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Food matrices – Impact on odorant partition coefficients and flavour perception

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Inaugural - Thesis for obtaining the Degree of Doctor in Natural Sciences (Dr.rer.nat.) at the Bergische Universität Wuppertal

The present work arose on suggestion and under the guidance of Mr. Prof. Dr. Helmut Guth

Wuppertal 2006

Die Dissertation kann wie folgt zitiert werden:

urn:nbn:de:hbz:468-20070067
[http://nbn-resolving.de/urn/resolver.pl?urn=urn%3Anbn%3Ade%3Ahbz%3A468-20070067]

The work described in this thesis was carried out in the Department of Food Chemistry, Bergische Universität Wuppertal, under the scientific supervision of Prof. Dr. Helmut Guth, during the period of January, 2001-November, 2006.

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sotului meu, Vasile

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ACKNOWLEDGMENTS

No work is ever the making of one single person. Hereby I would like to thank all the persons who have contributed directly or indirectly to my work and to this thesis.

First, I would like to thank my supervisor, Prof. Dr. Helmut Guth for offering me the PhD position in his group and for his guidance, and supporting me during all these years, making this study possible. I would like to express my gratitude for his patience, understanding and constructively critical eye; all these have always helped me find my way through the critical moments of my research.

I am very grateful to Prof. Dr. Walter Reineke for agreeing to take the time to evaluate this thesis as a co-referee.

Completing a PhD is truly a marathon event, and I would not have been able to complete this journey without the aid and support of my former and present colleagues over the past five years. I would like to thank them for their help and friendship, and for the pleasant work atmosphere.
I thank always Mrs. Henny Usmawati for some data she put me at disposal from her research project, accomplished in the framework of Cost Action 921.

A special thanks goes to families Hauser and Schulz who accepted me in their house. They have been beside me every time I need them, making me easier this period, here, in Wuppertal.

I would also like to thank my entire family for the support they provided, from the distance, through my period here, and for being spiritually always near by me.

My final, but not least, and most heartfelt acknowledgment must go to my daughter Delia and my husband Vasile, for their support and patience, during these years. We have endured our separation and I would not be at this point without their encouragement and understanding.

ABSTRACT

Foods are complex multi-component systems which are composed of volatile and non-volatile substances. The flavour profile of a food is an important criterion for the selection of our foodstuffs.

The main objective of this study was the clarification of the complex relationships of the flavour release as a function of the composition of the food matrix at molecular level. Therefore the influence of matrix effects onto the partition coefficients, odour activity values and sensory properties of selected flavour compounds, in model and in real food systems were investigated. Different matrices were selected to measure their influence onto the partition coefficients of odorants: water, water-ethanol-mixtures, matrices containing lipids and more complex samples, such as mixtures of water, oil, proteins and polysaccharides.
The studies included a series of lactones, esters and alcohols (γ-octalactone, γ-nonalactone, γ-decalactone, δ-octalactone, δ-nonalactone, δ-decalactone, ethyl hexanoate and ethyl octanoate, 3-methyl-1-butanol and 2-phenylethanol).

The vapour pressures and partition coefficients were determined using static headspace gas chromatography (HS-GC) techniques. The influence of the model systems on the adsorptions of the odorants at the gas-tight syringes were taken into account. The results obtained, showed that the vapour pressures of the flavour compounds are decreasing with the increasing of the molecular weight of the compounds. The comparison of water/air partition coefficients ($\log P_{W/A}$) with miglyol/air partition coefficients of selected odorants ($\log P_{M/A}$) showed that for miglyol system, the $\log P_{M/A}$ are higher than the $\log P_{W/A}$ for all flavour compounds studied. The measurement of the partition coefficients of selected aroma compounds in water-oil matrices (emulsions) revealed that the fat content of emulsions influence significantly the partition coefficients of odorants. The highest partition coefficients were obtained in emulsions where the portion between water/miglyol/emulsifier was: 47.5 + 47.5 + 5, w/w/w.

In the present study the flavour release of different aroma compounds (ethyl hexanoate and S-(-)-limonene) in carbohydrate-water solutions was examined. The static headspace method allows the measurement of the released odour components that interact with β-cyclodextrin. The HS-GC analysis of β-cyclodextrin-water/odorant mixtures showed a reduction of the odorant in presence of the carbohydrate.

The influence of the various matrices on the human biological response of odorants was investigated by an olfactometer (e.g. determination of the threshold values of odorants in air and in the presence of ethanol) and the headspace odour activity values (HOAV's) were calculated. The results showed that the threshold values in air in absence of ethanol were lower than the values in presence of ethanol, which means the presence of ethanol in the matrix increase the threshold value of the odorant.

The studies also included the influence of wine matrix onto the partition coefficients of important wine flavour compounds. The quantification of the aroma compounds in white wine samples was achieved by isotope dilution analyses and standard addition method. Odorants in the headspace above wines were analysed by HS-GC techniques and the partition coefficients (wine/air) calculated. The results pointed out that the presence of ethanol in wine matrix does not influence the partition coefficients of selected aroma compounds. The highest partition coefficients in wines were found for the two alcohols: 2-phenylethanol and 3-methyl-1-butanol.

Concerning COST Action 921 custard samples were investigated as real foodstuff and the aroma compounds were quantified in the matrix and in the headspace above the food. The research data indicated that the partition coefficients custard/air are located between the partition coefficients water/air and partition coefficients miglyol/air, but closer to the miglyol/air values. Furthermore the mass transfer rates of selected odorants were investigated in custard- and milk powder/water samples. The values of the mass transfer rate were found higher in milk powder/water systems than in custard model. Nevertheless the results indicated that the viscosity of the matrix did not significantly influence the values of mass transfer rate of selected flavour compounds.

Molecular Modelling methods have been used for the prediction of solvation free energies of the flavour compounds studied in different model solutions, e.g. water and water-oil systems. The results showed that the predicted values (Mopac 97) for γ-decalactone, γ-nonalactone and 2-phenylethanol in water are in good agreement with experimentally solvation free energies.

ABBREVIATIONS

AEDA	Aroma Extract Dilution Analysis
API-MS	Atmospheric Pressure Ionisation Mass Spectrometry
CI	Chemical Ionization
DHS	Dynamic Headspace
EPICS	Equilibrium Partitioning in Closed Systems
FID	Flame Ionization Detector
GC	Gas Chromatography
GLC	Gas Liquid Chromatography
GCO	Gas Chromatography Olfactometry
GCMS	Gas Chromatography-Mass Spectrometry
HLB	Hydrophilic-Lipophilic Balance
HOAV	Headspace Odour Activity Values
HP	Hewlett Packard
HS	Headspace
HSGC	Headspace Gas Chromatography
HS-SPME	Headspace Solid Phase Micro Extraction
IDA	Isotope Dilution Analysis
MC	Monte Carlo
MD	Molecular Dynamics
MEP	Molecular Electrostatic Potential
MSD	Mass Spectrometry Detector
MST	Miertus-Scrocco-Tomasi
MS (CI)	Mass Spectrometry / Chemical Ionization
MW	Molecular Weight
OAV	Odour Activity Values
PRV	Phase Ratio Variation
QM	Quantum Mechanical
QSAR	Quantitative Structure-Activity Relationships
PTI	Purge and Trap Injector
SCRF	Self-Consistent Reaction Field
SFI	Solid Fat Index
SIDA	Stable Isotope Dilution Assays

SHA	Static Headspace Analyse
SPME	Solid Phase Micro Extraction
SHA-O	Static Headspace Analysis-Olfactometry
SHS	Static Headspace
TCT	Thermal Desorption Cold Trap Injector
VPC	Vapor Phase Calibration
VOC	Volatile Organic Compound

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1. INTRODUCTION

1.1. Effect of food matrices on flavour release and perception

Foods are complex multi-component systems which are composed of volatile and non-volatile substances. Flavour is one of the major organoleptic characteristics of food; it depends only on the nature and quantity of aroma compounds involved.

As Bakker et al. (1996) reported, it is considered that the sensory perception of flavour of a food forms an important aspect of the enjoyment people get from eating, and hence influences consumers' acceptability.

Buck et al. (1991) explained that the flavour sensation is caused by flavour molecules released into the vapour phase during eating and subsequently transported to the olfactory epithelium. To perceive an aroma, the flavour compounds need to achieve a sufficiently high concentration in the vapour phase to stimulate the olfactory receptors.

As Taylor et al. (2000) showed, flavour perception can be defined as:

Flavour perception = aroma + taste + mouth feel + texture + pain/irritation

He mentioned also that's ideally, to characterise a flavour, it is necessary to measure all these parameters.

A food's characteristic flavour and aroma are the result of a complex construct of hundreds of individual constituent compounds interacting to produce a recognizable taste and aroma.

Milicevic et al. (2002) defined aroma as one of the sensory food characteristics provoked by physiological phenomena. According to the *British Standards Institution* definition, aroma is a combination of taste and odour caused by the experience of pain, heat, cold and sense. Therefore aroma is a complete and unique experience generated from not only the taste and odour stimula but from other sensorial receptors too.

Thus, if one or more flavour constituents are altered or diminished, food quality may be reduced. A reduction in food quality may result from the oxidation of aroma components due to the ingress of oxygen, or it may be the result of the loss of specific aroma compounds to the packaging material or environment. Aroma compounds are little molecules with a molecular weight generally lower than 400 g mol^{-1} (Souchon et al., 2004). They are characterised by two main properties: their hydrophobicity and their volatility.

The flavour of a food will be characterized by volatiles, the so-called odorants, which were perceived by the human nose (nasal) and in the mouth-nose space (retro nasal), respectively. The flavour profile of a food is an important criterion for the selection of our foodstuffs.

