CHEMISTRY OF NUTRACEUTICS, FLAVOURS, DYES AND ADDITIVES

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Contents

Summary

Food has always been considered for its nutritional value, thus for the energy and the building blocks it supplies, which are necessary for life, and which are afforded by the main components of food: carbohydrates, proteins and fats. This chapter, instead, regards all those minor components present in food which not necessarily have a nutritional value, but still are naturally present or added, and that may play fundamentals roles. Nice flavor and aspect are necessary to make a food attractive and eating a pleasure, preservatives and other additives are important to prevent food spoilage and contamination, compounds like sweeteners may allow the reduction of caloric intake, and the use of nutraceutical compounds may have potential beneficial effects on health. In this chapter an overview of all these minor compounds, along with their structure and chemistry is given. The chapter is divided in paragraphs, each regarding a different class of compounds. Paragraph two focus on flavors; in the first part describes compounds responsible for natural flavors and how they are generated, while in the second part gives an overview on artificial flavors. Paragraph three gives an

overview on coloring substances; again, in the first part the attention is for natural colorings, divided by their chemical structure, while the second part deals with artificial compounds defined as dyes. The fourth paragraph is the most heterogeneous since it deals with general additives. These have been classified in subclasses: sweeteners, preservatives, emulsifiers and stabilizers. Sweeteners, in their turn, have been classified in nutritive and non-nutritive and both comprise natural and artificial compounds. Information on their structures and characteristics are given. Preservatives have been separated according to their mechanism of action in antioxidants and antimicrobials. Before illustrating their structure and their application, the main causes responsible for food spoilage are discussed. Emulsifiers and stabilizers have been described together. What they are, why and where they are used is shown. Finally, the last paragraph deals with nutraceutics. A description of these compounds is given. Some examples of nutraceutical compounds divided by chemical structure in flavonoids, polyunsaturated fatty acids, amino acids and vitamins, are illustrated.

1. Introduction

The main object of chemists in the XVIII, XIX centuries was to understand the nature of food, thus the first components to be studied were the main constituents among which carbohydrates, fats and proteins. Nonetheless chemists have been interested also in other substance, which are present in food and beverages in fewer amounts but not for this are less important under their nutritional point of view, or for their properties of rendering food more attractive for their aspect, flavor and consistence, or for making them storable for long periods. All these minor substances are represented by colorings, flavors, preservatives and other additives. More recently nutritional supplements, functional foods or herbal supplements, all known as nutraceutics, are receiving a growing interest and international recognition as having potential beneficial effects on health, including prevention and/or treatment of diseases. Still there is a lot of confusion in the field, also due to the different legislation adopted in different countries or absence of it. In this chapter we will try to give some clear information on what nutraceutics are and what is known about them. All these minor substances present in food will be discussed in separate paragraphs, although keeping in consideration that most of these components have multiple function and characteristics and for this are present in different paragraph.

2. Flavors

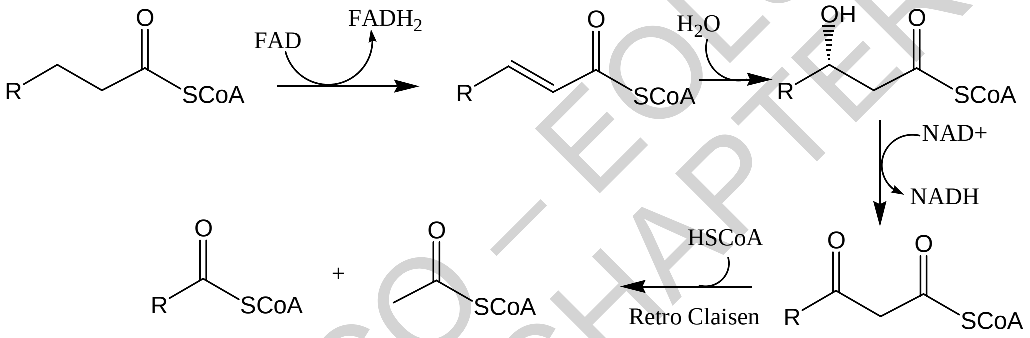
Flavors are more or less volatile substances produced by fruit or vegetables during different stages of ripening or during manipulation. In particular ripening is the main process through which fruit flavors are produced, while manipulation (like cutting or chewing) is the main cause of vegetable flavors generation. In this paragraph chemical aspects of these natural substances will be illustrated, along with the mechanism of their generation. An overlook of artificial flavorings will also be given.

