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Part B, chapter 7: Food flavors

Nathalie Cayot

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7

Food Flavors

N. Cayot¹

7.1. INTRODUCTION

The flavor of food products is one of the most important characteristics determining the choice by the consumer. Flavors are perceived through different ways: odors may be perceived before the consumer can see the product or at the opening of the food package. The product is then chewed, modified by saliva (dilution, enzymatic modification), modified by mouth temperature, and ingested. All these steps will modify the release of flavor compounds. Flavor perception is thus a multi-step and multi-factor process. During food manufacturing, both raw materials and process are contributing to the flavor profile of a final food product. Moreover the addition of exogenous flavoring substances is of common use in food manufacturing. In fact, food flavors are needed:

- to formulate a product with given sensory expectations. For example, the addition of cocoa in the paste gives a chocolate flavor to the cake.
- to hide some off-flavors (non-expected flavors). For example, vanilla can be added to soy products to hide the beany flavor of soya in a dessert cream.
- to enforce endogenous flavors and ensure the regularity of the product. For example, strawberry flavor can be added to strawberry ice cream and give a regular product whatever the season and the maturity of the strawberries.
- to compensate the modification of endogenous flavors occurring during manufacturing process. For example, orange juice is extracted from fresh oranges, concentrated, transported, and then diluted and stabilized to obtain orange juice. Orange flavor can be added to compensate flavor loss and modification during process.

¹ Équipe Procédés Alimentaires et Physico-Chimie, UMR A 02.102 PAM AgroSup Dijon/Université de Bourgogne, 1 esplanade Erasme, F-21000 Dijon

Food flavors added to products are submitted to an EU regulation. This regulation is based on a common authorization procedure for food additives, food enzymes and food flavorings (Regulation (EC) No 1334/2008 (OJ L 354, 2008). Additionally there is a positive list for approved flavoring substances; it means that substances that are specifically authorized can be used to the exclusion of other substances.

An expended list of flavoring substances has been adopted on the 1st October 2012 (Commission Implementing Regulation (EU) No 872/2012 (OJ L 267, 2012). New flavoring substances and certain complex flavoring substances must have an in depth risk assessment. The regulation indicates that flavorings must be used to improve or modify the odor and/or taste of foods for the benefit of the consumer, and not be used in a way as to mislead the consumer.

Flavoring substances are defined chemical substances, which include flavoring substances obtained by chemical synthesis or isolated using chemical processes, and natural flavoring substances.

The following definitions are given in the regulation text (Regulation (EC) No 1334/2008 (OJ L 354, 2008):

- ‘flavorings’ shall mean products:
 - (i) not intended to be consumed as such, which are added to food in order to impart or modify odor and/or taste;
 - (ii) made or consisting of the following categories: flavoring substances, flavoring preparations, thermal process flavorings, smoke flavorings, flavor precursors or other flavorings or mixtures thereof;
- ‘flavoring substance’ shall mean a defined chemical substance with flavoring properties;
- ‘natural flavoring substance’ shall mean a flavoring substance obtained by appropriate physical, enzymatic or microbiological processes from material of vegetable, animal or microbiological origin either in the raw state or after processing for human consumption. Natural flavoring substances correspond to substances that are naturally present and have been identified in nature;
- ‘thermal process flavoring’ shall mean a product obtained after heat treatment from a mixture of ingredients not necessarily having flavoring properties themselves, of which at least one contains nitrogen (amino group) and another is a reducing sugar (aldehyde or ketone function) ; many aroma compounds are produced this way by a pool of reactions named Maillard reactions;
- ‘smoke flavoring’ shall mean a product obtained by fractionation and purification of a condensed smoke yielding primary smoke condensates, primary tar fractions and/or derived smoke flavorings.

Flavoring preparations used in food manufacturing can be composed of two parts: a flavoring part (from the categories defined here above) and a non-flavoring part containing ingredients, antioxidants, preservatives, stabilizing agents, anticaking agents, water, ethanol, and other support materials.

The non-flavoring part is necessary to ensure good storage of the food flavor and to favor the application in the food product.

The flavoring part is mainly composed of volatile chemical compounds able to reach the olfactory epithelium (**Fig. 7.1.**) and be perceived as odorant.

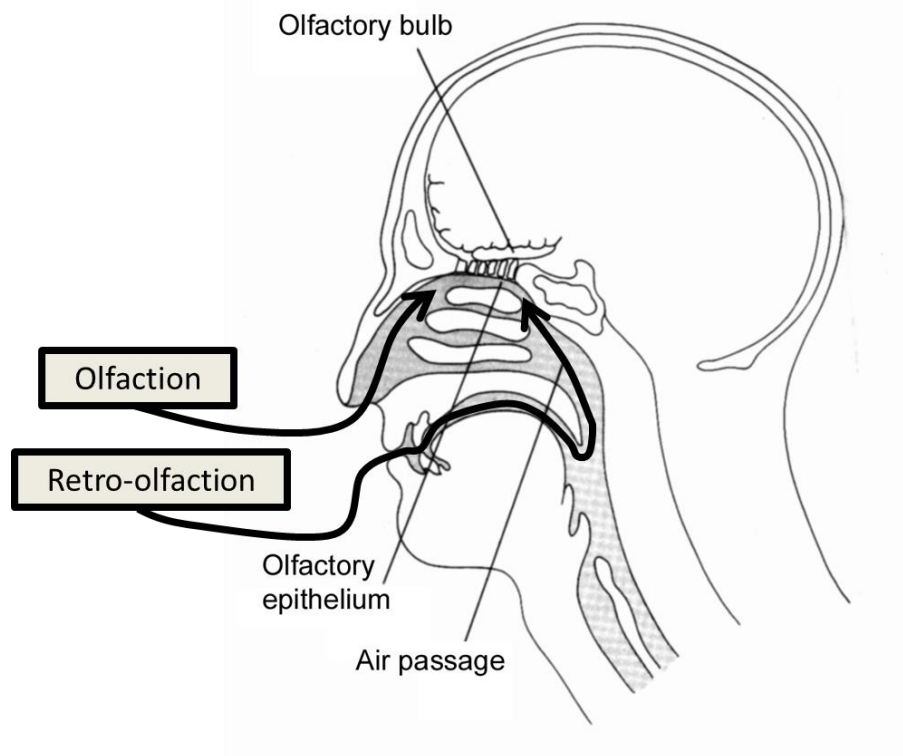


Figure 7.1. How volatile compounds can reach the olfactory epithelium?

An odor is generally composed of many different chemical compounds. The names used for the odor compounds are sometimes not clear. Commercial labels are used together with chemical names that may correspond to different nomenclatures. It is thus preferable to use CAS numbers or FLAVIS numbers given in the EU regulation to avoid mix-up.

The interactions among all these chemical compounds and also with the other ingredients within the flavoring preparation and within the food products give the final odorant profile of the product.

The aim of this chapter is to give the main keys of understanding of food flavor design and flavor release control.

7.2. THERMODYNAMICS RELATED TO FLAVOR INTERACTIONS, PARTITIONING AND KINETICS OF FLAVOR RELEASE

The release of volatile compounds may be described by three steps:

- transport within the food product towards the interface with the gaseous phase,
- partition between the food product and the gaseous phase,
- transport within the gaseous phase.

7.2.1. Thermodynamic equilibrium

Chemical potential in the gas phase

The gas phase above a product is considered as a perfect gas with water and volatile compounds. The chemical potential μ_i of a compound named i is written as a function of the partial pressure of i :

$$\mu_i = \mu_i^0 + RT \ln \frac{P_i}{P^0}$$

where μ_i^0 , the reference chemical potential for i in a gaseous state under standard conditions of temperature and pressure; P_i , the partial pressure for i and P^0 , the reference pressure.

Chemical potential in the liquid phase

In the liquid phase, the chemical potential varies with the composition of the solution :

$$\mu_i = \mu_i^0 + RT \ln x_i \cdot \gamma_i$$

where x_i , the molar fraction of i in the liquid phase, γ_i , the activity coefficient of i .

For an ideal solution, $\gamma_i=1$. It means that, chemical species are coexisting with no interaction. It is the case for infinite dilution (the activity coefficient γ_i^∞ is then constant).

For a real solution, $\gamma_i \neq 1$. $\gamma_i > 1$, if interactions between solutes and solvent are far lower than the interactions among solutes or among solvent molecules. $\gamma_i < 1$ in the opposite case.