The structure of our food, in particular the presence of macromolecules as for example proteins, fats and polysaccharides, influence the mouth feeling and the extend of the flavour release.

As Taylor et al. (2004) predicted, there are four levels of interaction that must be taken into account when analyzing flavour:

- chemical interactions occurring in the food matrix, that may directly affect flavour perception; physicochemical interactions can change flavour intensity or even generate new flavours;
- mechanical/structural interactions of the food and mastication with the release of compounds;
- peripheral physiological interactions; and
- cognitive interactions among tastes, odours and somato-sensations perceived together.

Kolb et al. (1997) showed that in headspace analysis, the use of the term "matrix" express the bulk of the sample that contains the volatile compounds to be measured. Usually the matrix is not a pure compound, but a complex mixture of compounds, some of which may be non-volatile.

The interaction of the matrix components with the analyte influences its solubility and partition coefficient. This is called the *matrix effect*.

If the matrix is a mixture of two (or more) compounds, the distribution of the analyte between the two phases will depend on the quantitative composition of the matrix, which plays an important role in controlling flavour release at each step of food product separation and consumption.

The chemical composition of a food matrix will influence perceived flavour, whether the food is primarily lipid, protein, carbohydrate or aqueous will affect release of flavour-active compounds from the matrix (Taylor et al., 2004). Flavours may be dissolved, adsorbed, bound, entrapped, encapsulated or diffusion limited by food components. Oil interacts with flavours, changing the concentration of free flavour in the solution and consequently increasing or decreasing the amount of adsorption.

Because many food products are emulsions of fat and water, such as milk and milk products, the fat content is an important variable in the food matrix.

Davidek et al. (1992) mentioned, that lipids, particularly fats and oils, are the only main components of foodstuffs which are not water-soluble.

Lipids often interact with water-soluble substances forming unstable products in which the lipids are bound to non-lipidic moieties mainly or exclusively by physical forces, such as hydrogen bonds with the polar groups of lipids or hydrophobic forces between non-polar groups of non-lipidic substances and hydrocarbon chains of lipids.

Lipids interact not only with proteins, but also with other hydrophilic biomacromolecules, for instance with carbohydrates, and particularly with starch.

The fat/oil content is often reduced in order to increase calorific intake to make food healthier. Removal or reduction of lipids can lead to an imbalanced flavour, often with a much higher intensity than the original full fat food (Widder et al., 1996; Ingham et al., 1996).

In fact, lipids adsorb and solubilize lipophilic flavour compounds and reduce their vapour pressures (Buttery et al., 1971; Buttery et al., 1973). This effect was confirmed by mathematical models (Harrison et al., 1997), headspace analysis (Schirle et al., 1994), and sensory analysis (Ebeler et al., 1988; Guyot et al.).

Extensive reviews of the effects of lipids (Hatchwell, 1994; de Roos, 1997; Plug et al., 1993) on the rate and amount of aroma released have been previously published.

De Roos (1997) reported that in products containing aqueous and lipids phases, a flavour compound is distributed over three phases: fat (or oil), water and air. Flavour release depends on oil content, which affects the partition of aroma compounds during the different emulsion phases (lipid, aqueous, and vapour). Flavour release from the oil/fat phase of a food proceeded at a lower rate than from the aqueous phase. This was attributed, first to the higher resistance to mass transfer in fat and oil than in water and, second to the fact that in oil/water emulsions flavour compounds had initially to be released from the fat into the aqueous phase before they could be released from the aqueous phase to the headspace.

In the case of emulsions the structure itself has been shown to affect the release rate of flavour (Overbosch et al., 1991; Salvador et al., 1994).

Overbosch et al. (1991) showed in their model that diffusion from a single phase system and release is independent of the emulsion type. Their data, using diacetyl at two levels (10 mg/kg and 20 mg/kg), indicated that the flavour release was twice as fast from oil-in-water emulsions than from water-in-oil emulsions, which they suggested was a consequence of using a different emulsifier for each system. In the oil-water emulsion, sodium dodecyl

sulphate was used, while in the water-oil emulsion, mono acyl glycerol, and lecithin were used.

In the investigations of Salvador et al. (1994), the emulsions were made from the same emulsifier (sugar ester emulsifier S-370, HLB =3) and diacetyl as a model flavour, because it is a common volatile in high-fat foods. In their experiments, with diacetyl at an initial concentration of 2 g/litre, the rate of release from the oil-in-water emulsion was 1.5 times greater than from the water-in-oil emulsion. This difference was due to the emulsifier.

The effects of the primary structural and compositional properties of emulsions on the release of aroma have been both systematically investigated (van Ruth et al., 2002; Miettinen et al., 2002).

Van Ruth et al. (2002) examined the influence of compositional and structural properties of oil-in-water emulsions on aroma release under mouth and equilibrium conditions. The impact of the lipid fraction, emulsifier fraction, and mean particle diameter on release was determined for 20 aroma compounds, included alcohols, ketones, esters, aldehydes, a terpene and a sulphur compound. The selection of the 20 compounds was based on the physicochemical and odor properties of the compounds. As emulsifier, Tween 20 (polyoxyethylene sorbitan monolaurate) was used. All the influences were evaluated statistically for the complete data set as well as for the individual compounds by MANOVA (multivariate analysis of variance). The results obtained showed that the decrease in lipid fraction and emulsifier fraction, as well as increase in particle diameter, increased aroma release under mouth conditions.

Miettinen et al. (2002) investigated the effects of oil-in-water emulsion structure (droplet size) and composition of the matrix (oil volume fraction and the type of the emulsifier) on the release of two chemical different aroma compounds: linalool (non-polar) and diacetyl (polar). Modified potato starch (starch sodium octenylsuccinate, E 1450) and sucrose stearate (E 473) were chosen as emulsifiers (1% w/w) because of their ability to form stable emulsions over a wide range of oil volume fraction. The results showed that the fat content strongly affected the release of linalool, but it was not as critical a factor in the release of the more polar compound, diacetyl. A slight effect of the emulsifier type on the release of aromas was observed with sensory and gas chromatographic methods. The reduced droplet size, resulting from higher homogenization pressure, enhanced the release of linalool but had no effect on diacetyl.

Flavour release depends on the ability of the aroma compounds to be in the vapour phase and therefore on their affinity for the product, which participates in their rate of transfer (Voilley et al., 2000).

Kinsella (1989) reported that several mechanisms might be involved in the interaction of flavour compounds with food components, mechanisms responsible for the release of volatile components from food:

- Diffusion phenomena influence the viscosity;
- Specific and unspecific binding of aroma compounds to macromolecules influence the intermolecular interactions.

In lipid systems, solubilization and rates of partitioning control the rates of release. Polysaccharides can interact with flavour compounds mostly by non-specific adsorption and formation of inclusion compounds.
In protein systems, adsorption, specific binding, entrapment, encapsulation and covalent binding may account for the retention of flavours.
Oil/fat has a major influence on flavour compounds (perception, intensity, volatility, etc.) and on the properties of packaging material.

An entire understanding of the matrix with its influence on the binding of the most different odour materials leads to a differentiated application of the suitable ingredients in the food industry.

1.2. State of the art

1.2.1. Methods for the determination of flavour release and partition coefficients (LogP)

Widder et al. (1996) showed that the binding of flavour and flavour release can be studied by different methods:

- On the one hand sensory methods, such as descriptive sensory analysis are used to describe and quantify the influence of the food composition on specific flavour attributes leading to flavour profiles;
- On the other hand flavour release can be investigated by analysing the volatiles in the gaseous headspace above the food sample.

Stevenson et al. (1996) showed that various techniques are used to separate and isolate mixtures of volatile flavour compounds from sample matrices.

These include:

- headspace sampling (static and dynamic);
- distillation followed by liquid-liquid extraction;
- simultaneous distillation-extraction;
- solid-phase extraction and;
- new methods of extraction such as solid-phase micro extraction and membrane-based systems.

Also, the authors specified that mass spectrometry coupled with gas chromatography is a major method used to identify volatile flavour compounds.

Atmospheric Pressure Ionisation Mass Spectrometry (API-MS)

The technique of Atmospheric Pressure Ionisation Mass Spectrometry (API-MS) is now commercially available for the trace analysis of volatile compounds and is fast and sensitive enough to measure breath-by-breath release of a wide range of aroma compounds (Taylor et al., 2000). It can detect volatile compounds at concentrations in the *ppb* to *ppt* (by volume) range, providing sensitivity to measure about 80% of volatiles at their odor threshold.

The collection of expired air involves resting one nostril on a small plastic tube, through which expired air passes, and from which a portion of air is continuously sampled into the API-MS (**Figure 1.1.**).