2.1. Natural Flavors

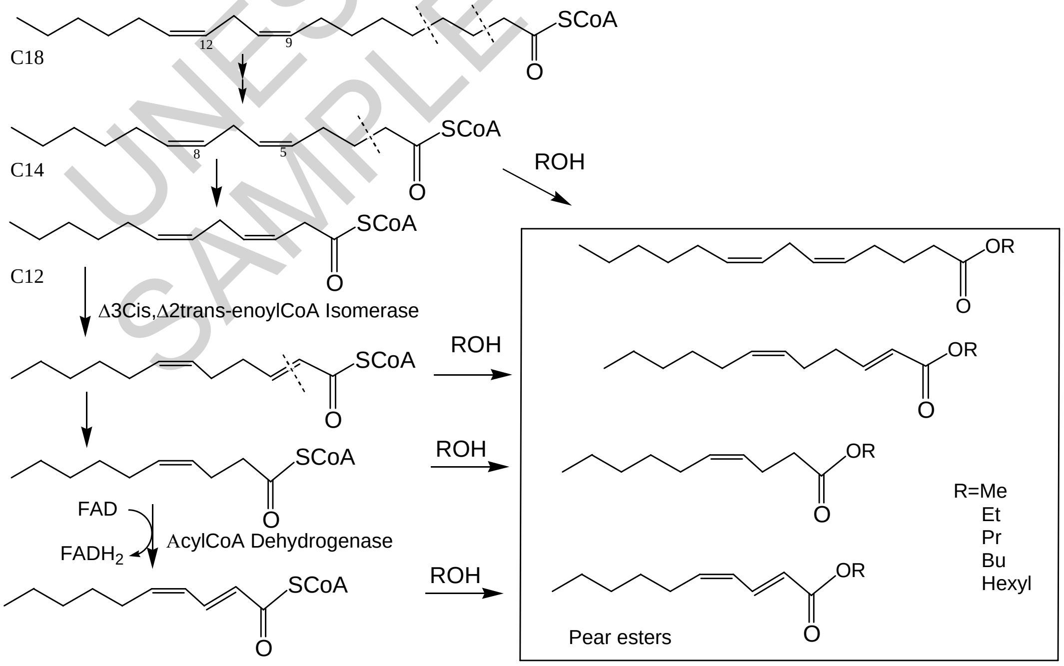
A natural flavor is a combination of naturally derived aroma chemicals and/or essential oils. Natural flavors are very often added to food stuff to enhance the taste of products while avoiding the consistency problems involved with using fruit or other whole flavoring ingredients. Furthermore, adding whole flavoring ingredients like fruit can be extremely costly and is limited by its seasonal availability. All food containing added natural flavors must carry the label declaration "natural flavors/aroma" among the ingredients. To better understand what flavors are and where do they come from, in this section an overview of how flavors are generated will be given, along with some structural and chemical characteristic of the specific flavoring compounds.

Fruit flavorings are mainly generated during ripening process and are produced by secondary metabolism and by the catabolism of small quantities of carbohydrates, lipids and amino acids. Also vegetables flavors are produced essentially by catabolic process but most of these are not due to ripening rather to manipulation.

2.1.1. Flavors derived from Lipid Catabolism



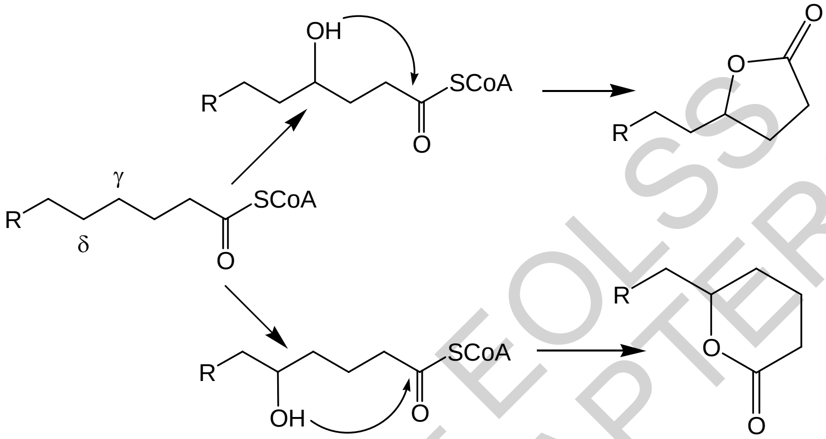
Scheme 1: β-oxidation mechanism.



