methylated thio ethers of certain strains. Unpleasing flavor or certain bitternesses may be eliminated by adding natural protein fractions containing lipoxygenases and peroxydases.

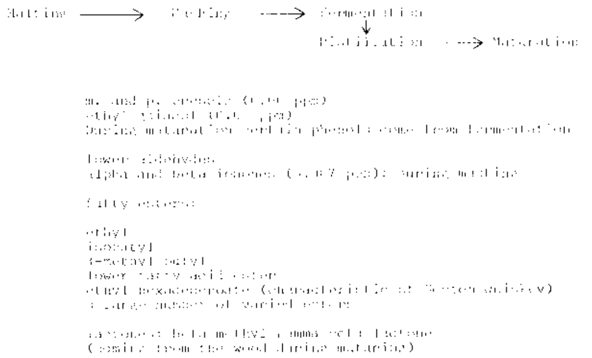


Figure 7

Thermal processes

Thermal degradation reactions applied to complex-nature food materials explain the formation of a large number of aromatic constituents, according to the type of precursors present in the thermolyzed substance and the conditions of the substance. In different cases this thermolysis follows equally important enzyme processes. Among thermal degradations, it is necessary to cite all of the so-called Maillard reactions, which come from heating a carbonylated compound (aldehyde or ketone) with an aminated compound; but more often they concern sugar and aminated acid.

We shall not give the details of these reactions, which have been described and illustrated elsewhere; we shall only say that they contribute to the aroma of numerous food products; among them cereals (bread crusts, biscuits, rusk), malt, peanuts, coffee, cocoa, and heated wine.

Judicious additions of aminated acids and of sugars before thermal treatment can give food products custom-made aromas or flavors as one wishes. The substances formed by these diverse reactions are many: aldehydes, reductions, oxygenated heterocyclic compounds (furans, pyrans), sulfurated heterocyclic compounds (thiophenes), and nitrogenated heterocyclic compounds (pyrazines, pyrroles). Some examples of thermal and mixed (enzymatic then thermal) processes and their results in various beverages and foods follow.

Tea

Several hundred minor components of tea have been identified, but it is to the equilibrium of a limited number of different constituents that the characteristic aroma of tea is related. In addition, the aromas are specific to their origins and the treatments to which they are subjected, which correspond to dehydrations, fermentations, and heating to temperatures that can reach 230°C. The precursors are free amino acids, linolenic acid, and the carotenes. Minor components of four distinct teas are listed below.

The characteristic components of Ceylon tea follow.

2-methyl-2-hepten-6-one
retro ionone
linalol oxides
geraniol
4-octanolide
cis-3-hexenyl-cis-3-hexenoate
4-nonanolide
theaspirone
2,3-dimethyl-2-nonen-4-olide
5-decanolide
jasmin lactone
methyl jasmonate
dihydroactinidiolide

Methyl jasmonate, jasmin lactone, linalol, and linalol oxides are the most important components. The characteristic components of Keemun black tea are light ethylene alcohols, beta ionone, and linalol oxides. The characteristic components of roasted tea (Hoja-Cha) follow:

furfural
acetyl furfural
methyl furfural
acetyl pyrrole
formyl pyrrole
1-ethyl-2-formyl pyrrole

And finally the characteristic component of Lotus tea is 1,4-dimethoxybenzene.

Beer

In the production of beer, fermentation and brewing are responsible for the creation of aliphatic aldehydes, alcohols, and furans by oxidation of lipids; pyrroles and pyrazines from a Maillard reaction during brewing; and phenols from thermal fragmentation of ferulic and coumaric acid. A few of the important minor components identified in beer are branched or non-branched aliphatic aldehydes and alcohols, C5 to C11; phenols: substituted guaiacols, cresols; arylaliphatic and aromatic aldehydes, dienals and trienals; lactones (see fig. 8); furans: hydroxy, formyl, and acetyl; sulfides and disulfides; and multiple pyrazines, alkylated formylated and acetylated pyrroles.