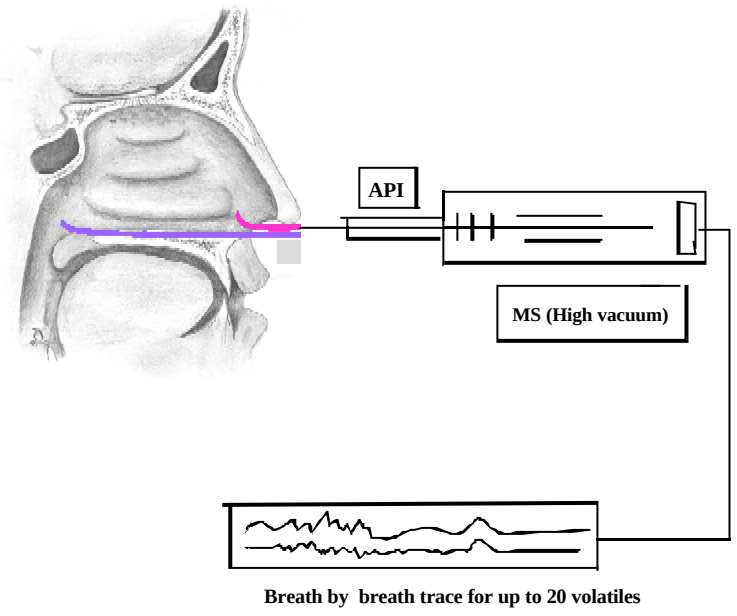


Fig.1.1*. Schematic diagram of API-MS and breath collection*

This technique has been used to follow release from strawberries (Grab et al., 2000) and from model confectionery gels (Linforth et al., 1999) as well as from yoghurt (Brauss et al., 1999), biscuits (Brauss et al., 2000).

In the modelling area, both model system release (Marin et al., 1999; Malone et al., 2000; Marin et al., 2000) and release from people eating foods (Linforth et al., 2000) has benefited from the availability of real data with which to validate the models.

The API-MS technique and other emerging techniques will be increasingly deployed to provide data to compare with the theoretical models and with which the effect of food matrix on flavour release can be determined.

Headspace-Gas Chromatography

Generalities

The term headspace gas chromatography (HS-GC) is applied for various gas extraction techniques, where volatile sample constituents are first transferred into a gas with subsequent analysis by gas chromatography (Kolb, 1999).

Headspace gas chromatography has been shown to be a mostly objective analytical, suitable and easy method for investigating food flavours (Bohnenstengel et al., 1993).

The same author remarked, after the experiments carried out, that there are strong interactions between substances in the headspace and between the volatiles and the sample matrix. Even small changes in the sample composition can cause drastic changes in the resulting headspace composition. Other influencing factors, such as the volatility and polarity of the analytes, their solubility in the sample matrix, are also difficult to estimate, especially in HS-GC with large sample volumes of complex samples.

The HS technique involves the equilibration of volatile analytes between a liquid phase and a gaseous phase; with only the gaseous phase sampled (Seto, 1994).

HS analysis involves a special sampling technique. The sample is placed in a vial, which is sealed vapour-tight with a septum cap. The vial is thermostated, and when equilibrium between the sample and the vapour in the headspace has been reached, a portion of the vapour is withdrawn and injected onto the analytical column.

The gas chromatographic headspace technique is therefore suitable for the analysis of components of relatively high vapour pressure in the presence of matrix components.

In this way, headspace analysis is a particularly useful analytical tool. It finds important applications in: clinical chemistry; in the quality control of foods and drinks; in industrial hygiene; in water analysis. In fact, anywhere trace volatile components or contaminants are to be determined.

The HS-GC technique can be divided into the two following categories:

- Static (equilibrium) HS and
- Dynamic (non-equilibrium) HS, also referred to the "purge and trap" method (Seto, 1994).

The static headspace method (SHS) involves the equilibration of volatile analyte within the sample with the vapour phase at a defined temperature. The vapour phase containing the analyte is then injected into the GC column.

SHS analysis is based on the theory that an equilibrium between a condensed phase and a gaseous phase can be established for the analytes of interest and that the gaseous phase containing the analytes can be sampled (Meyers, 2000).

Advantages:

- Simple;
- Minimizes the number of artifacts during analysis;
- Can provide precise quantification;
- Can effectively measure volatile substances with relatively low water solubility.

The method is useful for the analysis of highly volatile compounds.

Disadvantages:

- Low sensitivity

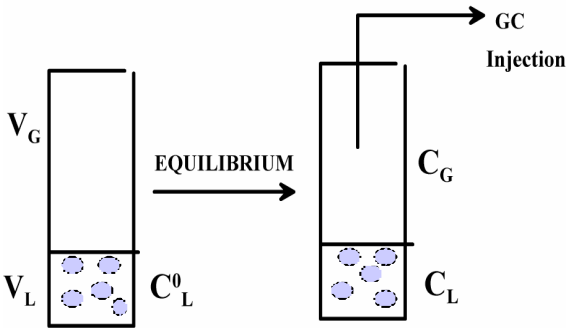


Fig. 1.2*. Schematic diagram of static headspace gas chromatography*

where: C_L and C_G represent the concentrations in the liquid and headspace, respectively, after equilibrium, C_L^0 represents the analyte concentration in the liquid phase prioir to HS equilibrium and V_L and V_G represent the volumes of the liquid and headspace. As illustrated in **Figure 1.2.** the following equation is valid:

$$C_L^{\,0} V_L = C_L V_L + C_G V_G \qquad (\mathbf{1\text{-}1})$$

The partition coefficient (**k**) and phase ratio (ß) are defined as C_L / C_G and V_G / V_L, respectively. **Equation (1-1)** can be transformed as follows:

$$C_G = C_L^{\,0} / (k + ß) \qquad (\mathbf{1\text{-}2})$$

The dynamic headspace method (DHS) involves passing a carrier gas over the sample for a specified period of time and trapping the analyte in a cryogenic or adsorbent trap. The concentrated analyte is then introduced using pulsed heating.

In general, the DHS method is effective for the measurement of volatile substances of moderate to high water solubility. In addition, this method offers increased sensitivity when compared with SHS, direct aqueous injection (DAI) and solvent extraction (SE) methods owing to the concentration after trapping of the volatile analyte (Seto, 1994).

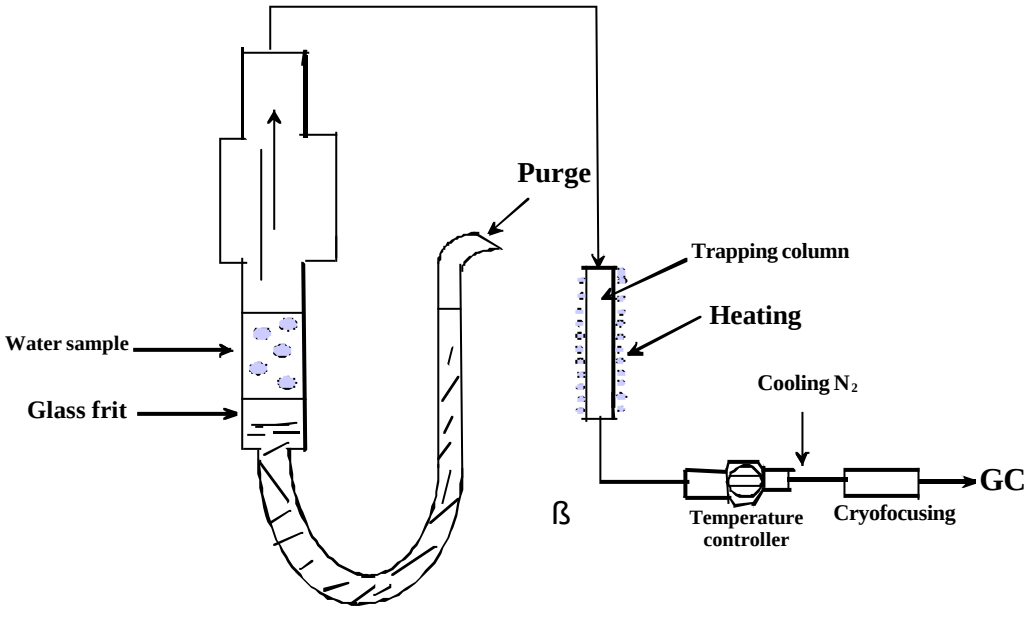


Fig. 1.3. *Scheme of purge-and-trap technique*

The dynamic headspace extraction is represented in **Figure 1.4**.

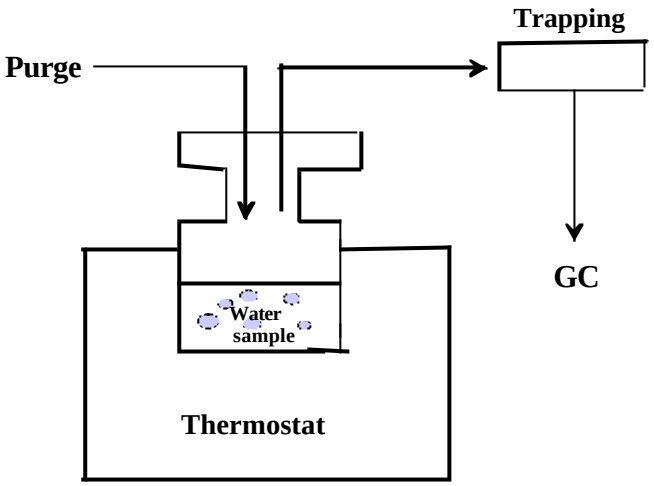


Fig. 1.4. *Scheme of dynamic headspace extraction*

Instrumentation

The HS-GC system consists of:

- HS element (pre-treatment) and
- GC element (measurement) (Seto, 1994).

The HS instrument can either be manual or automated and consists of:

- vaporization container where equilibrium is obtained;
- heating device which keeps the HS container at a constant temperature;
- injection device which transfers the vapour phase from the HS container into the GC column.

Initially, the HS instrument consisted of a glass vial sealed with a rubber septum with transfer through a gas-tight syringe (Purchase, 1963; Curry, 1962; Yammamura, 1966; Butler, 1967; Machata, 1964; Nanikawa, 1969; Goldbaum, 1964; Duritz, 1964). In general, a glass vial is recommended as container. The container is sealed by either a screw-cap or a crimped cap. A septum is necessary for sealing the container. Butyl rubber or silicone rubber septa were used but were found to introduce serious errors due to adsorption of the analyte on these materials, resulting in a time-dependent decrease in vapour concentration (Davis, 1970). Currently, septa are coated with, either polytetrafluoroethylene (PTFE; Teflon) or aluminium foil to prevent adsorption. All components of the HS container and injection equipment which contact the sample must be composed of chemically inert materials (Lansens, 1989).