Scheme 2: Production of pear esters from linoleic acid.

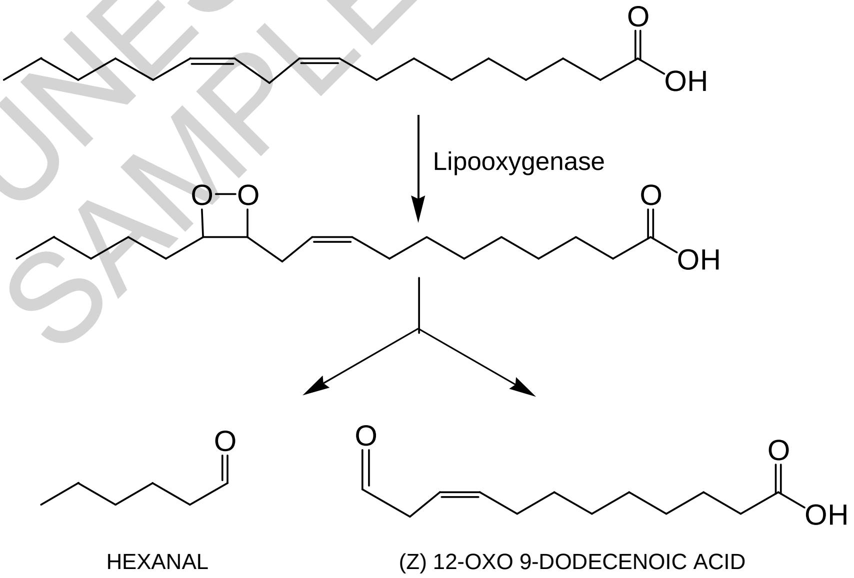
Lipid catabolism may follow three different mechanisms: 1) β-oxidation, 2) lactones formation, 3) lipooxigenase mediated oxidation. The β-oxidation mechanism result in a two carbon unit cut of the lipid activated as thioester, and is illustrated in scheme 1. As example of the β-oxidation catabolism the production of pear esters flavors from linoleic acid is reported in Scheme 2.

Lactones originated from lipids are among the main flavors of peach, apricots and coconuts, there is no known mechanism for the formation of these compounds but it has been suggested that they are originated during oxidation that involve positions γ or δ to the carboxylic function (Scheme 3)



Scheme 3: Lactones formation.

The action of the enzyme lipooxygenase is another oxidative process that generates aliphatic esters, alcohols, acids and carbonyl compounds. The generation of some tomatoes flavors follows this process and is illustrated in scheme 4.



Scheme 4: Production of tomatoes flavors from linoleic acid through lipooxygenase.

2.1.2. Flavors of the Terpene Family

Terpenes are responsible for a great numbers of flavors and are among the main components of essential oils extracted from many different sources. Table 1 reports a selection of flavoring substances, mainly terpenes, isolated from essential oils.

Flavoring substance	**Odor**	**Possible Source**
Anethol	Herbaceous-warm, anisic	Anise (Pimpinella anisum) Fennel (Foeniculum vulgare) Star anise (Illicium verum)
Allyl isothiocyanate	Pungent, stinging	Black mustard (Brassica nigra)
Benzaldehyde	Bitter almond	Bitter almond (Prunus amygdalus var. amara)
S (+)-Carvone	Warm-herbaceous, bread like, spicy, floral, caraway, dill	Caraway (Carum carvi)
R (-)-Carvone	Warm-herbaceous, bread like, spicy, spearmint	Spearmint (Mentha spicata)
1,8-Cineole	Fresh, camphoraceous-cool	Eucalyptus (Eucalyptus globulus)
Cinnamic aldehyde	Warm, spicy, balsamic	Cassia (Cinnamomum cassia) Cinnamon (Cinnamomum zeylanicum)
Citral	Lemon	Lemongrass (Cymbopogon citratus, C. flexuosus) Litsea cubeba
Citronellal	Fresh, green, citrus	Eucalyptus citriodora
Decanal	Orange peel	Orange (Citrus sinensis)
Dimethyl sulfide	Sharp, green radish, cabbage	Commini (Mentha arvensis)
Eugenol	Warm-spicy	Clove (Syzygium aromaticum)
Geraniol	Floral, rose	Palmarosa (Cymbopogon martini) Citronella (Cymbopogon nardus)
Geranyl acetate	Sweet, fruity-floral, rose, green	Lemongrass (Cymbopogon citratus)
(Z)-3-Hexenol	Green, grassy	Corn mint (Mentha arvensis)
D-Limonene	Fresh, Orange peel	Citrus (Citrus species)
Linalool	Refreshing, floral-woody	Basil (Ocimum basilicum) Bois de Rose (Aniba rosaeodora) Camphor tree (Cinnamomum camphora)
Linalyl acetate	Sweet, floral-fruity	Bergamot mint (Mentha citrata)
Massoia lactone	Coconut	Massoia tree (Cryptocaria massoia)
Methyl chavicol	Sweet-herbaceous, anise,	Basil (Ocimum basilicum)