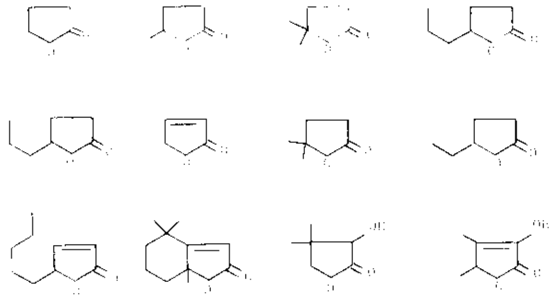
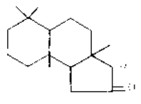


Figure 8

Tobacco

The constituents of the tobacco aroma are particularly numerous and are a function of the more or less important treatments they undergo with enzyme and thermal processes. There are several hundred constituents, but only certain groups bring to bear an important odoriferous contribution in spite of their low contents. These include aliphatic and arylaliphatic acids (4-hydroxy phenyl acetic acid); lactones, alcohols, and diterpene oxides (ambrettolide, norambreinolide, and dehydro-)(38); aliphatic, cyclic and polycyclic ketones (solanone(39), ionone, beta damascone(40)); and certainly the nitrogenated heterocyclic compounds (nicotine and derivatives, pyrroles).



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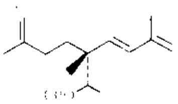

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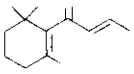


(40)

Bread

More than 150 volatile components have been identified in the aroma of bread, which shows itself to be extremely complex inasmuch as its aroma is a function of the ingredients and of the process of bread-making, fermentation, and baking. The enzymes contribute to the overall aroma while the Maillard reactions that take place during baking contribute more specifically to the aroma of the crust. The carbonylated compounds, and likewise a certain number of oxygenated, nitrogenated, and sulfurated heterocyclic compounds, make the most important contribution to the aroma, while the acids, alcohols, and esters have a more limited role. Thiophenes (furfuryl- and acetyl), pyridines (acetyl and formyl), pyrazines (miscellaneous alkyl pyrazines), and substituted pyrroles are important in the aroma of bread, while furans and furanones (2,5-dimethyl furanone (2H)) are found in bread crust.

Cocoa

The roasted cocoa flavor is the summation of a complex mixture of several hundred components resulting from biological and technical treatments that give rise to fermentations and to nonenzyme burnishing reactions (Maillard, Amadori, Strecker). The most important components are aldehydes (5-iso- and phenylacetaldehyde and condensation products for flowery notes, as well as a large number of pyrazines, oxazoles, acetylpyridine, and methylvinylthiazole for the roasted cocoa notes. A few sulfurated and phenolated products are likewise created during roasting. Purines, theobromine, and caffeine with the diketopiperazines bring the bitter and stimulant taste. The aroma precursors found in the nut are carbon hydrates, flavoroids, catechols, phenolic acids, and amino acids, which are specifically implicated during thermal treatments (see fig. 9). The aliphatic sulfurated compounds should also be mentioned.

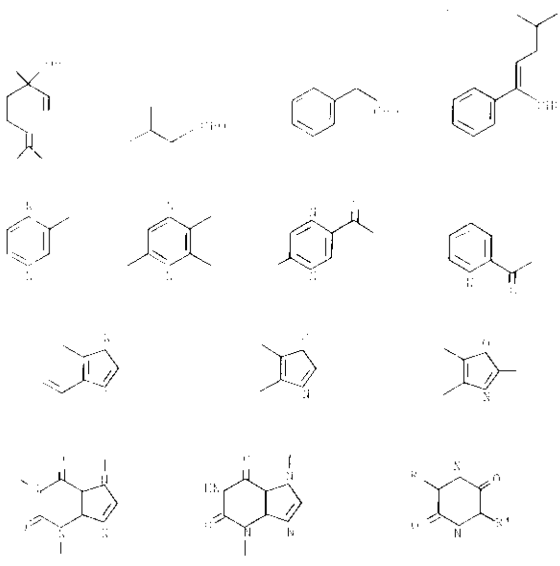
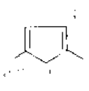