The vapor-liquid equilibrium

For an ideal solution, the partial pressure for i is given by the Raoult law:

$$P_i = P_i^S \cdot x_i$$

where P_i^S , the saturating pressure vapor of i

For a real solution, the equilibrium between phases is given by the Henry law:

$$P_i = x_i \cdot H_i$$

H_i is defined as follows: $H_i = P_i^S \cdot \gamma_i$

If the gas phase is a perfect gas,

$$P_i = y_i \cdot P_T$$

where y_i , the molar fraction of i in the gas phase and P_T , the total pressure of the system.

The activity coefficient is then

$$\gamma_i = \frac{y_i}{x_i} \cdot \frac{P_T}{P_i^S}$$

The ratio y_i/x_i is the gas-liquid partition coefficient expressed with molar fraction. This thermodynamical parameter stands for the volatility of the compound i . Under conditions of infinite dilution, K_i is constant.

From the analysis of the gas phase and the liquid phase at equilibrium, it is then possible to measure the gas-liquid partition coefficient, with molar fraction or weight fraction:

$$K_i = \frac{C_V}{C_L}$$

Where C_V and C_L are weight concentrations, respectively in the vapor phase and in the liquid phase (g.l^{-1}).

When some interactions exist between the compound i and the other constituents of a solution, K_i is lower than its value in water. A retention or release ratio can then be calculated:

$$R = \frac{K_{\text{water}} - K_{\text{solution}}}{K_{\text{solution}}} \times 100 \text{ (\%)}$$

A positive value corresponds to retention of the volatile compound by the liquid phase; a negative value corresponds to release as compared to pure water.

7.2.2 Mass transport

Transport phenomena within the different phases can be described by two main mechanisms of mass transfer: diffusive transport in motionless phases and convective transport in well-stirred phases.

Diffusive transport

In a macroscopic, motionless (without internal movement and deformation), homogeneous (made up of one phase) and isotropic (properties independent of the

direction considered) medium, aroma compounds diffuse in the direction of their decreasing chemical potentials, until thermodynamic equilibrium is reached, i.e., when chemical potentials are the same on both sides of the medium. To solve transfer equations, a simplification is generally made, considering chemical potential as a concentration or partial pressure (in the gas phase).

Diffusion corresponds to mass transfer from one place to another place due to random agitation of molecules.

The diffusive transport model is based on Fick equations.

Fick's first law applies to a permanent rate of flow or steady state (no accumulation, i.e., no change of concentration with time) with unidirectional diffusion and in the absence of any chemical reaction.

$$J_i = \frac{dm_i}{dt} = -A \cdot D_i \frac{dC_i}{dx}$$

$$\frac{dC_i}{dx}$$

where $\frac{dC_i}{dx}$, the concentration gradient of i on the distance dx;
 D_i , the diffusion coefficient of i ($\text{m}^2 \cdot \text{s}^{-1}$).

In case of transient flow rates (variation of mass flux with time), Fick's second law, which is a derivative of the first law, applies:

$$\frac{\partial C_i}{\partial t} = D_i \frac{\partial^2 C_i}{\partial x^2}$$

where D , the aroma compound diffusion coefficient or diffusivity ($\text{m}^2 \cdot \text{s}^{-1}$); C , the aroma compound concentration ($\text{kg} \cdot \text{m}^{-3}$).

Diffusion coefficients varying from 10^{-5} to $10^{-14} \text{ m}^2 \cdot \text{s}^{-1}$ can be found in scientific literature. The highest values are found for the diffusion of small molecules in air ($D = 2 \cdot 10^{-5} \text{ m}^2 \cdot \text{s}^{-1}$ for water vapor in air).

Mass transfer model (convective transport)

- Mass transfer between a well-stirred fluid (forced convection) and a homogeneous surface

In a biphasic system with a well-stirred fluid, while mass transfer occurs between the two phases, the existence of a thin regular boundary layer with constant thickness δ and a laminar rate of flow is assumed near the interface, in which mass transfer resistance ($1/k$) occurs.

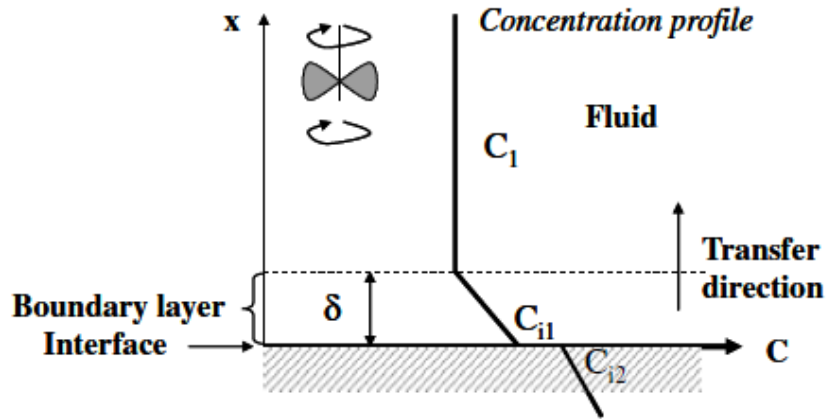


Figure 7.2.2. Mass transfer between well-stirred fluid and homogeneous surface.

Outside this thin boundary layer, mass transfers are fast enough for the fluid to be considered homogeneous at a given time and the mean concentration of the diffusing molecule to be considered constant (C_1).

In these conditions, the rate of aroma compound diffusion is given in the following equation:

$$J_i = \frac{dm_i}{dt} = -A \cdot D_i \frac{d\bar{C}_i}{dx} = -A \cdot D \frac{(\bar{C}_1 - C_2)}{\Delta x}$$

The mass transfer coefficient is defined as: $k = \frac{D}{\Delta x}$

We obtain

$$J_i = \frac{dm}{dt} = A \cdot k \cdot (C_2 - C_1)$$

- Mass transfer between two well-stirred fluids

Each phase presents a boundary layer. Mass transfer resistance only occurs in boundary layers. At the interface, both fluid concentrations reach equilibrium.

The partition coefficient K_i of compound i between fluid 2 and fluid 1 is defined as follows:

$$K_i = \frac{C_2^*}{C_1^*}$$

Mass transfer from fluid 1 to fluid 2 is given by:

$$J = A \cdot k_{app} (\bar{C}_1 K_i - \bar{C}_2)$$

where, k_{app} the apparent mass transfer coefficient :

$$\frac{1}{k_{app}} = \frac{1}{k_2} + \frac{K_i}{k_1}$$

7.2.3. Transfers between phases

Partition and transfers occurred between the different phases of a food product all along its shelf life and during food consumption. Different phases can be listed, for example:

- Food and saliva layer on tongue
- Food product and gas phase above it in the food package
- Different phases of the food product that undergoes modifications and is no more at equilibrium.

Volatile compounds move as a function of their affinity for the different phases. Transfers of volatile compounds are often coupled with transfers of other small molecules such as water, coloring molecules, protons...

Various factors are affecting release and listed hereafter.

- The properties of volatile compounds

For pure compound: chemical class, saturating vapor pressure, melting temperature, molar mass.

In a homogeneous phase: solubility, partition coefficient (K), activity coefficient γ_i .

In a heterogeneous phase: hydrophoby (logP), apparent partition coefficient (K_{app}).

Hydrophoby is often defined as logK between water and n-octanol. It generally varies from -3 to 7. Compounds with logP < 1 can be considered as relatively polar. Negative values correspond to hydrophilic compounds.

- Food products

Effects are observed through composition (that can induce specific molar interactions) and structure (that can hinder transport phenomena).

Generally speaking, for interactions between a macromolecule (M) and a ligand (L), the following equilibrium is observed:



$$K_a = \frac{[ML]}{[M][L]} \quad \text{Where } K_a \text{ the association parameter.}$$

Molecular interactions are due to existing attractive or repulsive forces between groups of molecules whose valences are satisfied.

The attractive forces include several types of interactions: interactions of van der Waals, hydrogen bonds and hydrophobic interactions. They depend in particular on the presence of electric charges and polarity of the various connections involved.

Such interactions may influence the release of volatile compounds, modifying their partition coefficient, and, as a consequence the mass transfer.

Depending on the composition of the food product, volatile compounds can be trapped, absorbed or adsorbed, covalently bound, or encapsulated.

The release of volatile compounds is influenced by dry matter, pH, and ionic strength. For instance, the presence of solutes such as salts or sucrose may induce an increase of release known as « salting out » effect.

Food structure may also have an impact on flavor release. Liquid systems are relatively simple to understand considering partition and release. For viscous or solid systems, additional parameters such as diffusion must be taken into account. More complex structures such as foams and emulsions were also studied.