	fennel	
Methyl cinnamate	Fruity-balsamic	Eucalyptus campanulata
Methyl N-methyl anthranilate	Musty-floral, sweet	Mandarin (Citrus reticulata)
Nootkatone	Fruity, sweet, citrus, grapefruit peel	Grapefruit (Citrus paradisi)
Terpinenol-4	Warm-peppery, earthy-musty	Tea tree (Melaleuca alternifolia)
Thymol	Sweet-medicinal, herbaceous, warm	Thyme (Thymus vulgaris) Origanum (Origanum vulgare)

Table 1: Flavoring substances isolated from essential oils.

Terpene flavors are generated from the first member of the mevalonic acid pathway, geranyl pyrophosphate, through multiple chemical mechanisms which involve carbocation formation, dehydratation, oxidations, intramolecular cyclizations, reductions, transpositions and hydratation reactions. The structures of some of these open chain or cyclic compounds are represented in Figure 1. Most of these terpenes are chiral compounds, and generally only one of the two possible enantiomers is produced by a specific plant or microorganism; the two enantiomers may have similar or completely different flavors. This is the case, for example, of S (+)-carvone and R (-)-carvone, the first is present in caraway while the second is the main flavor of spearmint.

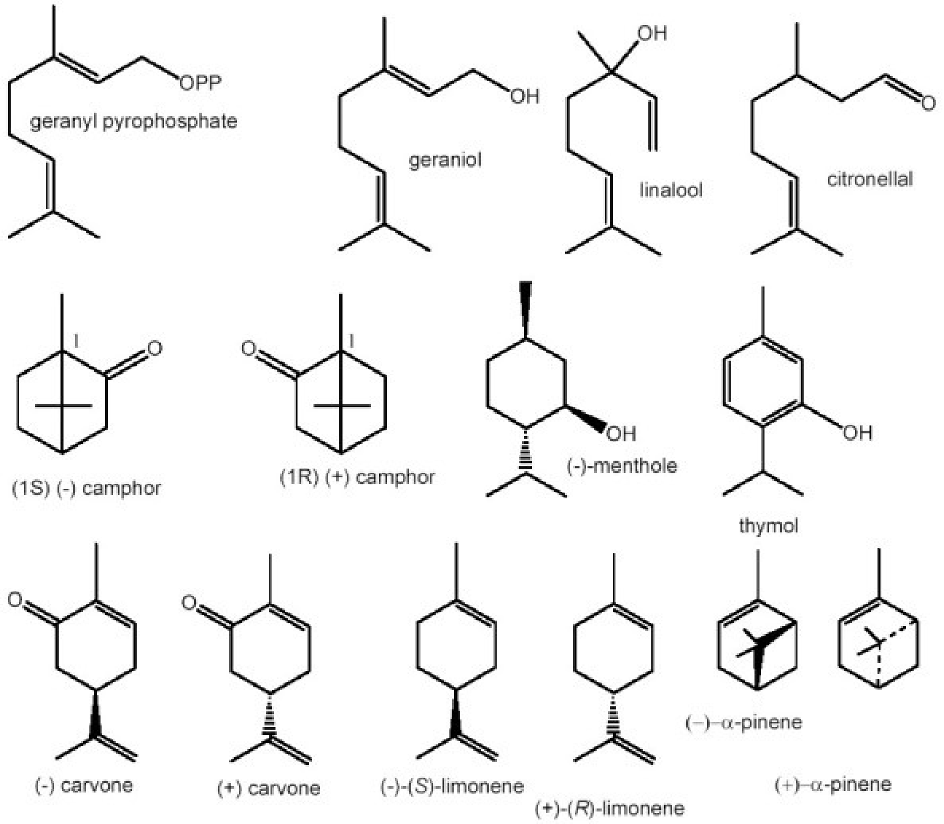
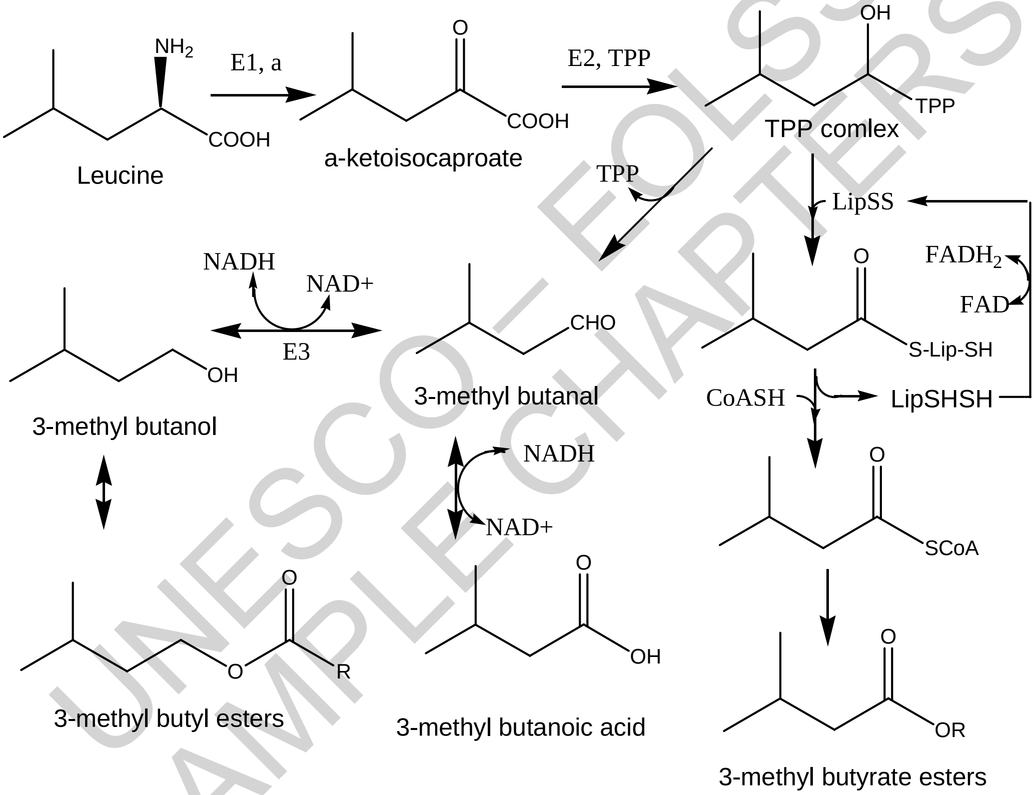


Figure 1: Selected cyclised and open chain terpene flavors.