Figure 9

Coffee

The number of components identified in the flavor of roasted coffee is considerable—there are several hundred. Those components whose contribution is essential are carbonylated diones and cyclopentonones; miscellaneous phenols; oxygenated heterocyclic compounds: alkyl furans, furyl sulfides, and 5-acetyl-2-methyl oxazole (41); sulfurated heterocyclic compounds: benzo (b) thiophene, 4,5-dihydro-2-thiophenone (3H) (42), 2-acetyl-4-methyl thiazol, and 6-methyl-2,3-dihydro thieno 2,3-c furan (43); and nitrogenated heterocyclic compounds. There are numerous pyrazines: 2-isobutyl-3-methoxy pyrazine and 2-(2-furyl) pyrazine (44). There are numerous pyrroles and numerous pyridines.



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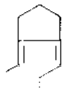

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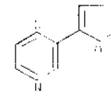

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Cooked meats

The flavors of cooked meats are attributed to complex mixtures obtained from the action of heat on heterogeneous systems containing odorless precursors that can develop volatile or nonvolatile minor components with odoriferous and gustatory properties and synergistic potentializers. Among the precursors are diverse nucleotides, nucleic acids, peptides, glucopeptides, amino acids and amino saccharines, sugars, amines, and organic acids.

Heating in an aqueous medium at 100°C and thermolysis at 150-160°C are the two techniques used that supply numerous minor components. The diverse Maillard reactions, Amadori rearrangement, and Strecker degradation are responsible for certain of these conversions. Refer to the remarkable work of the Ohloff team on the heterocyclic compounds of the meat aroma, whose basic skeletons are shown in figure 10.

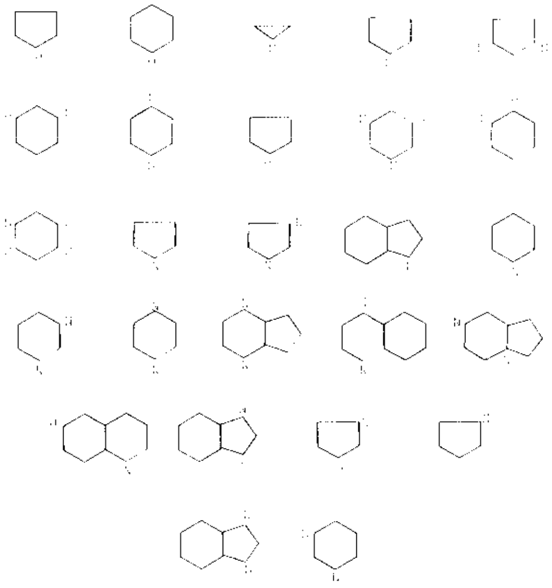


Figure 10

Nevertheless, let us cite a few important minor components, of which certain ones are more characteristic of the origins of meat.

1,2,4-trithiolane and 3-methyl-5-ethyl-3-methyl-5-isopropyl
4,5-dimethyl-4-cyclopentene-1,3-dione
hydantoins
4-acetyl-2-methyl pyrimidine
miscellaneous furans and furanones—hydroxydimethyl-2,3-dihydro-3,5-dihydroxy-6-methyl-4-furanone (4H) and 4,5-dihydro-4-methylthio-2-furanone (3H) (roasted meat)
aliphatic lactones
substituted thiophenes
1-ethyl-2-pyrolidone (pork)

2,4-dimethyl-5-vinyl thiazole
pyrazines—1-(2-pyrazinyl)-2-propanone (boiled beef)

Miscellaneous processes

Certain minor components can be created during various treatments following errors or insufficiency of technology. It is during a poorly-conducted steam distillation for the manufacturing of essential oils that substituted furans can form with a burnt, acrid odor. This same note can be brought by dimethylsulfide and methylpropanal, which are formed by thermal degradation when fruit juices are concentrated. It is also the bitterness of cheeses, related to the presence of cut peptides or of burnt caramel. It can be the byproduct of the rancidness of oils or of beer.