The diffusion of molecules is inversely proportional to viscosity, as described by the Stokes-Einstein law:

$$D_i = \frac{kT}{6\pi \eta r}$$

where k, Boltzman parameter; T, temperature (K) ; η , dynamic viscosity of the solution (Pa.s) and r, radius of the molecule (m).

It is noticeable that temperature as a direct influence on diffusion.

Is also has a strong influence on volatility (K) (Voilley & Roques, 1987) :

$$K = A \cdot \exp\left(-\frac{H_v}{RT}\right)$$

where H_v , volatility energy ; R, perfect gas parameter, and T, temperature (°K).

When the gas phase is renewed by a gas flow, the concentration of the volatile compound in the gas phase is the result of equilibrium between its release in the gas phase and its extraction by the gas flow.

When the food product is stirred, mass transfers are accelerated. Mastication of the food product also has an impact on the exchange surface and thus modifies the concentration gradient of the volatile compound.

7.3. VOLATILE COMPOUNDS AND AROMA COMPOUNDS

Volatile compounds are chemical molecules of small molecular mass (below 250). Being volatile does not mean being odorant. To be odorant, the compound must not only reach the olfactory epithelium, but also interact with the olfactory receptor cells, give a signal transmitted by the olfactory nerve, and finally be interpreted by the brain as an odor.

7.3.1. Physicochemical characteristics of volatile compounds

An odor can be given by one molecule, for example vanilla odor is mainly given by vanillin, but, more frequently, an odor is the result of the action of numerous volatile compounds. The odor of coffee was analyzed to be the results of above 650 compounds coming from coffee beans, compounds produced by the fermentation of coffee beans, and compounds produced by thermal treatments such as roasting (Richard, 1992).

In fact, volatile compounds are of various origins (**Figure 7.3.1.**).

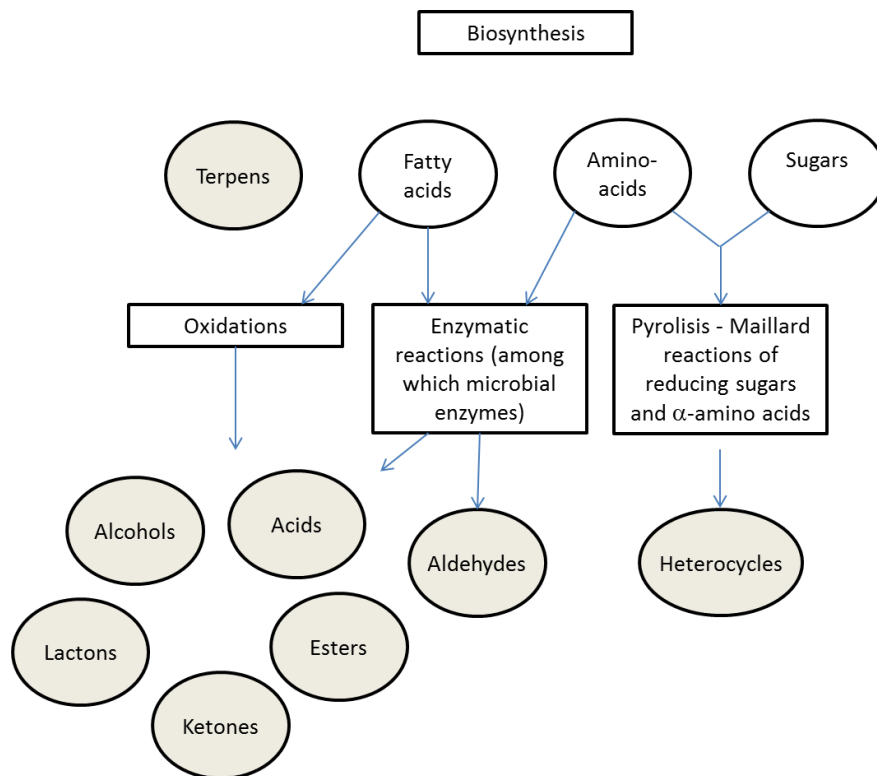


Figure 7.3.1. Biogenesis of aroma compounds: main simplified pathways

Some precursors exist in raw materials and are transformed due to reactions occurring during the product life: oxidations, microbial metabolism, enzymatic reactions, Maillard reactions.

Volatile compounds may be produced from fatty acids, from amino acids (Strecker degradation), from acetic acid (to give terpenoides), from sugar (caramelization) or from a combination of reductive sugar and molecules that contain amino groups (Maillard reactions).

Autoxidation gives carboxylic acids with short chains, aldehydes, and ketones, which can give a rancid odor. This type of odor is generally described as off-flavors.

The result of all these reactions implied in the formation of flavor is that volatile compounds may be from all types of chemical classes.

Some physicochemical characteristics of these compounds are important for their use, such as vapor pressure of the pure compound, solubility in water or in other solvents, hydrophobicity (generally given by the partition ratio between octanol and water), and volatility (partition between air and water).

Some data are given in **Table 7.3.1.** to show the wide range of these parameters.

Table 7.3.1. Some usual volatile compounds and their physicochemical characteristics.

Compounds	Solubility in water (25°C) g/L	Solubility (others)	Log water	P _{oct.}	Vapor pressure (25°C) Pa	K _{air-water} (30°C)
Ethyl-butyrate	5.65	Miscible alcohol, ether	1.80		1885	1.83. 10 ⁻²
Furaneol	< 10%	1g/ 4ml ethanol at 95%	0.82		-	
Cis-3-hexenol	<1%		1.61		-	5.36 10 ⁻⁴
Vanillin	10		1.21		0.003	
Hexanal	2.60		1.80		1420-1517	1.16 10 ⁻²
β-ionone	very low		3.57		-	

(Savary, Doublier, & Cayot, 2005)

These characteristics are the basis to understand how each molecule will behave in a food product and mainly how it will interact with other ingredients. For example, ethyl butyrate is more soluble in water and more volatile than hexanal despite the fact they have the same hydrophobicity.

7.3.2. Reactivity and storage of volatile compounds

As many volatile compounds are necessary to prepare a formula used to flavor a manufactured food product, a first step is to be able to mix together different volatile compounds in a solvent. Various solvents can be used depending on the final use of the food flavor. Some frequently used solvents are propylene glycol, triacetin, alcohol.

Mixing molecules of different chemical classes together in a solvent allow them to react with each other's. For example, aldehydes such as hexanal and vanillin may interact with alcohol such as propylene glycol to form hemiacetals or acetals (**Figure 7.3.2.1.**). The formation of such compounds is an equilibrium that depends on pH conditions (notice that food products are mainly acidic). Therefore, a volatile compound can be modified when added to the mixture and give no odor or a different one. Conversely, some new compounds may appear in the mixture.

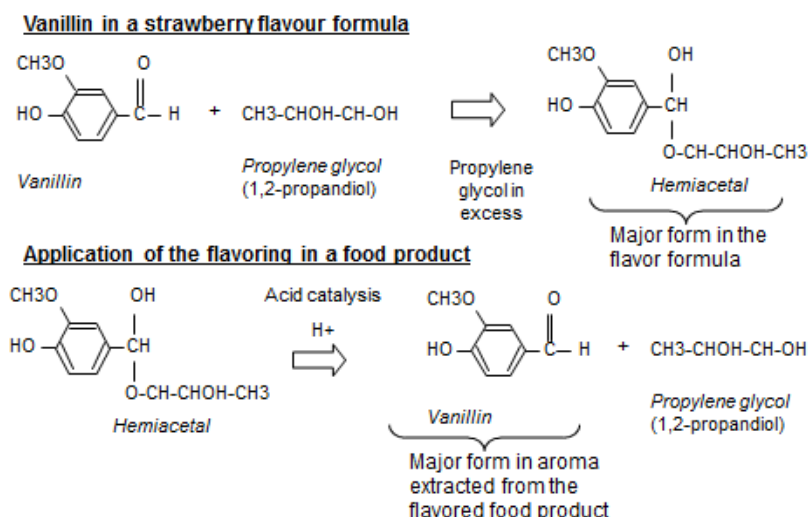


Figure 7.3.2.1. Reactivity of volatile compounds in mixture. Example of vanillin in a strawberry flavor formula (Savary, 2006).

As seen from **table 7.3.1.**, volatile compounds may be of very different solubility and hydrophobicity. Therefore, it is sometimes impossible to store the different compounds in mixture. For example, citrus or orange flavors are often in two separated phases,

- one aqueous phase which was obtained from the juice containing for example aldehydes among which acetaldehyde,

- one apolar phase which was obtained from the orange peel and which is the bulk of orange essential oils containing terpens such as limonene. (Rega, 2003)

Both phases are mixed together in the final product for juices produced with concentrated extracts.