The most popular device for headspace sampling is a gas syringe. Besides the risk of sample carry-over and significant memory effects there is the inherent problem that the internal pressure in the vial extends into the barrel of the syringe and after withdrawal from the vial, the headspace gas then expands through the open needle to the atmosphere. Part of the headspace gas will thus be lost. This drawback may be avoided by using a gas-tight syringe equipped with a valve. Such syringes may be adequate for manual sampling, but are hard to automate (Kolb, 1999).

Manual injection with gas-tight syringes (**Figure 1.5.**) is the transfer method of choice. Unless special pressure corrections are employed (Seto, 1993), the use of pressure-lock-type syringes is recommended to prevent the loss of sample vapour.

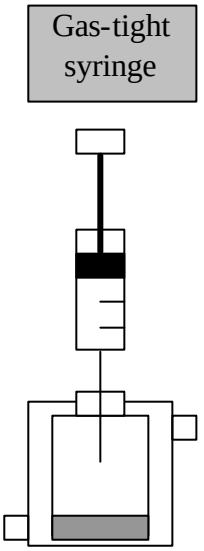


Fig. 1.5*. Scheme of gas-tight syringe (Guth and Sies, 2001)*

Contamination of the syringes is a major concern as it can lead to non-quantitative results (Bassette, 1968). It is possible to minimize contamination by cleaning the syringe with hot water and drying with hot air at high temperature between analyses.

The following procedure was applied: appropriate carrier gas flows and temperature zones are established. Additional carrier gas flow is initiated to sweep the lines, sample loop, and needle. A sample in a sealed vial is allowed to equilibrate at an elevated temperature for a specified length of time. The sealed sample vial is raised onto a needle that punctures a septum and pressurization gas fills the vial to a predetermined level. The vial is allowed to equilibrate for a relatively short time to ensure complete diffusion of the pressurization gas with the sealed sample vial's atmosphere. A vent valve is opened and the pressurized contents

of the sealed sample vial exit the system through a thermally controlled sample loop of previously selected volume, usually = 1 mL. The vent valve is then closed and the contents of the loop allowed equilibrating for a specified time. Next a multiple port valve is activated, placing the sample loop in the carrier gas stream. The carrier gas then sweeps the contents of the loop through the heated transfer line and into the GC. Usually upon initiation of sample transfer to the GC, instrumentation software is employed to automatically begin the chromatographic separation and data collection (Meyers, 2000).

A peculiar problem in static HS-GC is the internal pressure in the headspace vial generated during thermostated by the sum of partial vapour pressures from all volatile sample constituents, from which in general the humidity of the sample is predominant (Kolb, 1999).

Thus, the vapour pressure of water contributes mostly to the internal pressure. Moreover, some sampling techniques pressurize the vial prior to sample transfer with the inert carrier gas. For these reasons it is necessary to close the vial pressure tight by a septum (preferably PTFE-lined) and to crimp-cap it by an aluminium cap.

HS-GC can be performed with both packed and capillary columns.

1.2.2. Studies on the physico-chemical parameters of flavour compounds in model systems and in real food - models of flavour release.

As Taylor (1998) explained, food contains a number of different phases (e.g. oil, water, air), and the partition of volatile flavour molecules from the food phases into the air phase gives the characteristic volatile profile, sensed as aroma by humans. In this situation, the volatile profile in the gas phase is largely dependent on partition. During eating, the nature of the food changes as additional water is mixed into the food and/or the temperature of the food is adjusted nearby the physiological temperature of 37°C. In this case, equilibrium is not achieved and factors such as mass transfer also play a role, along with partition, in generating the chemical signal that is perceived as flavour.

Therefore, to understand the relationship between flavour perception and the nature of the chemical signal that produces it, many studies have been performed to develop methods and produce data on the partition of flavour molecules between the phases in model systems and in real food (Taylor, 1998).

As Guyot et al (1996) presented, reconstructing the interactions between the volatile and the non-volatile compounds requires the evaluation of the behaviour of aroma compounds in model systems similar to the original product. Moreover, while studies dealing with vapour-

liquid partition phenomena may have reported the effects of medium composition on the headspace concentrations at equilibrium, they have not connected the physical properties with sensory scores by model equations (Van Boekel et al, 1992; Land, 1979).

With complex foodstuffs, it is useful to have some model systems to relate to. Studies of these model systems can at least give an approximation of the behaviour we might expect in the actual practical system (Buttery et al., 1973).
Various models for predicting flavour release have been proposed, based either on partition (De Roos & Wolswinkel, 1994) or on an understanding of the physical processes involved in the mouth during eating (Harrison & Hills, 1997).
Three types of model systems are mentioned in the literature:

- First model system: *pure water* (e.g. Buttery et al., 1969, 1971);
- Second model system: *vegetable oil* (Buttery et al., 1973);
- Third model system: *water-vegetable oil mixtures* (Buttery et al., 1973).

Several reviews of flavour release studies (Overbosch et al., 1991; Bakker) emphasized the need for a better understanding of food-flavour interactions and under more complex food consumption conditions.
Most detailed studies on flavour release have been made on simple liquid systems, and little research has been done on the release from solid or semi-solid foods, having different structures (Bakker et al., 1996).
The same authors mentioned that the perceived quality and intensity of the flavour of a food is related to the concentration of volatile components released into the airspace of the mouth while eating. It was assumed that the concentration of a flavour released into the airspace is quantitatively and qualitatively related to the sensory perception.

Models of flavour release

A review of the literature on flavour release (Overbosch et al., 1991; Plug et al., 1993) reveals two main mechanisms for release which are then adapted for the particular food matrix under investigation.

- *The first mechanism (convective model)* (**Figure 1.6.a**) assumes that the phases are well mixed so that the concentration of volatile is constant throughout both phases.

Mass transport across the interface occurs by diffusion in very thin layers (the boundary or interfacial layers) either side of the interface.

- *The second mechanism (diffusive model)* (**Figure 1.6.b**) occurs when one or both phases are not well mixed. Mass transport between the phases in this case also depends on diffusion but the distance over which it occurs is much greater than in the convective model and changes with time. To simplify at this stage, the schematics in **Figure 1.6.a and b** refer to a simple liquid-air situation:

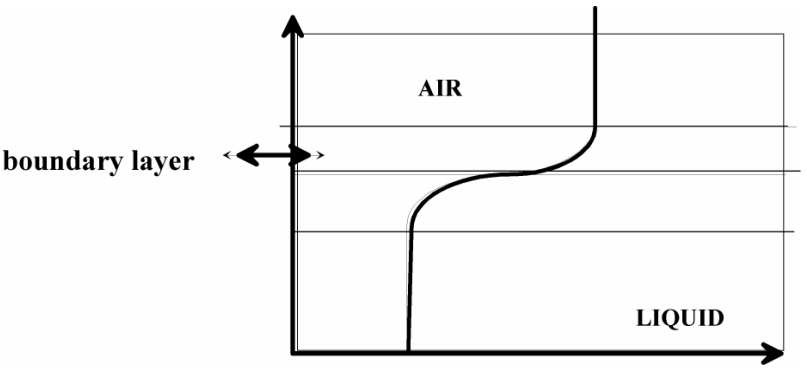


Fig. 1.6.a*. Schematic of convective type mass transfer mechanism between two phases*

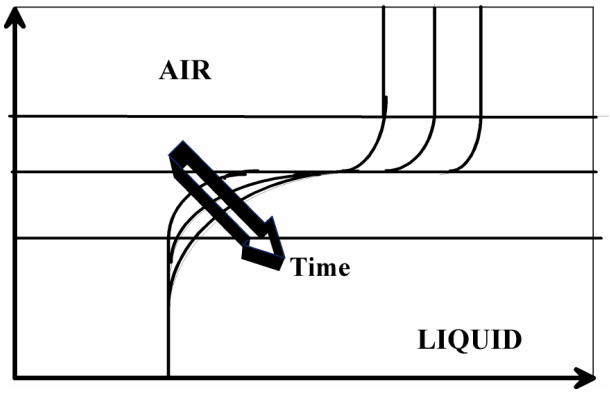


Fig. 1.6.b. *Schematic of diffusive type mass transfer mechanism between two phases*

Overall transport across the interface can be generally described (Marin et al., 1999) by the following equation:

$$\frac{1}{k} = \frac{1}{k_g} + \frac{K_{gl}}{k_l} \qquad \textbf{(1-3)}$$

where: k is the overall mass transport across the phases;

k_g and k_l refer to mass transport in the gas and liquid boundary layers, respectively;

K_{gl} is the partition coefficient between the gas and liquid phases.

Because k depends on the mass transfer in the liquid and gas phases, plus a contribution from the air-water partition coefficient **(1-3)**, the values for these parameters and the effects of flow rate on these parameters (when appropriate) were determined (Marin et al., 1999).

In the model proposed by Marin et al.(1999) (an air-water system at equilibrium, for which the air-water partition coefficient (K_{aw}) and temperature are the determining factors for volatile release) the authors reported that release depended almost entirely on the air-water partition coefficient for values of K_{aw} less than 10^{-3}. When K_{aw} was greater than 10^{-3}, the model predicted that the conditions in the gas phase (exemplified by the Reynolds number), would become significant. The Reynolds' number (a dimensionless parameter) is the ratio of inertial to viscous forces and determines the type of flow (Roberts et al., 2000).