2.1.3. Flavors derived from Amino Acids Catabolism

Amino acid catabolism affords different compounds present in essential oils, again these are mainly esters, aldehydes and alcohols, which can be branched aliphatic, is the case of compounds produced from leucine or valine, aromatic, when obtained from phenylalanine or tyrosine. Sulfur containing compounds, responsible of the unpleasant smell of cooked cauliflower and other cabbages are generated from cysteine precursors. Some of the catabolic mechanism have been elucidated and may involve many reaction steps and different enzymatic complexes, for example radioactive labeling studies have shown that leucine or valine is transformed in branched chain compounds which are essential for banana flavor. As illustrated in scheme 5, the initial step is the deamination of the amino acid followed by decarboxylation. Reductions and esterificantions lead to different volatile flavors.



Scheme 5: Conversion of leucine into banana flavors: E1 (leucine aminotransferase), a (glutamate), E2 (pyruvate decarboxylase), TPP (thiamine pyrophosphate), E3 (aldehyde dehydrogenase), LipSS (lipoic acid oxidized), LipSHSH (lipoic acid reduced), NAD+/NADH (nicotinamide-adenine dinucleotide oxidized/reduced), FAD/$FADH_2$ (flavin-adenine dinucleotide oxidized/reduced), CoASH (coenzyme A).