Some pure aroma compound may also be in a solid state at ambient conditions. It is the case for vanillin (CAS number: 121-33-5; melting point 81-83°C) that needs to be heated to be solubilized in a liquid flavor formula to be applied during the production of a liquid product, or remain as solid in a powder formula to be applied in a powder mixture.

Another important aspect of reactivity is the sensitivity to oxidation in presence of oxygen. As oxidation is favored by light (with pigments) and temperature, flavor formula must be stored at cold temperature and in the dark. Another way to prevent compounds from oxidation is to use encapsulation.

7.3.3. Perception of volatile/odorant compounds

As a function of their physico-chemical characteristics, volatile compounds will reach more or less easily the olfactory epithelium and be perceived or not as odorant. For each compound, the quantity necessary for it to be detected as odorant can vary a lot. Moreover, higher concentrations are necessary for it to be recognized and named. Some examples of detection thresholds are given in **Table 7.3.3.1.**

Table 7.3.3.1. Some examples of detection thresholds of odorant compounds

Volatile compounds	1 g in ...m ³	concentration
Ethanol	0.01	1·10 ⁻⁴
Bis(2-methyl-4,5-dihydrofuran-3-yl)disulfur	50	2· 10 ⁻⁸
α-ionone	250	4· 10 ⁻⁹
Nor-isoborneol	29000	3.5· 10 ⁻¹¹
Geosmine	67000	1.5· 10 ⁻¹¹
β-ionone	140000	7· 10 ⁻¹²
Methyl-1-bicyclo[3,3,0]-dithia-2,4-oxa-8-octan	2500000	4· 10 ⁻¹³
Trichloro-2,4,6-anisol	33000000	3· 10 ⁻¹⁴

Therefore some odorant compounds may be at very low concentration in a product and be the main compound from a sensory point of view.

The sensory descriptor of an odorant compound is not strictly linked with its chemical class. Nevertheless some general classification has been reported and can be useful to have an idea of the expected odor of a mixture. For example, esters often have fruity descriptors. Such classification is given in **Figure 7.3.3.1.**

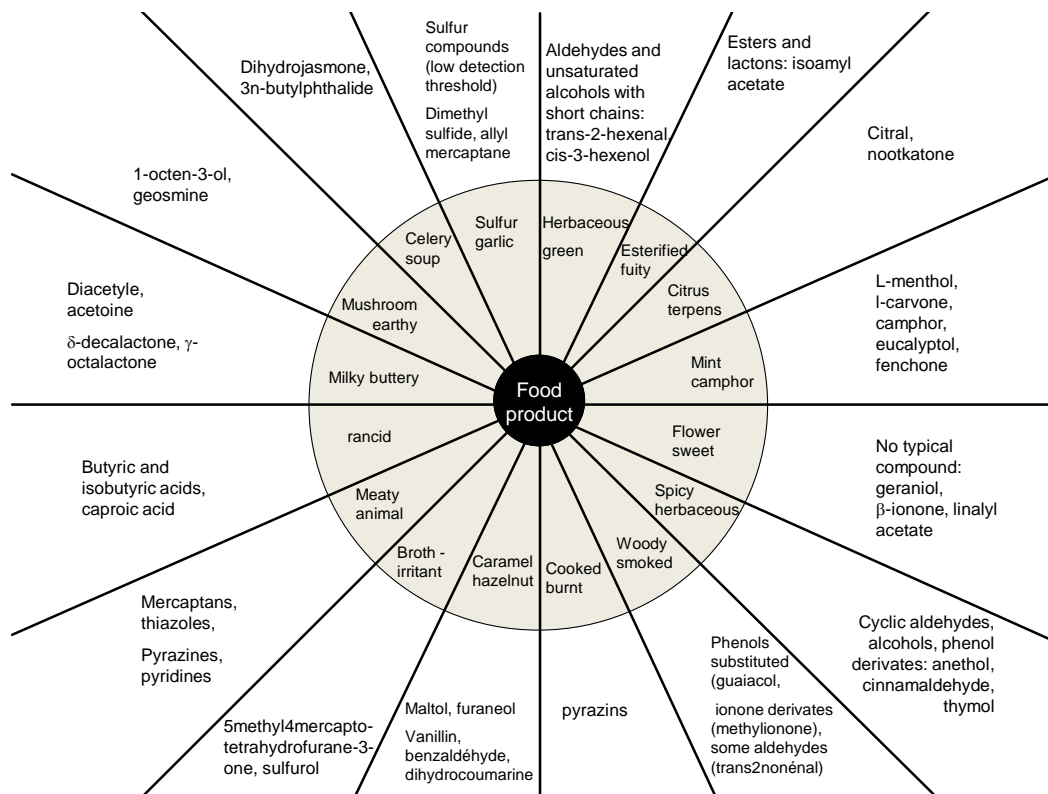


Figure 7.3.3.1. Classification of odor as a function of chemical class

It must be noticed that the sensory descriptor of a pure compound can vary with its concentration, and can become disgusting when too concentrated. For example, 4-methoxy-2-methylbutanethiol was reported as very important for blackcurrant odor, but in high concentration was described as “cat pee” (Latrasse, Rigaud, & Sarris, 1982). The easy oxidation of thiol ($2\cdot\text{R-SH} + \text{O}_2 \rightarrow \text{R-S-S-R} + \text{H}_2\text{O}$) could be another explanation: the disulfide could smell cat pee.

The formulation of a food flavor, as the formulation of a perfume, is therefore a question of equilibrium between different compounds in an appropriate solvent. Some example of food flavor composition is given in **Table 7.3.3.2.**, showing that slight variations in the composition lead to different overall perceived odor.

Within such a list of compounds, some molecules are more important as others for the overall aroma perceived. They are named “key aroma compounds”. Some authors analyzed the aroma of food products using a technique named Gas Chromatography olfactometry (GC-O) and used it with different dilutions of the extract to determine the relative odor potency of the compounds. An odor active

value (OAV) is calculated as the ratio of the concentration of the compound to its odor threshold. The odor potency is said to be proportional to the OAV. Such methods allowed the determination of key aroma compounds of various products (W. Grosch, 2007).

Table 7.3.3.2. Example of food flavor formula

odor	Apple	Banana	Pear	Pineapple
1-butanol	30	5	30	1
2-methyl butanol	50	5	50	5
1-Hexanol	30	5	40	1
Amyl acetate	0	50	10	20
Isoamyl acetate	5	150	5	5
Ethyl butyrate	5	40	10	10
Amyl butyrate	5	30	20	20
Heptyl acetate	5	5	100	5
Ethyl-2-methyl butyrate	5	10	5	20
Allyl caproate	5	5	5	120
Citronellyl acetate	5	5	40	1
Hexanal	100	1	5	1
(E)-2-hexenal	100	10	30	5
Benzaldehyde	0.1	0.2	0.2	0.1
Vanillin	1	30	1	30
Eugenol	0.1	2	0.2	0.1
Ethanol	693.8	686.8	638.6	770.8
total	1000	1000	1000	1000

From (Ziegler, 2008)

Beyond these key aroma compounds, the other volatile compounds present in the flavor formula are very useful to give authenticity to the flavor and to make the flavor more subtle.

For example, a strawberry flavor composed of 10 major compounds will be perceived as chemical or candy-like strawberry as compared to a strawberry flavor composed of 100 compounds with more subtle notes and that will be perceived as closer to the aroma of ‘the strawberry of my grandmother’s garden’ or the aroma of ‘the wild strawberry’.

Gas chromatography-olfactometry is a technique based on sensory evaluation of the eluate from a chromatographic column. The concentration of the extracts used for GCO analysis must be chosen in order to ensure correct detection by instrumental detection and perception by panelists.

The column is connected to both a detector (for example, flame ionization detector) and a sniffing port. Humidified air is added via the sniffing port to avoid dehydration of nasal mucosa of the panelists. Panelists are asked to smell the effluent of the column and to give the start time and a verbal description of each perceived odor. The responses of the panelists are analyzed in parallel with the instrumental results.