The Reynolds' number is given by:

$$\mathrm{Re} = \frac{\rho v l}{\eta} \qquad \textbf{(1-4)}$$

where: ρ = fluid density [kg/m^3];

v = fluid velocity [m/s];

l = some typical dimension [m];

η = fluid viscosity [kg/ms].

<u>Oil water partition models</u>

Models for oil-water/air partition were published from McNulty and Karel, (1973c); McNulty and Karel, (1973b) and McNulty and Karel, (1973c) and summarized more recently (McNulty, 1987). The models are based on oil-water/air partitioning.

Using these models, release of volatile flavours from emulsions, representing the extremes of the oil fraction, were tested. Oil fraction (**f**) is the percentage of oil in the system (f = 1 corresponds to 100% of oil) and the examples used by McNulty and Karel (McNulty, 1987) were milk (**f = 0.035**) and mayonnaise (**f = 0.80**).

By modelling these two systems, they predicted that flavour release on dilution would depend entirely on the oil-water partition coefficient ($K_{o/w}$). They assumed that C_{owi} = 100 ppm (C_{owi} = initial emulsion flavour concentration), DF_{em} = 2 (i.e. a 1:1 dilution) (DF_{em} = emulsion

dilution factor) and V_{owi} = 10 ml (V_{owi} = initial emulsion volume), and then evaluated the flavour concentrations in the aqueous phases of milk and mayonnaise after dilution with saliva. The results obtained showed that, with increasing of $K_{o/w}$, the potential extent of flavour release increases slightly for milk and appreciably for mayonnaise. $K_{o/w}$ was given by:

$$K_{o/w} = \frac{C_{oe}}{C_{we}} \qquad \textbf{(1-5)}$$

where : $K_{o/w}$ = oil-water partition coefficient;

C_{oe}, C_{we} = flavour concentrations in the oil and aqueous phase at equilibrium, respectively [μg/ml].

McNulty and Karel summarized the experimental evidence to validate their model in their 1987 review (McNulty, 1987). Some experiments were carried out with a simple oil-water system, others with an oil-water-surfactant (Tween 60) system. The effect of fat melting point on the oil-water partition was then studied.

In the case of n-hexanol, $K_{o/w}$ decreased slightly with an increase in solid fat index (SFI) in the presence and absence of surfactant (cf. **Table 1.1.**, McNulty, 1987):

Table 1.1. *Effect of the solid fat index (SFI)[a] of vegetable oils and fats on transfer rates of n-hexanol at 24°C in a stirred diffusion cell (ϕ_i = 0.5)[b]*

	no surfactant		Tween 60	
SFI	$K_{o/w}$	rate x 10^4 (s^{-1})	$K_{o/w}$	rate x 10^4 (s^{-1})
0	9.00	0.539	7.33	0.464
8	8.09	0.225	6.69	0.188
24	9.53	0.263	8.09	0.158

[a]SFI measures the solidity of fat i.e., ratio of fat in crystalline form to liquid, at various specified temperatures;

[b]ϕ_i = initial oil volume fraction .

Using synthetic and natural oils, to achieve a wide range of viscosities, the effect of viscosity on oil-water partition ($K_{o/w}$) was also measured. Values of $K_{o/w}$ dropped by a factor of 2 for both systems, with the Tween-containing system, showed lower values again.

The influence of proteins, polysaccharides, and droplet size on flavour release of an oil-in-water emulsion was determined using aroma compounds with different hydrophobic characteristics (Charles et al., 2000). Flavour release of lipophilic compounds (ethyl hexanoate and allyl isothiocyanate) was influenced by three factors: the structure of the emulsion; the nature of the protein used as emulsifier, and the presence of polysaccharides.

Emulsions

There are many other published reports on flavour release from a variety of emulsion system (Dickinson, 1992; Salvador et al., 1994; Guyot et al., 1996; McClements, 1998; Charles et al., 2000; van Ruth and Roozen, 2000). Release has been measured using both sensory and instrumental methods and the effect of oil fraction on flavour release has been verified. The majority of work describes theoretical models with little experimental validation.

Flavour release from model emulsions has been followed *in vitro* using Atmospheric Pressure Ionisation Mass Spectrometry (API-MS) monitoring which allows real time measurement of gas phase concentrations at levels around 10 ppb (volatile per litre air). Using dynamic headspace dilution and following the release with time it was possible to compare actual release data with theoretical convective and diffusive models.

Overbosch et al. (1991) pointed out that the time course of release from the convective and diffusive models was different and this could affect flavour perception. If both systems are allowed to come to equilibrium and the headspace is then diluted, the diffusive model predicts an exponential release, while the convective model will show an initial decrease, followed by a steady state, followed by a further decline as the volatile flavour in the liquid phase becomes depleted.

Emulsion systems contained various lipids, water and dilute solutions of the emulsion (up to 1.92% oil) were used to minimize any viscosity effect.

For the emulsions, Malone et al. (2000) pointed out that the "Harrison models" were developed for simple model systems (water systems). Harrison et al. (1997) propose a mathematical model for the release of flavour (one hydrophilic: diacetyl and the other hydrophobic: heptan-2-one) from a liquid emulsion based on the assumption that the rate-limiting step was the resistance to mass transfer across the emulsion-gas interface and that this could be described by penetration theory. The authors assumed that partitioning of flavour molecules between oil and aqueous phase was extremely rapid compared to the transport of flavour across the emulsion-gas interface. They took also into account the dependence of mass transfer coefficients on viscosity, oil fraction and droplet size. The results showed that

the mass transfer coefficient was expected to increase with decreasing oil fraction, leading to faster flavour release in low fat systems. However, partitioning can also influence the flavour release rate, depending on physical/chemical properties of the flavour. Experimental data for heptan-2-one agreed reasonably well with theoretical predictions, suggesting that the rate limiting step for flavour release was the mass transport across the emulsion-gas interface.

To apply this model to the situation that occurs when a sample of emulsion is placed in mouth, masticated, and swallowed, required some estimate of other factors, like the degree of mixing in mouth, as well as the extent of dilution with saliva and air.

In vivo release mesurements of butanone, heptan-2-one and ethyl hexanoate were studied to cover a range of compounds with differing lipophilicity. Butanone represented a relatively hydrophilic molecule and its release was largely unaffected by the oil fraction of the emulsion used. For heptan-2-one and ethyl hexanoate (relatively lipophilic compounds), oil fraction affected both the maximum intensity of release as well as the duration of release, which is in agreement with theory (Taylor, 2002).

Malone et al. (2000) pointed out that the overall release of the three volatiles *in vivo* was related to their partitioning behaviour in static model systems.

Van Ruth et al. (1999) concluded that the pH of emulsions influences the aroma profiles of emulsions through effects on aroma generation and aroma release.

Real food systems

While all the models described in the previous chapter relate to well-defined model systems, flavour release from a system closer to a real food product, namely composite dairy gels, has been modelled (Moore, 2000).

The gel contained protein, fat and water, as the main constituents, but the gel also contained structural elements in the form of composite particles, composed of an inner layer of fat with an outer coating of protein.

The model proposed by Harrison and Hills (1997) was used with minor modifications and using direct numerical solutions. The mathematical model was compared with experimental data obtained by sensory time intensity measurements of flavour release. Good agreement between the model and the experimental data was obtained when the model contained appropriate terms for flavour removal by swallowing and breathing. The model predicted correctly that flavour diffusivity and fat particle size had little effect on perceived intensity of flavour, nor on the timing of maximum intensity. The prediction, that perceived flavour

intensity depended on flavour concentration in the gel, was also found to be valid, although the relationship between the two parameters was not exactly as predicted.

The theoretical models to predict the effect of food matrix on flavour delivery have not been validated to any great extent (Taylor, 2002). The suitable analytical methods are available to measure dynamic flavour release *in vivo* and *in vitro*.

Dynamic flavour release methods have been developed over the last 10 years and have been reviewed recently (Taylor et al., 2000).

The model proposed by Nahon and Roozen (2000) described the dynamic flavour release for five volatile compounds (ethyl acetate, methyl butanoate, ethyl butanoate, hexanal and octanal) from aqueous sucrose solutions. By determination of the viscosities and the partition coefficients, the model provided an acceptable fit to the experimental data obtained with instrumental analysis. The model description revealed that at low sucrose concentrations the partition coefficient primarily controls the flavour release, whereas at higher sucrose concentrations the mass transfer coefficient has more influence.

Banavara and Berger (2002) developed a mathematical model, derived from the convective mass transfer theory, to predict dynamic flavour release from water. The model was based on physicochemical constants of flavour compounds and on some parameters of an apparatus used for validation. The model predicted a linear pattern of release kinetics during the first 30 s, and large differences of absolute release, for individual compounds.

Rabe et al. (2002) measured dynamic flavour release from liquid food matrices using a fully computer-controlled apparatus. The concept of the apparatus presented was developed from the idea to represent an idealized situation of food consumption. Flavour compounds from different chemical classes were dissolved in water to achieve concentrations typically present in food (µg-mg/L). Most of the compounds showed constant release rates. The entire method of measurement including sample preparation, release, sampling, trapping, thermodesorption, and GC analysis, showed good sensitivity and reproducibility (mean standard deviation = 7.2%).

Analogous methods for following non-volatile release in-mouth are also available (Davidson et al., 2000).

1.2.3. Studies on Vapour Pressure

The saturated vapour pressure is one of the most important physico-chemical properties of pure compounds (Boublik et al., 1973).