Most of the cinnamic acid derivatives and other aromatic flavors have been shown to come from the catabolism of tyrosine and phenylalanine, while other are synthesized directly from shikimic acid, which is the biosynthetic precursor of the two amino acids. Most of these compounds, along with the previously cited terpene flavors, are present and characterize many essential oils. Figure 2 illustrates some of the structures, and Table 2 reports the essential oils containing mainly these aromatic flavors.

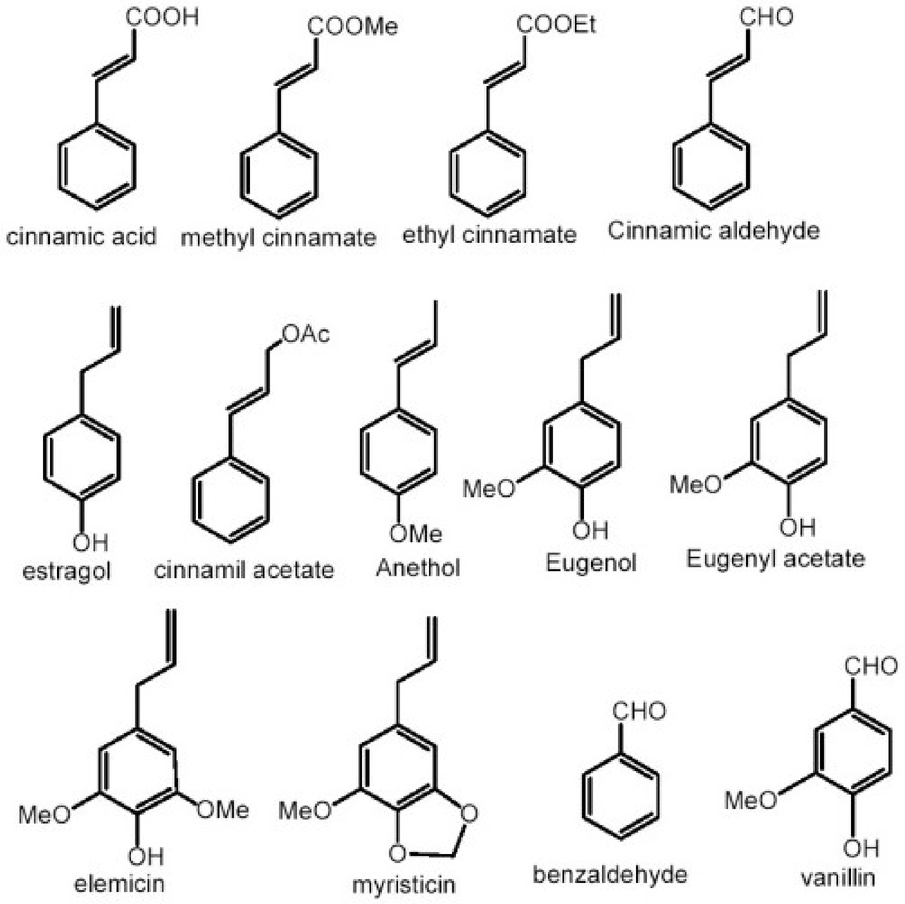
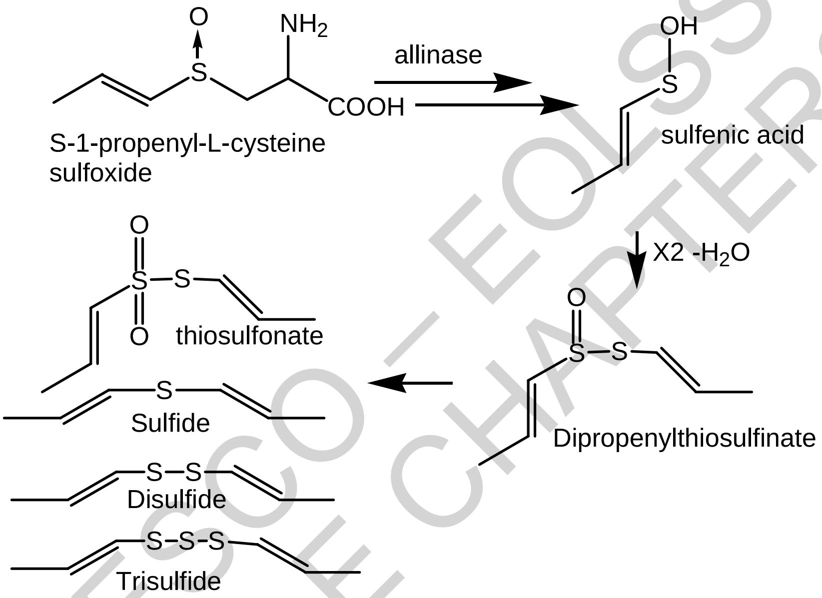


Figure 2: Aromatic flavors.