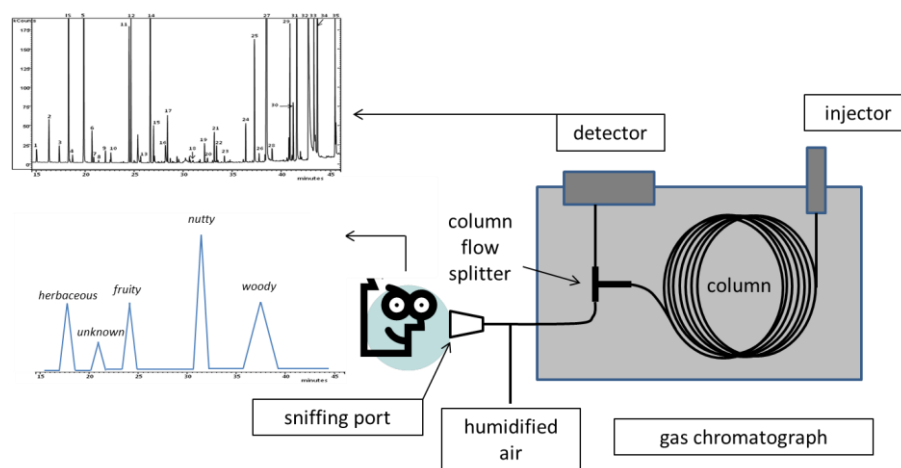


Figure 7.3.3.2. Gas-Chromatography-olfactometry: principle of the method

This method has been used to identify key aroma compounds and off-flavors in several food products (W. Grosch, 1994). Different methods can be used to characterize the odor activity of the volatile compounds: detection frequency, dilution to threshold, and direct intensity (Delahunty, 2006).

7.4. IMPACT OF INTERACTIONS, STRUCTURE, AND TEXTURE ON AROMA PERCEPTION

Once the correct equilibrium is obtained in the flavor formula, it must be tested in the food product to be flavored. Given the fact that other ingredients of the food product may interact with each of the volatile compounds, this step is not the easiest to handle.

7.4.1. Interactions between volatile and non-volatile compounds

Lipids, polysaccharides and proteins may all interact with volatile compounds, but in different manners. The result of interactions can be measured on the partition coefficient of the volatile compounds between the vapor phase and the product.

7.4.1.1. Interactions between volatile compounds and lipids

Lipids contained in food products are mainly composed of triglycerides which can solubilize great amounts of volatile compounds, which are mainly hydrophobic or at least partly hydrophobic compounds. From a chemical point of view, interactions with lipids depend on the composition in fatty acids, the chain length, and the unsaturation rate. The higher the chain length, the lower the solubility of aroma compounds in lipids. The more the lipid is unsaturated, the more the volatile compounds are retained in the lipid fraction. Partition coefficients (vapor / lipid) of some volatile compounds at 25°C are reported in **Table 7.4.1.1**.

Table 7.4.1.1. Partition coefficients (vapor / lipid) of some volatile compounds in various lipids at 25°C

Type of lipid	Ethyl butyrate	Ethyl hexanoate
Triolein	$2.2 \cdot 10^{-4}$	$3.0 \cdot 10^{-5}$
Tricaprin	$2.1 \cdot 10^{-4}$	$4.9 \cdot 10^{-5}$
Sunflower oil	$8.5 \cdot 10^{-3}$	$2.5 \cdot 10^{-4}$

From (Meynier, 2012)

Benjamin et al. ((Benjamin, Leus, & Everett, 2011)) studied the release under equilibrium conditions of volatile compounds from oil-in-water emulsions stabilized by β -lactoglobulin.

The effects of lipid type and mass fraction were investigated using soy oil and anhydrous milk fat. Gas-matrix partition coefficients were measured at 40°C by static headspace gas chromatography for five volatile compounds: 1-propanol, ethyl butyrate, heptanal, octanol and 2-decanone.

The more hydrophobic volatile compounds were retained to a higher degree in the oil and emulsion systems than in the aqueous solution. The increase in lipid content in the emulsions caused a significant reduction in K values.

However, a change in lipid type (did not have such a pronounced effect on the release, except for octanol and 2-decanone which were more strongly retained by the AMF emulsion

Table 7.4.1.2. Partition coefficients (vapor / liquid, $K \times 10^3$) of some volatile compounds in aqueous solution, oil, and 40% o/w emulsion stabilized by 1% β -lactoglobulin at 40°C

Volatile compound	Log P	Aqueous solution	Oil	Emulsion
1-Propanol	0.25	0.71	9.89	1.41
Ethyl butyrate	1.85	17.74	1.00	1.53
Heptanal	2.29	29.30	0.60	0.59
Octanol	3.00	5.65	0.31	0.47
2-Decanone	3.73	50.87	0.05	0.61

From (Benjamin, Leus, & Everett, 2011)

At this temperature, all the tested lipids are considered liquid. This is important to notice because the physical state of lipids is of great importance ((Relkin, 2004)). In fact, viscosity, density and solid fraction at given temperature have an impact on volatile compounds retention and release. At temperatures below their melting point, lipids are crystallizing. The formation of crystals can induce the release of volatile compounds.

When eating a fatty food product, lipids containing aroma compounds are melting in the mouth and, thanks to temperature increase and to chewing, aroma compounds can be easily released in the vapor phase and be perceived.

7.4.1.2. Interactions between volatile compounds and polysaccharides

Among polysaccharides, starches and their derivatives are frequently used in food products. We also found cellulose, galactomannans, agars, carrageenans, alginates, pectins, microbial polysaccharides, gums, and mucilages...All of them are often used in mixture to give well defined viscosity or texture properties. There are widely used as thickener, gelling agent, stabilizer and fat replacer in many products such as dressings, dairy products, and candy products. They are also used to encapsulate aroma compounds and to stabilize flavor for soft drinks.

Texturing agents are modifying the viscosity, which induces a significant decrease in perceived flavor above the critical concentration (c^*) at which entanglement begins.

However thickened solutions of similar viscosity do not induce the same flavor perception, indicating that both viscosity and binding affect flavor release and perception. Some of the polysaccharides not only have an impact on the physical properties of food but can also induce a drastic decrease of the volatility of aroma compounds.

From this point of view, starch is the most studied polysaccharides. Natural and processed starches occur under various partially crystalline types defined by their X-ray powder diffractograms. The corresponding structures result from different

helical conformations and packing arrangements. The ability of starch to interact with some ligands, particularly aroma compounds, has been extensively studied. It may entrap aroma compounds simply by hydrogen bonding with hydroxyl groups of starch. More particularly, the amylose fraction of starch is able to form complexes with hydrophobic compounds of small steric hindrance (**Figure 7.4.2.1.2.1.**). The so-called V-types are obtained after addition of complexing agents.

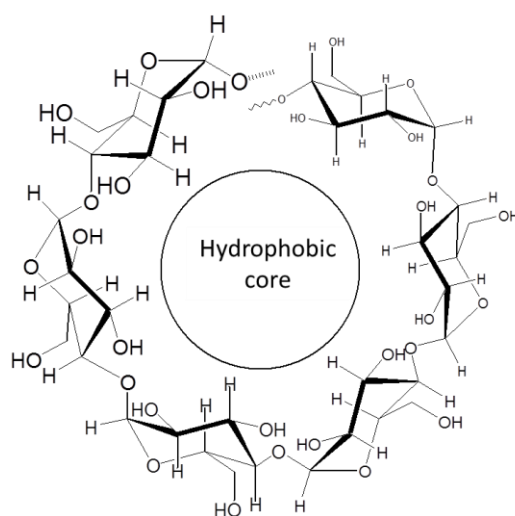


Figure 7.4.2.1.2.1. Structure of the amylose helical chain

In fact, amylose is one of the starch polymers. It has a helical structure and is able to form semi-crystalline complexes which are known for their high aroma retention (Jouquand, Ducruet, & Le Bail, 2006).

Depending on the ligand, the structure of V amyloses can be classified into two families: V_6 and V_8 where 6 and 8 represent the number of D-glucosyl units per turn. In V_6 amylose, three types of crystalline packing (V_{6I} , V_{6II} and V_{6III}) may be obtained depending on the nature of the complexing molecule which can be trapped either in the single helix of amylose (V_{6I}) or both within and between amylose helices (V_{6II} and V_{6III}) (Helbert & Chanzy, 1994) (**Figure 7.4.2.1.2.2.**).

Some starch derivatives such as maltodextrins and β -cyclodextrins are exhibiting a structure identical to the one of amylose with a hydrophobic core and a hydrophilic external surface. This structure gives them the ability to trap aroma compounds as well.