The vapour pressure of a liquid or solid is the pressure of the gas in equilibrium with the liquid or solid at a given temperature (Verschueren, 1983). The same author explained that vapour pressure values provide indications of the tendency of pure substances to vaporize in an unperturbed situation, and thus provide a method for ranking the relative volatilities of chemicals.

Vapour pressures are expressed, either in mm Hg (abbreviated mm), or in atmospheres (atm.).

The thermodynamic expression of phase equilibrium for a pure substance is given by the Clapeyron equation:

$$\frac{dP^0}{dT} = \frac{\Delta H}{T\Delta V} \qquad \textbf{(1-6)}$$

where: P^0 denotes the vapour pressure [Pa], T the absolute temperature [°K], ΔH [J/mol K], the heat absorbed at constant temperature in transferring one mole from phase (‘) to phase (“) in equilibrium and ΔV the volume change per mole transferred [m^3] (Boublik et al., 1973). This equation is applicable only over narrower temperature ranges in which the enthalpy of vaporization is relatively constant (Mackay et al., 1981).

A whole series of semiempirical equations (correlation equations) has been proposed. One suitable equation, published by Antoine (Antoine, 1888), has the following form:

$$\log P^{\,0} = A - B/\left(t + C\right) \qquad \textbf{(1-7)}$$

where P^0 is the vapour pressure [Pa], t the temperature [°C], and A, B, C are constants characteristic of the substance and the given temperature range.

The Antoine **Equation (1-7)** represents well the behaviour of most substances over large temperature intervals (Willingham et al. (1945)). The authors did measurements of vapour pressures for 52 purified hydrocarbons over the range 47 to 780 mm Hg at 12°C.

As de Roos (2000) described, vapour pressures in the product medium can be influenced by many factors, such as:

- Temperature;
- Composition of the aqueous phase;

- Flavour binding / Complex formation;
- Acid-Base equilibria;
- Phase partitioning between aqueous and lipid phase;
- Sorption to suspended particles;
- Crystallization.

As Widder et al. (1996) showed, the fat content is an important variable in a food matrix. One important point is that fat is a good solvent of flavour compounds and influences the vapour pressure of the volatiles, thereby affecting the perceivable aroma profile. Hence, good fat based flavourings tend to become unbalanced or even off-flavoured in aqueous or reduced fat systems (Hatchwell, 1994; Plug et al., 1993).
Vapour pressure measurements can be made directly through the use of a pressure gauge (e.g., a diaphragm gauge), or by indirect methods based on evaporation rate measurements or chromatographic retention times (Bambord et al., 1998).
Mackay et al. (1981) explained that accurate measurements of vapour pressure have been possible for many years using standard isoteniscopic techniques which are applicable down to approximately 1 mm Hg or 100 Pa. The authors noted that it is difficult to estimate the accuracy of much published data since the values reported are usually the fitted data or the regression constants.
The preferred experimental technique for determination of low vapour pressures is similar in principle to that of the "generator column" solubility technique except that a gas stream is saturated with solute (Mackay et al., 1981). Methods have been described by Spencer and Cliath (1968), Sinke (1974), and Macknick and Prausnitz (1979), in which a standard error better than 3% is attainable.

In the method described by Spencer and Cliath (1968) to determinate the vapour density of dieldrin, the apparent vapour pressures were calculated from the vapour density, W / V (W = the weight of the given volume of gas [g], V = volume of gas [m^3]), with the equation:

$$P = (W/V)(RT/M) \qquad \textbf{(1-8)}$$

where: R is the molar gas constant [8.314 J/mol K], T the absolute temperature [K], and M the molecular weight [g/mol] of dieldrin assuming a monomer gaseous species.

Macknick and Prausnitz (1979) used the same method to obtain experimental data at near-ambient temperature for selected high-molecular-weight hydrocarbons.

Buttery et al. (1969) determined the vapour pressures for nonanal, undecan-2-one, and methyl octanoate using gas-chromatography method. These values at 25°C were determined in the following way. The pure compound (10 ml) was placed in a dry Teflon bottle and equilibrated in a 25° C water bath for 30 minutes. Vapor samples were introduced into the GLC apparatus by connecting an 18-inch length of Teflon capillary tube (0. 04-inch I.D.) from the GLC gas sampling valve to the Teflon bottle and then transferring the sample to the valve by squeezing the flexible bottle.
The concentration of the compound in the vapour was determined by comparing the vapour GLC peaks to those obtained by injecting a standard solution of the compound in hexane.

Le Thanh et al. (1993) determined the vapour pressures for six aroma compounds (ethyl acetate, ethyl butanoate, ethyl isobutanoate, ethyl hexanoate, 2,5-dimethyl pyrazine, octen-1-ol-3) using a static measurement.This method is adapted for measuring the vapour pressure and consists of measure the pressure above the product for which the thermodynamic equilibrium is attended.
A smoothing of the experimental points was made with the semi-empirique equation of Antoine, which links up the vapour pressure with the temperature T (in K):

$$\log P^s = A - \frac{B}{C + (T - 273)} \qquad \textbf{(1-9)}$$

where: P^s = the vapour pressure of a pure compound [mm Hg];
T = absolute temperature [K].

The three coefficients A, B, and C from this equation were calculated for all the compounds.

Van Boekel et al (1992) reported that most flavour compounds have a lower vapour pressure (and higher odour thresholds) in oil than in aqueous solutions.

1.2.4. Studies on Partition Coefficients

Since partition is a fundamental parameter describing the distribution of a volatile flavour compound between two phases at equilibrium, it has been widely studied (Taylor, 2002).

The work presented by Land & Reynolds (1981) emphasized the importance of the partition coefficient as this is the underlying principle governing the release of flavours from the food matrix into the gas phase (the so-called *headspace*).

Andriot et al. (2000) and van Ruth et al. (2002) explained that the influence of specific physicochemical properties (composition, structure, concentration) of a food matrix on the volatility of an aroma compound can be observed using static headspace analysis and quantified in terms of the air-to-matrix partition coefficient for that compound.

The flavour volatiles partition between the phases depending on their relative affinity for the phases. Unfortunately, the theory of partition can be applied only to relatively simple systems and, as Land (1996) pointed out, the physical laws which describe the processes of diffusion and the equilibration concentration ratios are understood for simple single-phase bulk systems such as water or oil, although there is much less data for many of the solid materials which are present in foods.

Partition coefficients describe the thermodynamic component and the extent of aroma release under equilibrium conditions.

Partition coefficients are based on many parameters, e.g., polarity, volatility and molecular mass. In general, partition coefficients of volatile substances in water increase with increasing water solubility and therefore the following order among chemical classes is observed: aromatics > cycloalkanes > alkenes > alkanes (Seto, 1994). Within each chemical class, a decrease in partition coefficient occurs as the molecular mass increases (Buttery et al., 1969).

Verschueren (1983) gave a definition for the partition coefficient and said that the partition coefficient ***P*** is defined as the ratio of the equilibrium concentration ***C*** of a dissolved substance in a two-phase system consisting of two largely immiscible solvents, for example n-octanol and water:

$$P = \frac{C_{octanol}}{C_{water}} \qquad \textbf{(1-10)}$$

In addition to the above, the partition coefficient is ideally dependent only upon temperature and pressure. It is a constant without dimensions. It is usually given in the form of its

logarithm to base ten (**logP**). LogP values are indicator variables for lipophilicity (Buhr et al., 2001).

Taylor (1998) pointed out that if a single volatile is dissolved in a solvent (the binary system), the relationship between the concentration of volatile compound in the liquid phase and in the air phase can be expressed by Henry's law. This states that "the mass of vapour dissolved in a certain volume of solvent is directly proportional to the partial pressure of the vapour that is in equilibrium with the solution" (Morris, 1968).
The same author found when the concentrations in the water and gas phases are plotted against each other a linear relationship signifies that Henry's law is applicable for data in this range.
Land (1996) stated that "most published data at realistically low concentrations show that such (model) systems do obey Henry's Law".

As Hansen et al. (1993) remarked, an innovative static headspace method referred to as equilibrium partitioning in closed systems (EPICS) has been used frequently to measure the Henry's law constants of volatile organic compounds. The EPICS method is based on a comparison of the headspace concentration of a volatile compound in two systems at equilibrium which are identical in the mass of the compound but not identical in the volumes of the gas and liquid phases.

Robbins et al. (1993) presented a new method for determining Henry's law constants, applicable to the static headspace method. Experimentally, this method involves measuring by gas chromatography the equilibrium headspace peak areas of one or more compounds from aliquots of the same solution in three separate enclosed vials having different headspace-to-liquid volume ratios. A plot of the reciprocal of the peak areas versus headspace-to-liquid volume ratios gives a straight line. The slope of that line divided by its *y*-intercept, as determined by linear regression, gives a value for the dimensionless Henry's law constant:

$$H_i = slope / y - \text{int}\, ercept \qquad \textbf{(1-11)}$$

A similar headspace gas chromatographic method for the determination of liquid/gas partition coefficients of gases and volatile substances of low and intermediate solubility was described by Vitenberg et al. (1975) ; Guitart et al. (1989).

Taylor (1998) remarked that water is frequently used as the solvent, but the same principles can be applied to other solvents, provided that they are pure solvents. In studying food, the partition between oil and air is also of interest, with each solvent having a different partition coefficient depending on the relative affinity of the volatile for the solvent.