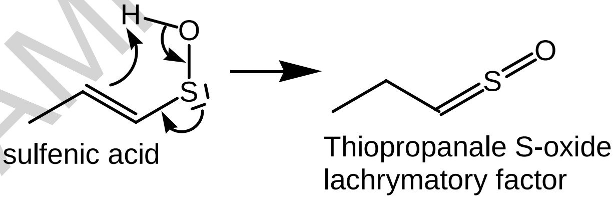
Flavoring substance	**Part used**	**Source; (%) in essential oil**
Anethol	Dry fruits Dry fruits Dry fruits	Anise (Pimpinella anisum); (80-90) Fennel (Foeniculum vulgare); (50-70) Star anise (Illicium verum); (80-90)
Estragol	Dry fruits Dry fruits Dry fruits	Anise (Pimpinella anisum); (1-6) Fennel (Foeniculum vulgare); (3-20) Star anise (Illicium verum); (1-6)
Benzaldehyde	Dry fruits	Bitter almond (Prunus amygdalus var. amara)
Cinnamic aldehyde	Dry bark Dry bark	Cinnamomun cassia; (70-90) Cinnamomum zelyanicum; (70-80)
Eugenol	Dry bark Leafs Dry flowers, sprouts	Cinnamomum zelyanicum; (1-13) Cinnamomum zelyanicum; (70-95) Syzygium aromaticum [cloves]; (70-95)
Cinnamil acetate	Dry bark	Cinnamomum zelyanicum; (3-4)
Eugenyl acetate	Dry flowers, sprouts	Syzygium aromaticum [cloves]; (10-15)
Myristicin	Seeds	Myristica fragrans [nutmeg]; (4-8)
Elemicin	Seeds	Myristica fragrans [nutmeg]; (2)

Table 2: Aromatic flavors and their source.

S-Alkyl/alkenyl cysteine sulfoxide are compounds present in vegetable like garlic, onion, cauliflower and other species of cabbage, are biosynthesized from cysteine and are the precursors of the typical unpleasant flavors generated by the manipulation (cutting, chewing, cooking) of these foods. It is well known that intact onions have virtually no characteristic flavor until cellular disruption. Only seconds after cell damage the onion develops full flavor, this is caused by the fact that cellular damage brings the S-1-propenyl-l-cysteine sulfoxide in contact with the enzyme allinase which catalyses the formation of the sulfenic acid (scheme 6). This compound, extremely reactive, readily forms a thiosulfinate species by reaction with a second molecule of sulfenic acid. The thiosulfinate is again very unstable and decomposes to stable thiosulfonate and mono- di- and trisulfides. Also the lachrymatory factor of onions is generated from the sulfenic acid as illustrated in scheme 7.



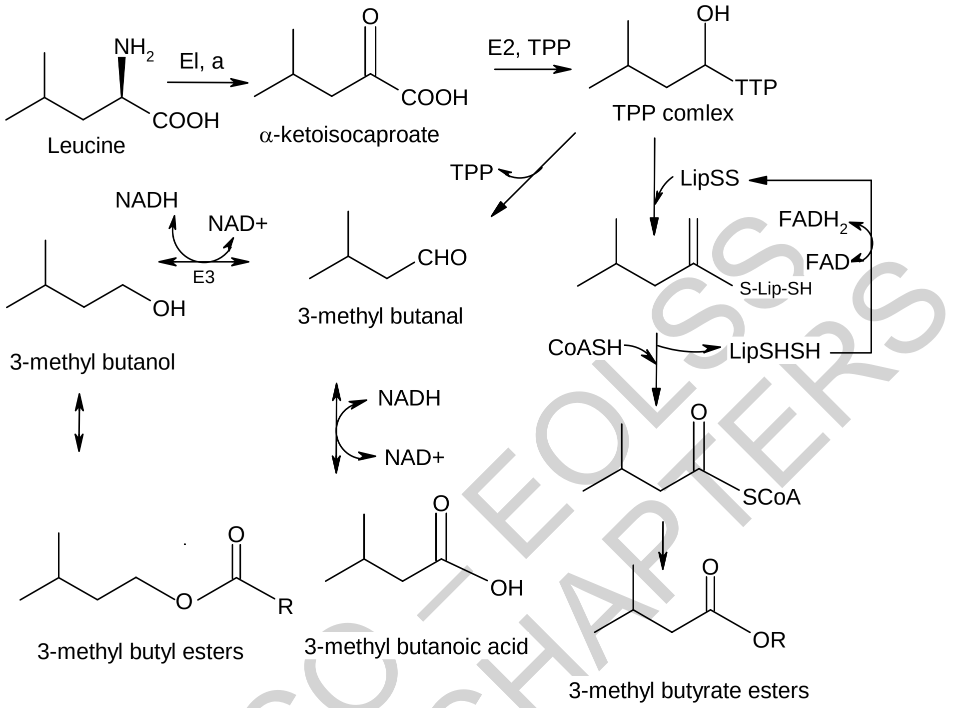
Scheme 6: Flavor formation in onion.