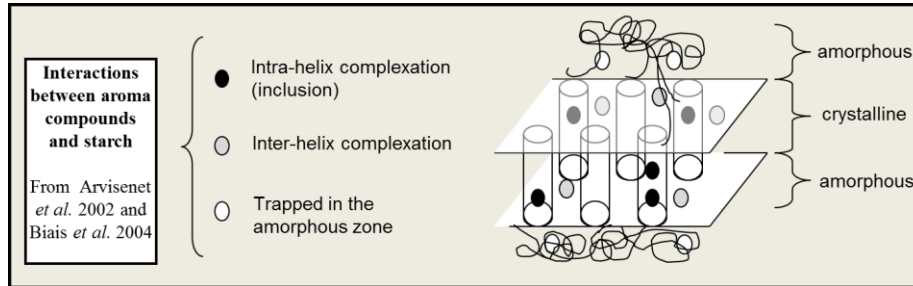


Figure 7.4.2.1.2.2. Different type of interactions of volatile compounds with starch and amylose (Arvisenet, Le Bail, Voilley, & Cayot, 2002; Biais, Le Bail, Robert, Pontoire, & Buléon, 2006)

Some part of the aroma compounds trapped by amylose (inclusion complexes) cannot be easily release and thus are lost for perception. Amylases present in saliva can help the release of aroma compounds during chewing of a food product.

In the example used in **Figure 7.4.2.1.2.3.**, the aroma compound is encapsulated using β -cyclodextrin, a starch derivate composed of 7 glycosyl units forming a cylinder of internal Ø 0.60-0.65 nm (hydrophobic cavity). The solubility in water at 25°C is 1.85% (w/v). The more hydrophobic the compound, the easier the trapping by β -cyclodextrin.

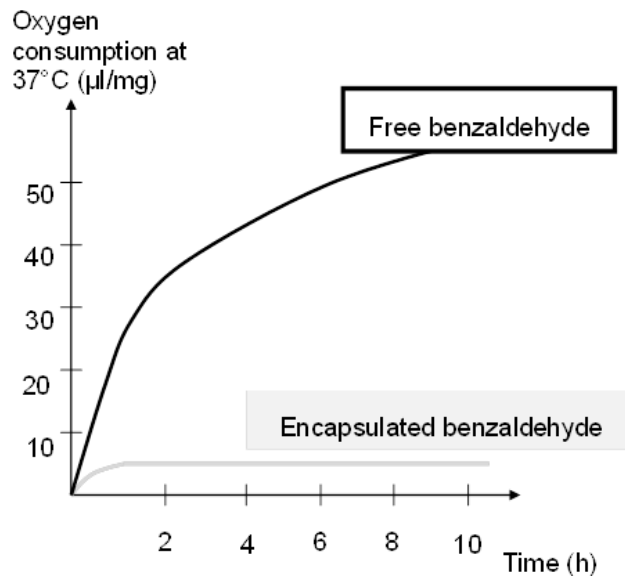


Figure 7.4.2.1.2.3. The role of encapsulation to prevent oxidation of volatile compounds. Example of benzaldehyde (CAS number 1477-19-6). (Matheis, 2008)

Figure 7.4.2.1.2.3 allows comparing the oxygen consumption, that means oxidation, of benzaldehyde, a volatile compound with bitter almond odor, as free compound and as encapsulated in β -cyclodextrin. It is clear from this example that encapsulation is a good way to slow down oxidation.

Encapsulation is also used to obtain a controlled-release of the flavor. For that purpose many biopolymer-based materials can be used, such as Arabic gums, starches, alginates. Proteins (gelatin, casein, soy proteins) and lipids are also used. (van Soest, 2007)

Considering polysaccharides others than starch, studies are far less numerous. The impact of the polysaccharides is above all a physical effect on aroma release, i.e. an effect via the modification of the structure and of the texture of the food product.

7.4.1.3. Interactions between volatile compounds and proteins

The majority of the studies concerning interactions between aroma compounds and proteins were done in aqueous solutions, with varying conditions of pH, ionic strength, and temperature. The most studied protein is β -lactoglobulin, which is a well-known soluble milk protein widely used in the food industry due to its emulsifying properties. Other proteins were also studied, such as caseins and soya proteins.

β -lactoglobulin was reported to interact with a great variety of compounds such as aldehydes, ketones, ionones, and esters (Tromelin, Andriot, & Guichard, 2006). Within a given homologous series, the affinity of volatile compounds for β -lactoglobulin is increasing with the length of the aliphatic chain i.e. the hydrophobicity. It is then generally admitted that hydrophobic interactions are existing between volatile compounds and β -lactoglobulin. Different binding sites have been identified (**Figure 7.4.1.3.**)

It must also be noticed that aldehydes may form covalent binding with proteins (NH_2 residues). All these interactions or reactions are sensitive to pH because this parameter influences the conformation of the proteins, and, as a consequence, the accessibility of binding sites. For example, an increase of retention of methylketones, esters, and terpenes was measured between pH 3 and 9, as compared with conditions between pH 9 and 11 when the protein is denatured.

Studies done with other proteins confirmed that the most hydrophobic molecules were the most retained ones (Damodaran & Kinsella, 1980).

Bovine serum albumin was reported to have high retention capacity (Jasinski & Kilara, 1985). This protein contains 17 cysteyle residues, and is thus very reactive towards sulfur compounds such as allyl isothiocyanate (Kawakishi & Kaneko, 1987). This was also observed in a lesser extent with ovalbumin and lysozyme.

The retention of volatile compounds by soya proteins was found to be intermediate between the one obtained with bovin serum albumin and β -lactoglobulin. One of the soya proteins, β -conglycinin, was reported to have higher retention capacity (O'Neill & Kinsella, 1987).

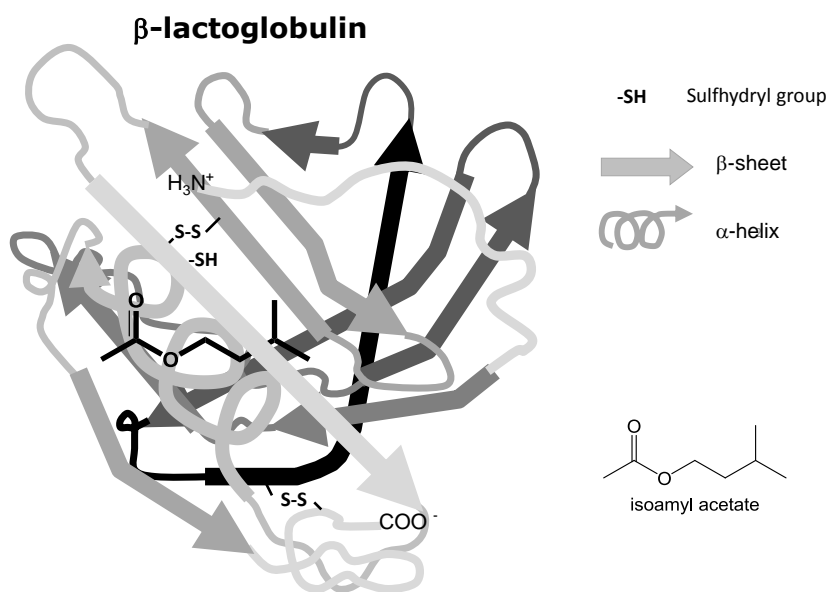


Figure 7.4.1.3. Tertiary structure of β -lactoglobulin with trapping of isoamyl acetate in the hydrophobic cavity

To perceive an effective decrease of odor intensity due to protein addition, the retention percentage must be at least of 20%. The formation of covalent binding between proteins and aldehydes can be used to eliminate some off-odors because the resulting chemicals are often odorless (Meynier, 2012).

7.4.2. Effect of structure and texture on diffusion and perception

The understanding of how flavor compounds can interact with other ingredients is indispensable but not sufficient to fully understand what is occurring in a real food product. Food products are mixtures containing all the ingredients cited here above, and moreover these ingredients together with physical treatments lead to a specific structure and a perceived texture of the food.

Food can be suspensions, emulsions, gels, foams or all of them together. Considering the impact of such structures on flavor release, studies have been focused on emulsions and gels.

7.3.2.1. Diffusion and perception of aroma compounds in gels

In fact, every thickener or gelling agent, if above a given concentration, can induce the decrease of the aroma release by reducing the diffusion of the small molecules in the thickened or gelled system (**Figure 7.4.2.1.**).