The relationship between concentration in the aqueous phase and the gas phase is no longer a simple linear relationship but can still be described mathematically (Taylor, 1998). A special case of non-ideality was reported by Buttery et al. (1971) for volatiles that had a high affinity for the solvent. The activity coefficients for non-polar volatiles in lipid and for hydroxyl-containing volatiles in water were less than 1, demonstrating interactions between these volatile solutes and the solvents.

As Leo et al. (1971) showed, the most extensive and useful partition coefficient data were obtained by simply shaking a solute with two immiscible solvents and then analyzing the solute concentration in one or both phases.

To determine the air-water partition coefficient many workers have used simple sealed vessels (normally a glass bottle) containing a solution of the compound, which is allowed to equilibrate with the air phase at a defined temperature. Samples of the gas phase are then taken and analysed by GC (Taylor, 1998).

Chaintreau et al. (1995) described a convenient experimental method in which the phases were equilibrated in a glass syringe and portions of the air phase were then injected onto a GC by moving the syringe plunger to determine the gas-phase concentration. The method developed by him is based on a combination of static headspace sampling and dynamic headspace traps. This method has the following advantages:

- determination of the partition coefficients does not require calibration;
- quantification is achieved without adding a standard;
- combination of static headspace with traps allows components with low vapour phase concentrations to be analyzed;
- small changes in the aroma profile due to nonvolatile constituents can be investigated.

Amoore and Buttery (1978) determined the air/water partition coefficients of many odorants from available data on their vapour pressures and solubilities at 25°C. They specified that if a liquid odorant is added to water, and shaken with air in a closed vessel, the odorant dissolves and evaporates, distributing itself between the air and water in a constant ratio (at a given pressure):

$$K_{AW} = \frac{c_A}{c_W} \qquad \textbf{(1-12)}$$

The constant K_{AW} is the air / water partition coefficient; c_A: the concentration of the odorant in the air phase (grams of odorant per litre of air); c_W: the concentration of the odorant in water phase (grams of odorant per litre of water).

Buttery et al. (1969) evaluated this constant for a series of lower aliphatic aldehydes, by measuring their concentrations in samples, drawn from the air and water phases, injected into a gas chromatograph.

Also Mackay et al. (1981) determined the Henry's law constants (air/water partition coefficients) from vapour pressures and solubility data for chemicals of environmental interest, and Fendinger et al. (1989) measured experimentally the Henry's law constants of several pesticides, that have been reported in environmental samples, using a wetted-wall column and a fog chamber.

As a conclusion described by Gossett (1987), Robbins et al. (1993), and Poddar et al. (1996) in their papers, there are three basic methods cited by Mackay and Shiu (1981) for measuring Henry's law constants:

- use of vapour pressure and solubility data;
- direct measurement of air and aqueous concentrations in a system at equilibrium;
- measurement of relative changes in concentration within one phase, while effecting a near-equilibrium exchange with the other phase (batch air stripping).

Ramachandran et al. (1996) remarked that reliable *H* data are crucial in quantitative investigations in diverse areas as:

- the estimation of the flux of VOCs from surface waters to air (Fendinger et al., 1989);
- design of air-stripping towers (Hansen et al., 1993);
- partitioning of gas-phase atmospheric species into cloud and fog droplets (Kames et al., 1992);
- transfer of chlorinated organic solvents from tap water to indoor air (Tancrede et al., 1990) and;
- transfer of inhaled anesthetic gases to the brain (Lockhart et al., 1990).

Henry's law constant is a strong function of temperature (Poddar et al., 1996). The author specified that at a constant pressure, the temperature dependence of Henry's law constant can be expressed by (Robbins et al., 1993):

$$H_i = \exp\left(\frac{B_{Hi}}{T} - A_{Hi}\right) \qquad \textbf{(1-13)}$$

where: T is the absolute temperature [K] and A_{Hi} and B_{Hi} are constants which depend on the solvent-solute combination and need to be obtained from the experimental data.

The partition coefficient for a volatile compound between headspace and water ($K_{HS/water}$) can be related to its hydrophobicity, volatility, and solubility, and the presence of nonvolatile constituents in solution can subsequently change the thermodynamic behaviour of the volatile compound (Jung et al., 2003).

Buttery et al. (1968) determined air to solution partition coefficient using the following equation:

$$K_{as} = \frac{c_a}{c_s} \qquad \textbf{(1-14)}$$

where: K_{as} = the partition coefficient; c_a = solute concentration in air; c_s = solute concentration in solution.

Since many flavours are hydrophobic, fat as a food ingredient is an excellent solvent of many food flavours and even the addition of a small amount of fat to a flavour solution has a considerable effect on the food-air partition coefficient. Binding of flavours to food ingredients (proteins and also carbohydrates) can also lessen the concentration of free flavour, and hence will affect partitioning of flavour (Bakker et al., 1996).

Behaviour of the odorous compounds

Guyot et al (1996) studied the relationships between odorous intensity and partition coefficients in model emulsions for some aroma compounds, among them being δ-decalactone.

He observed that the δ-decalactone has a very strong hydrophobic behaviour that makes not possible to measure vapour-liquid partition coefficients in media containing paraffin oil.

Because δ-decalactone is highly retained by the oily phase, its concentration in the gaseous phase decreases and leads to weaker odour intensities.

Guyot also remarked that, unlike δ-decalactone has a higher affinity for the aqueous phase, when the oil content increases, the concentration in the gaseous phase increases and leads to higher odour intensities and vapour-liquid partition coefficients.

Partition in real foods

Although partition coefficients can be defined and measured, their relevance to real food systems has often been questioned (Taylor, 1998). The argument is that partition values measured at equilibrium do not reflect the situation in real foods where equilibrium is rarely achieved. The same author remarked that in many instances, foods are undergoing a dynamic process, where the gas phase is not constrained and equilibrium between the food and the gas phase is never attained. This is the situation for the majority of foods, where flavour volatiles are lost from the food into the surrounding gas phase. When food is eaten, the situation is even more complex and equilibrium is not achieved, since both aqueous and gas phases are undergoing dilution, due to saliva flow and breathing, respectively. To model this behaviour, partition coefficients need to be combined with other factors such as mass transfer and dilution (Taylor, 1998).

The rate at which equilibrium can be achieved is determined by the mass transfer coefficient (kinetic component). The mass transfer coefficient k is a measure for the velocity at which the solute diffuses through the phase (De Roos, 2000).

Mass transfer is always described as a succession of diffusion steps from the aqueous boundary layer, through the stripping phase filled membrane pores, to the stripping phase boundary layer (Souchon et al., 2004).

When phase equilibria are disturbed, mass transport will take place resulting in concentration gradients in the product and vapour phases, as is presented in **Figure 1. 7.** (de Roos, 2000).

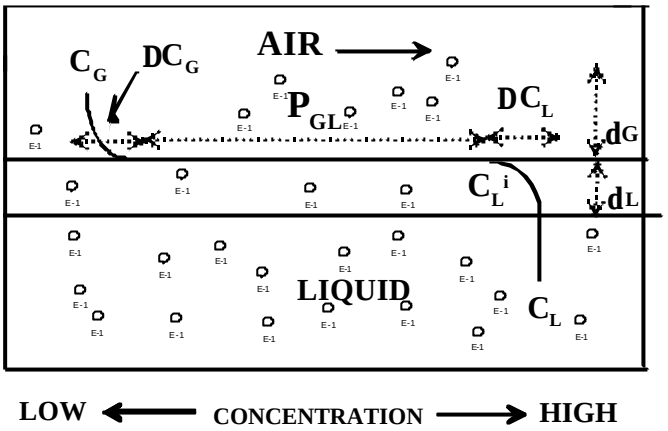


Fig.1.7*. Schematic diagram of flavour concentrations at the liquid-gas interface during release from the liquid phase (C_L: concentration in the liquid phase; C_G: concentration in the headspace; P_{GL}: gas-liquid partition coefficient; $\mathbf{d}_G$: the thickness of the gas layer; $\mathbf{d}_L$: the thickness of the liquid layer)*

1.2.5. Odor activity values (OAV)

Generalities

Odor consists of many kinds of compounds which are present in low concentration that the applicability of chemical analysis is limited. Therefore, a method based on the usage of *human nose* as a sensor, is developed.

Olfactometer is one of the techniques used to deliver odorants to panelists.

With the olfactometer, can be measured:

- Odor threshold (odour concentration);
- Odor intensity;
- Hedonic quality.

Odor intensity and odor concentration are the two most important properties of an odor (Zhang et al., 2002).

Measurement procedure

The human nose smells odor only when a certain number of molecules is present in the air.

A human nose can detect odor at concentrations well below the sensitivity levels of chemical analytical methods.

An olfactometer is an instrument that dilutes sample (odor) air with fresh air and presents the diluted sample to a sniffing port.

The concentration, at which an odorous substance can be smelled by the human nose, is called as odor threshold. In this case, the difference between an odorous and an odourless air can be detected.

As the sensitivity of different human noses differs greatly among the people, every sample is tested by a number of fixed persons at the same time.

In the olfactometer, the gas samples are diluted and are smelled by the testers. The testers smell different samples of odourless air and odorous air without any information about the type of the sample air. At the beginning, odorous air is diluted with odourless air so that the testers notice no difference between the odorous and clean air (concentration below the odor threshold). With the decrease in dilution step by step, the concentration of the odorous air increases. Independent from each other, the testers give signals when they smell any odor .

The dilution threshold is established when 50% of the panellists have correctly identified the odorous sample from the odor free samples (Feddes et al., 2001). This dilution threshold is equivalent to the odor concentration described as odor units. A sample diluted by a factor of 100 at the detection threshold has an odor concentration of 100 odor units (Feddes et al., 2001).