Scheme 7: Lachrymatory factor generation.

The mechanism for the formation of garlic flavors and of the members of the Brassica family flavors (cauliflower, sprouts, cabbage and other) is similar to that described for onion, and are originated by S-allyl-l-cysteine sulfoxide and S-methyl-l-cysteine sulfoxide respectively.

Glucosinolates are another class of non volatile flavor precursors present in some vegetables, mainly belonging to the Cruciferae or the Brassica family, which are enzymatically hydrolyzed to volatile flavors when cellular structure is disrupted. The volatile products are initially isothiocyanates and nitriles which are responsible for the spicy taste of these vegetables. Secondary reactions lead to the formation of other sulfur containing compounds. Scheme 8 illustrates the mechanism proposed for the formation of radish root flavors from the glucosinolate there present. In figure 3 are reported the structures of some glucosinolates present in different vegetables.



Scheme 8: Formation of flavors in radish root.

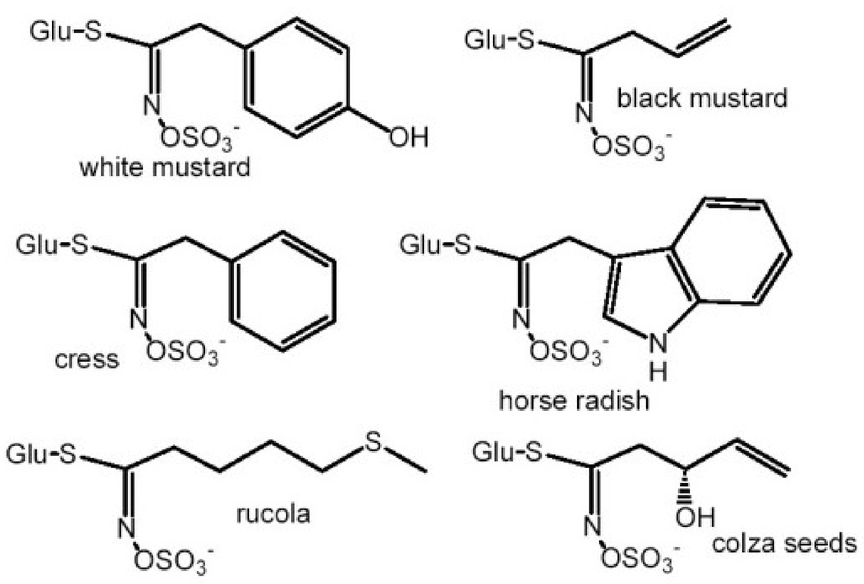


Figure 3: Glucosinolates present in different vegetables.

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Biographical Sketch

La Ferla Barbara was born in Milan in 1972, graduated in Chemistry at the University of Milan in 1997, in 2000 received her Ph.D in Chemistry at the University of Milan (Mentor Prof. G. Russo). From 1997 to 2002 she worked as a post-doc fellow at the University of Milano-Bicocca (Supervisor Prof. F. Nicotra).

In March 2003 she joined the Biotechnology and Biosciences Department of the University of Milano-Bicocca as researcher. Co-author of twenty three publications, six review articles on international scientific journals, three book’s chapters on carbohydrate and peptidomimetic chemistry, one patent and more than 40 communications at national and international meetings.

Current scientific interests focus on the synthesis and biological activity of carbohydrate analogues and glycoconjugates.