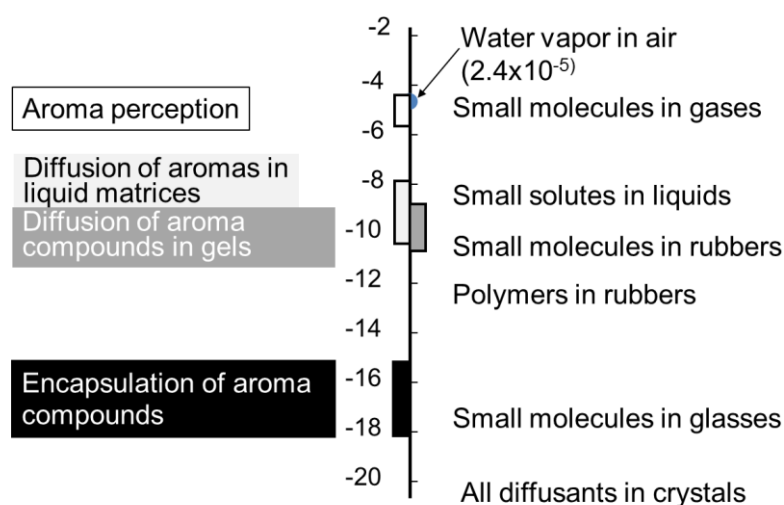


Figure 7.4.2.1.: The effect of the state of the matrix on mass transfer. Typical values of D, the diffusion coefficient (10^{-m²/s}) of flavor compounds in different matrices.

7.4.2.2. Diffusion and perception of aroma compounds in emulsions

An emulsion is a non-stable mixture of two non-miscible liquids where droplets of one liquid are dispersed in the second liquid (the continuous phase). In food products, the continuous phase is mainly an aqueous phase, and the dispersed phase is mainly an oil phase. The contact surface between both liquids is named interface and contains some surfactants added to stabilize the emulsion. In food products, surfactants are often proteins, lecithin, mono and di-glycerides, and sucro-esters.

The perceived aroma compounds i.e. the amount of aroma compounds present in the vapor phase depends on the location of the aroma compounds in the emulsion.

The relative concentrations of aroma compounds within the different phases of the emulsion (dispersed phase, continuous phase, interface between continuous and

dispersed phases, interface between the emulsion and the vapor phase) depend on the nature of the different phase, on the physical state, and on their affinity for aroma compounds.

The higher the volume fraction of the oil phase in the emulsion, the more important the retention of hydrophobic aroma compounds in the emulsion.

Because of their interactions with aroma compounds, the proteins located at the oil/water interface will slow down the transfer of aroma compounds from oil phase to water phase and, as a consequence, slow down the transfer from the emulsion to the vapor phase (Charles, Rosselin, Beck, Sauvageot, & Guichard, 2000).

If the size of the droplets is decreasing, the surface of the interface is increasing. McClements (McClements, 1999) reported a theoretical relationship between the volatility of an aroma compound and the size of the droplets in the emulsion. Above a 10 μm diameter, partition coefficient remains constant. This theoretical relationship is hardly verified experimentally due to variations of behavior from a volatile compound to another.

7.4.2.3. Diffusion and perception of encapsulated aroma compounds

To prevent aroma compounds from oxidation or from other types of degradation, they can be encapsulated. Various techniques are employed to form the capsules, including different types of drying, coating, liposome entrapment, coacervation, inclusion complexation...

Fats, starches, dextrans, alginates, protein and lipid materials can be employed as encapsulating materials.

Many patents are existing using various matrices to entrap volatile compounds. For example:

- Formation of spheres of a solid suspension from a solid fat matrix and a non-fat solid substrate with an active ingredient
- Use of an oil-in-water emulsion from a vegetable oil, an aqueous medium and a water soluble carbohydrate film forming agent. The oil-in-water emulsion is sprayed onto a soluble beverage powder, whereupon the aqueous layer of each droplet evaporates to form capsules. The soluble beverage is dissolved in hot water to release the aroma flavor.
- Another method for encapsulating volatile aroma compounds involves dissolving an inert gas in an aromatized edible liquid, then co-extruding the gas-liquid mixture with a molten carbohydrate material. The result is a continuous stream of carbohydrate outer shell surrounding an inner core of aromatized, gasified liquid. When these capsules, which can be incorporated directly into an instant consumer product, contact hot water the inert gas in the inner core expands and ruptures the capsule wall-releasing the aromatized liquid.
- More simple process are also used such as aroma solution mixed with a carbohydrate matrix, and then dried (for example, vanilla sugar).

In such systems, the aroma compounds are protected when kept in correct storage conditions. The use in food product implies heating or hydration that will allow aroma release. The aroma delivery can then be controlled, depending on the solubility or melting of the capsule walls. Here again, thermodynamics can be used to understand aroma release.

7.5. FLAVORING AND FOOD PROCESS: MAIN FACTORS TO TAKE INTO ACCOUNT

Once you know the different types of interactions that can occur and the role of food structure, several questions are remaining:

- What about interactions in complex systems? What about competitions among flavor compounds and towards other ingredients?
- When is the best moment to flavor the product? Is there an order to follow to put the ingredients in the food formula?

To answer this last question, you also have to consider the process the product will undergo with its different operating units.

In fact food manufacturing process generally comprises operating units such as mixing, thermal treatments to remove water or to stabilize the product, particle size modification, and dilution.

Important parameters are modified during these steps: temperature, pressure, water content and activity, gas composition. All of them are of great impact on volatile compounds behavior, as shown before.

Finally, the phenomena occurring must be taken into account throughout the lifetime of the product from manufacturing to consumption, and throughout storage.

Some applications of such principles and reasoning can be evoked through the production of an orange juice from concentrate.

Juice from concentrate used squeezed fruit juice as raw material, to which about 80% of its original water has been removed. The concentrate thus obtained is easier to store and transport. Arrived at the place of bottling, the juice is reconstituted with the same amount of water as the original juice. The legislation authorizes restitution of flavor and pulp lost during the process of concentration, from fruits of origin or from other fruits, but the same variety. A simplified production chart is given in Figure 7.5.

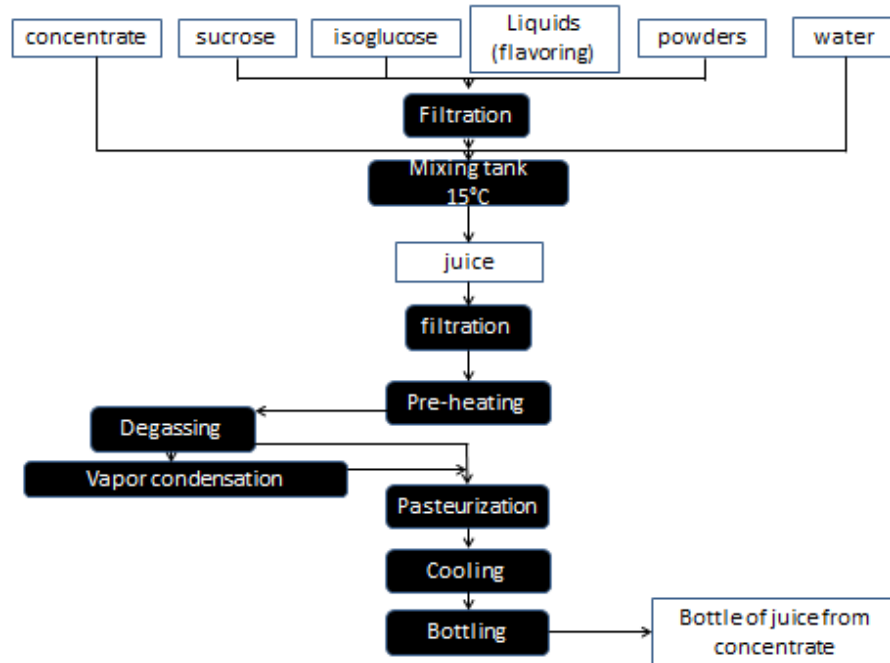


Figure 7.5. Simplified process for the production of fruit juice from concentrate.

Formulation of the orange juice

In this product, some aroma compounds are present in the concentrate. They were coming from the juice directly obtained from fruits. This juice underwent centrifugation and pasteurization. Thus some aroma compounds were lost, or oxidized, or modified, due to temperature increase during process that will have an impact on volatility. Other raw materials added in the product (sugars) are poor in volatile compounds but give dry matter that can be a good support for aroma compounds.

Flavoring preparations can be added in a liquid form. For example an orange flavoring with an aqueous phase and a lipid phase is often used. All the volatile compounds will be mixed together. Partition and transfer will be modified.

Limiting aroma modification through the process

After mixing of all the ingredients and filtration, the obtained juice is pre-heated. This pre-heating allows the de-gassing of the juice. Indeed, gas will expand when temperature is increasing. This step is interesting to preserve the flavoring that was previously added because oxygen is removed and then further oxidation will be limited.

Nevertheless, some aroma compounds will also be lost because volatility will increase together with temperature. That is why, at this step, the vapor phase is collected by condensation. The vapor phase will contain water but also some volatile compounds, probably the most hydrophilic compounds. The condensate will then be re-introduced in the juice to limit aroma loss.