Conversely, the formation of disagreeble flavors related to the presence of minor components can be prevented by preliminary destruction of the responsible enzymes. For example, heating of soybean proteins or adding amino acids or protein hydrolysates to the oxidizable lipids that uses the antioxidant effect of the Maillard reactions.

Finally, let us recall that certain minor impurities are responsible for the typical odor or flavor that has been wrongly attributed to products assumed to be pure, but whose further purification demonstrated the absence of odor.

Synthetic minor components identical to natural substances

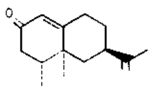
The difficulty of isolating certain minor components starting from natural media in sufficient quantities and at worthwhile prices in view to using them in perfumery compositions or in food flavors has motivated the preparation of numerous components by synthetic or semisynthetic chemical or enzymatic routes. These components had properties that were recognized as particularly interesting.

This type of substance, called "nature identical," makes possible formulations close to natural products (reconstitution and others) having important economic outlets, by giving them a more natural note or by reinforcing the flavor itself. These substances have also made possible original odoriferous formulations that have had great success, such as the jasmin note.

The problems raised by the extension of the idea of natural minor components are numerous. First of all, there are the complex questions of official regulation and international recommendations for use (prohibition or dosage limits), and for labeling, for example, natural, nature-identical, or artificial products. There are also questions of harmlessness with respect to the health of users and of very costly and lengthy obligatory tests before marketing, more specifically for artificial substances. There are, too, delicate questions of supply guarantees and of formulations. Finally, there are questions of patent rights and expertise. We shall not deal with the first two groups of problems, but we shall examine the last two.

The supply of minor components gives rise (for numerous possible users) to delicate problems by assuming that they know the exact structures and the specific properties. This is not always the case. This is an esoteric field for many, where the concerned molecules with complex and asymmetric structures require lengthy chemical synthesis steps, often with modest yields. The use of semisyntheses starting from natural origin intermediates is sometimes advantageous, such as the use of biomimetic syntheses taken from models of nature or from enzyme processes. The concept of manufacturing cost is secondary since the quantities used in formulations are low, but the guarantee of supplies is imperative. This means that there is an interesting business for dynamic laboratories that are highly specialized in the manufacturing and sale of minor components. Their upgrading in all kinds of formulations is delicate, since these products can only be evaluated in terms of great dilutions, which are unusual in conventional composition methods. In addition, the organoleptic effects are sometimes variable when passing from one dilution to another. Furthermore, the original structures of certain minor components constitute an excellent inspiration for the synthesis of closely-related or altogether new dirivatives, said to be "artificial." A few examples of minor components identical to natural substances obtained by conventional chemical means as well as biological routes, such as microorganisms and enzymes, follow.

Compounds with oxygenated heteroatoms:
  cis-3-hexen-1-ol and acetate
  cis-3-hexen-1-al
  (+) nootkatone (grapefruit) (1 ppb) (45)
  2,5-dimethyl-4-hydroxy-3-furanone (2H) (0.04 ppb) (Furaneol) (46)
  damascone and derivatives (beta-damascenone) (47)
  15-pentadecanolide (Exaltolide) (48)
  rose oxide (49)
Compounds with sulfurated heteroatoms:
  allyl isothiocyanate (mustard)
  para mentha-8-thiol-3-one (Buchu)
  methyl-1-thiopropanol (wine, beer)
Compounds with nitrogenated heteroatoms:
  3-Methoxy-2-sec butyl pyrazine (galbanum)
  3-Methoxy-2-isobutyl pyrazine (green pepper)
  2-isobutyl thiazole (tomato) (50)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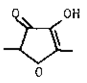
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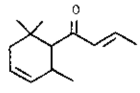
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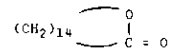