Then pasteurization occurs. In modern production plants, flash pasteurization is used in order to limit the effect of temperature. Thus, Maillard reactions are drastically limited. The aroma compounds produced by Maillard reactions can thus be limited.

A correct storage of the bottled juice must then be observed in order to avoid further degradation of the aroma. Despite all the precautions taken, the two phases of the orange flavoring are often demixing. The separation of the lipid phase from the juice is thus resulting in the apparition of a yellowish annulus at the top of the bottle. In other processes, come pulp can be added to the juice and can be a good support material for the aroma compounds.

7.6. KEY FACTS

- ✓ Raw materials and process are contributing to the flavor profile of a final food product, but the addition of exogenous flavoring substances is often necessary in manufactured products.
- ✓ Food flavors added to products are submitted to EU regulation (1331/2008/EU).
- ✓ Flavoring preparations can be composed of two parts: a flavoring part and a non-flavoring part to ensure correct application and storage.
- ✓ Volatile compounds are chemical molecules of small molecular mass.
- ✓ To be odorant, the volatile compound must not only reach the olfactory epithelium, but also interact with the olfactory receptor cells, give a signal transmitted by the olfactory nerve, and finally be interpreted by the brain as an odor.
- ✓ An odor is generally the result of the action of numerous volatile compounds.
- ✓ Volatile compounds are of various origins and of all types of chemical classes.
- ✓ The formulation of a food flavor is a question of equilibrium between different compounds in an appropriate solvent.
- ✓ Lipids, above all in liquid state, can solubilize great amounts of volatile compounds.
- ✓ The amylose fraction of starch is able to form complexes with hydrophobic compounds of small steric hindrance which are thus lost for perception; the impact of other polysaccharides is an effect via the modification of the structure and of the texture of the food product.
- ✓ The most hydrophobic volatile molecules were the most retained ones by the proteins; aldehydes may form covalent binding with proteins.

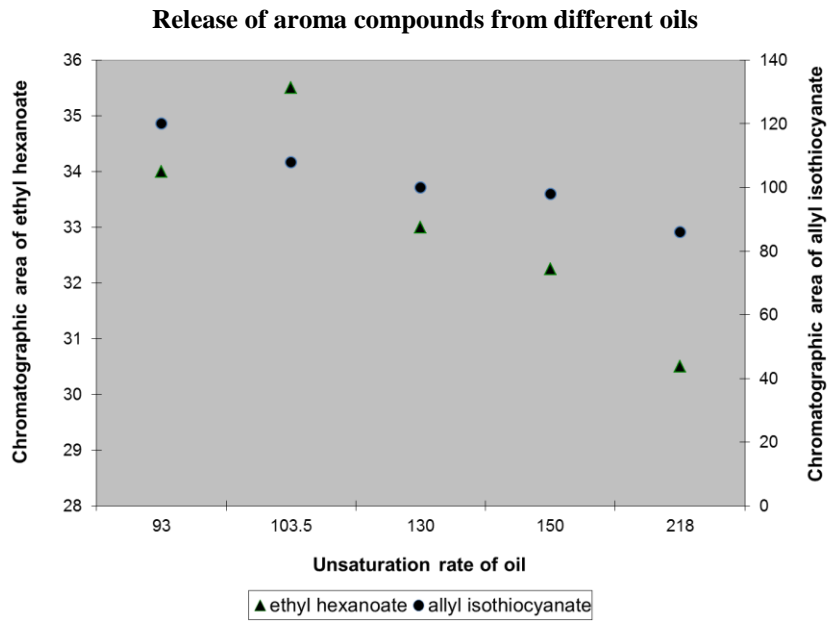
- ✓ Every thickener or gelling agent, if above a given concentration, can induce the decrease of the aroma release by reducing the diffusion of the small molecules in the thickened or gelled system.
- ✓ The perceived aroma compounds above an emulsion depend on the location of the aroma compounds in the emulsion.
- ✓ Important parameters are modified during food process: temperature, pressure, water content and activity, gas composition. All of them are of great impact on volatile compounds behavior.

7.7. EXERCISES

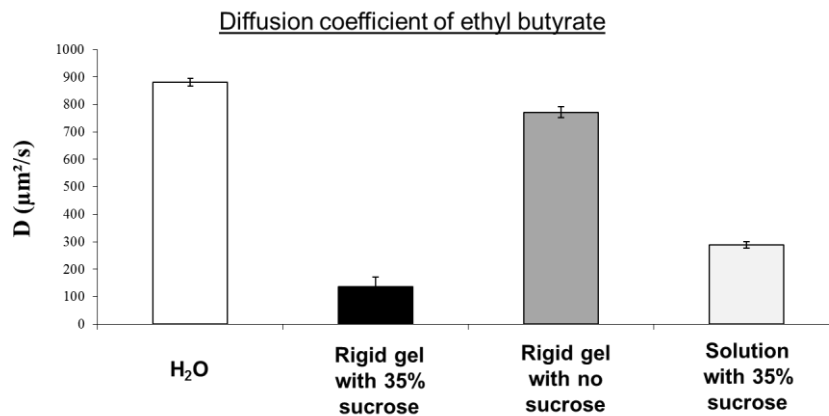
- Vanilla aroma is produced via two different processes. Process A is an extraction of the vanilla beans with ethanol / water; Process B is a bio-conversion of lignin by a microorganism. Which processes give 'natural flavoring substance' as defined by the EU regulation? Explain why.
- The physicochemical characteristics of two volatile compounds are given:
 - Isoamyl Acetate (MW= 130 g.mol⁻¹; log P = 2.13; P_i^s at 25°C = 733 Pa; solubility in water at 25°C = 2.4 g.L⁻¹)
 - Linalool (MW= 154 g.mol⁻¹; log P = 3.54; P_i^s at 25°C = 27 Pa; solubility in water at 25°C = 2.6 g.L⁻¹). Which compound is the most volatile? Which one is the most hydrophobic?
- The composition of a flavoring formula is given in the following table. What can you tell about the most probable sensory descriptors of this formula?

CAS No.	Name	Amount [mg/g]
3658-77-3	Furaneol	5
121-33-5	Vanillin	5
103-26-4	Methyl Cinnamate	24
123-66-0	Ethyl Hexanoate	20
105-54-4	Ethyl Butyrate	90
140-11-4	Benzyl Acetate	2
93-92-5	Styrallyl Acetate	1
706-14-9	gamma-Decalactone	20
134-20-3	Methyl Anthranilate	1
108-64-5	Ethyl iso-Pentanoate	10
66-25-1	Hexanal	1
3681-71-8	cis-3-Hexenyl Acetate	5
928-96-1	cis-3-Hexenol	15
24851-98-7	Methyl Dihydrojasmonate	5
14901-07-6	beta-Ionone	1
102-76-1	Triacetin (Solvent)	795
	SUM	1000

4. Observe and comment the following figure



5. Observe this figure and explain why aroma diffusion is less important in gels than in solutions.



6. You want to make a food product with a reduced amount of fat. What will occur with flavor compounds? What strategy will you adopt to keep flavor in the product?

7.8. SOLUTIONS

1. Both processes give 'natural flavoring substance' as defined by the EU regulation i.e. flavoring substance obtained by appropriate physical, enzymatic or microbiological processes from material of vegetable.
2. Isoamyl Acetate is the most volatile compound. Linalool is the most hydrophobic compound.
3. The great amount of esters probably gives a fruity odor.
4. The release of both aroma compounds is decreasing as the unsaturation rate of the oil is increasing. At a given temperature, the higher the unsaturation rate, the more important the liquid fraction of the oil. Thus, for higher rates of unsaturation, the oil is able to solubilize higher amounts of aroma compounds which are then more retained.
5. The diffusion coefficient of ethyl butyrate is varying with the nature of the matrix. It is high in water, lower in a gelled product because the mobility of molecules is lowered in a structured media. The presence of high amount of sucrose also decreases a lot the mobility of molecules because the main role of water is then to solubilize sucrose. The lowest diffusion coefficient is observed for a gelled matrix containing high amount of sucrose.
6. When food products are made with a reduced amount of fat, aroma compounds are less solubilized in the product. Then aroma loss and deterioration can easily occur, and the food product is less odorant when melting in the mouth. This will be particularly the case for the most hydrophobic compounds of the flavor formula. The common strategy used is to try to compensate this phenomenon by using other means to trap aroma such as entrapment in polysaccharides and/or by changing the aroma profile using less hydrophobic compounds. The moment of addition of the flavoring during the process can also be adapted to ensure better preservation of the aroma.

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