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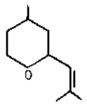
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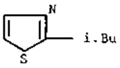

(50)

The formation of terpenoids and of lactones, important components of flavors, by means of microorganisms is presently intensively being studied, but industrial production of definite components is still limited. On the one hand, this is so because the use of plant meristematic cells that frequently divide rarely leads to the manufacture of terpene, phenol, or alkaloid compounds. On the other hand, this is so because the conversions of terpenoids by means of miscellaneous microoganisms are still poorly studied. Nevertheless, a few types of usable microbiological reactions include reduction (ethylenic-carbonylated bonding); oxidation (oxygenation of tertiary carbon or ethylene bonding, dehydrogenation of alcohol or ethane bonding); isomerization, methylation, condensation, breaking of carbon-carbon bond; esterification, hydrolysis, dehydration; acetylation, transglucosidation; and amination, disamination.

Minor components identical to natural substances that are prepared by the biological route include maltol (3-hydroxy-2-methyl-4H-pyran-4-one) (caramel, malt).

Patent rights, that is the patentability of the components of natural products and proof of fraudulent practices, are complex and poorly resolved. Let us recall the recent decision of German and American judges who consider all constituents isolated from a natural medium as protectable, whether this constituent had not been identified before or had been mentioned for example in an overall list of components in an aroma from a natural origin, its specific organoleptic properties had not especially been shown.

In the case of minor components, proof of fraudulent practice is difficult, given the problems of isolating sufficient quantities for a valid identification, starting from complex media where the substance is highly diluted. The expertise in artificial components, that is not identified up to now in natural products, comes from traditional isolation and identification methods, for example the vapor phase chromatography/mass spectroscopy couple. But to suitably differentiate the components extracted from nature or prepared synthetically requires certain refinements and adaptations whose success is not always guaranteed.

The presence or absence of characteristic impurities in certain natural or synthetic substances was an interesting element, but since purification technologies have become more and more refined, the detection of these impurities has often become lengthy and delicate.

The determination of the relative contents in radioactive carbon 14 or in the stable isotopes C13, O18, N15, D of a component is an interesting method of expertise since these components are subjected to relative enrichments or impoverishments as a function of the biosynthetic or synthetic process of their formation. The absence or presence of radioactive carbon 14 makes it possible to distinguish components obtained by synthesis using fossil products (coal, fuel) which practically no longer contain any C14 with a limited life, from those extracted or prepared by semi-syntheses using contemporary plants that utilize atmospheric CO_2 that is sufficiently rich in C14. Since isotopic fractionation takes place during plant biosynthesis, the determination of C13/C12 ratio makes it possible to differentiate components synthesized by plants with a Calvin carbon cycle from those coming from plants with other cycles (for example, vanilla).

Conclusion

Fragrances and flavors are most often constituted by numerous components, of which certain particularly powerful ones are detected in dilutions of the order of a gram per metric ton and even lower. The increasing importance of these minor components is not always realized by most specialists in our profession. Yet, these minor components constitute a future field for fundamental research and for industrial applications with economic consequences. Certain companies have understood this well, which gives them a definite headstart in the marketing of flavors and fragrances, along with mastery in this field. Although our present knowledge has made it possible to isolate and identify numerous minor components, we can hope to know many more with the assured development of analytical means in future years.

These future developments will be the result of work done by well-staffed, multidisciplinary teams that are motivated by the certainty of their scientific abilities within the context of companies convinced of the economic interest of such work. Perfumers and flavorists can only be happy about such prospects, which will bring them an era of prosperity along with original means of creation.

Acknowledgement

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