In 1963, Rothe and Thomas proposed the Odor Unit (concentration in food / odor threshold) to quantify the potency of odorants in a flavor system, i.e., beverages, foods, fragrances, and other natural products. This relationship has been used to distinguish constituents that may contribute to aroma (potent odorants) from the vast majority of volatiles in natural products that are at concentrations below their odor threshold (Grosch et al., 1990; Grosch, 1993; Schieberle, 1995; Audouin et al., 2001; Mistry et al., 1997; Munch et al., 1997).

Among the techniques used to deliver odorants to panelists are also food models, GCO, and water, as predicted Huyer (1917); Chaplet (1936); Dravnieks (1974); Lawless (1998); Ong et al. (1999); Gygax et al. (2001). The use of gas chromatography olfactometry (GCO) to correlate variation in the human olfactory response in both quantitative and qualitative terms can clarify the relationship between patterns of odorant mixtures and the perceptions they invoke (Acree et al., 2004).

Quantification and calculation of odor activity values (OAV)

As Grosch (2001) described, due to the complexity of the volatile fraction and the large differences in concentration, volatility and reactivity of the odorants, it is not possible in most cases to quantify the odorants precisely by using conventional methods (Grosch, 1993; Schieberle, 1995). Precise quantitative measurements of the odorants can be performed by the use of stable isotopomers of the analytes as internal standards in the so-called: stable isotope dilution assays (SIDA). As Grosch (2001) explained, each assay consists of the following steps: after the food sample or its extract has been spiked by the addition of known amounts of the corresponding labelled odorants, the volatile fraction is distilled off; the volatiles are then enriched by column chromatography; finally, the subfractions containing the mixture of the unlabelled analytes and their isotopomers are analysed by capillary GC in combination with MS. The precision of SIDA has been confirmed in model experiments (Guth and Grosch, 1990). To approach the situation in food, OAV's of the odorants were calculated.

1.2.6. Matrix effects - b-cyclodextrin / wine containing models and wine samples

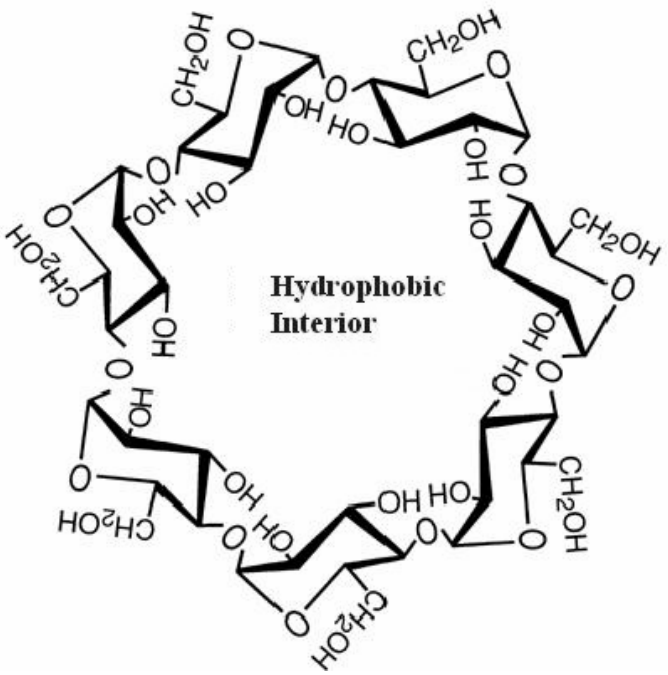
b-Cyclodextrin

Cyclodextrins are macrocyclic carbohydrate compounds, obtained from the enzymatic degradation of starch. They represent one of the simplest encapsulant systems (Kant et al., 2004).

First of all, amylase from starch decomposed in maltodextrins. Then with the help of an enzyme, cyclodextrin glycosyl transferase (CGT), from *Bacillus macerans,* the suitable cyclodextrins are produced. Besides, under splitting, the enzyme transfers an α-1, 4-binding glycosyl moiety to the non-reducing end of maltodextrins, under formation of cyclic glycosides, with 6-12 glucopyranose units.

There are three naturally occurring forms:α, β and γ, which have 6, 7 and 8 glucose units respectively.

β –Cyclodextrin (oligosaccharide), the principal product, is a cyclic heptamer composed of seven glucose units joined "head-to-tail" by α -1,4 links. The chemical structure of β –cyclodextrin is shown below:



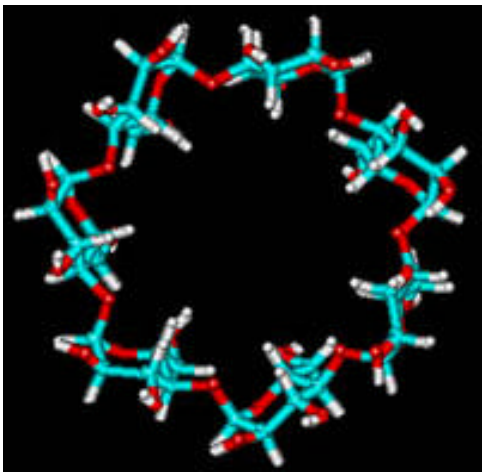


Fig. 1.8*. Chemical structure of* **b***-cyclodextrin*

The molecular weight of β–cyclodextrin is 1135 Da.

The molecule is a cylinder which is limited on one side by a crown primary (C_6) and on other side of a crown secondary hydroxyl groups (C_2, C_3), while the superficial surfaces, composed of pyranose rings, are hydrophob.

The hydroxyl units on the top of the cyclodextrin are chirally positioned and have different interactions with solute enantiomers when they are included into the cyclodextrin cavities.

The hydroxyl groups can be chemically derivatised to give a range of neutral and ionic derivatives which give different chiral separations and are more water soluble that the naturally occurring forms.

From the hydrophob hollow cavity the hydrate water is lost out very lightly from the sterisch suitable apolar compounds (e.g., to aroma compounds) which are disguised (masked) in this way.

Therefore, β-cyclodextrin is used in the food processing to the stabilization by vitamins and aromatic compounds as well as to the neutralization of bitter taste of the substances introduced.

As a result of its cyclic structure, β–cyclodextrin has the ability to form inclusion compounds with a range of molecules, generally of molecular mass of less than 250.

Micro encapsulation is a process by which a substance or a mixture is coated or entrapped in another material (Goubet et al, 2001). It is widely used in the food industry to protect flavour compounds and to control their release.

Matsui et al. (1994) have previously determined the spatial conformation of β-cyclodextrin complexes obtained with ethyl hexanoate. The authors used NMR studies for analysis of the β-cyclodextrin – ethyl hexanoate inclusion complex.

They proved that only one molecule of ethyl hexanoate can be included in each cavity. The maximal retention of 0.91 mole of aroma per mole of carrier observed in the case of ethyl hexanoate can also be explained by the fact that this aroma compound is retained in the cavity of β-cyclodextrin, whereas molecules initially present in excess are removed during freeze drying.

Figure 1.9. illustrates retention of ethyl hexanoate, hexanal, hexanol and hexanoic acid, after dehydration of a mixture in which increasing amounts of an equimolar mixture of these four compounds were initially added (Goubet et al., 1999).

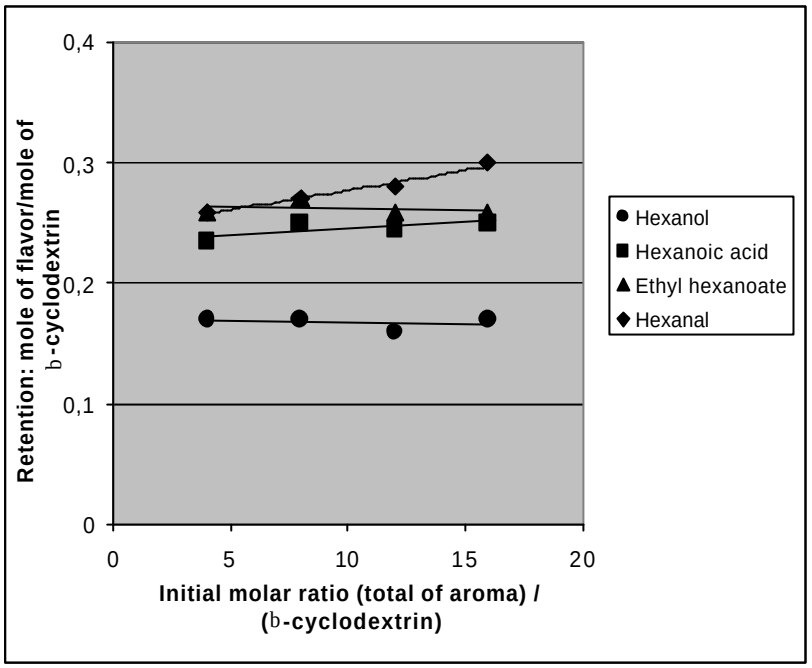


Fig.1.9*. Retention of volatiles, after dehydration of mixtures initially composed of water,* **b***-cyclodextrins and increasing amount of four aroma compounds*

There was no significant difference between retention of ethyl hexanoate, hexanoic acid and hexanal but in all cases, retention of hexanol was significantly lower than ethyl hexanoate and hexanal. Since these four compounds all have a hexyl group but differ in their other functional group, it can be deduced that there is an effect of chemical function of aroma compounds on their retention by β-cyclodextrins.

Competition between ethyl propionate and ethyl hexanoate, representing two esters with different chain length, were also studied (Goubet et al., 1999; Goubet et al., 2001).

When β-cyclodextrin was initially saturated by two moles of ethyl propionate per mole of carrier and rising amounts of ethyl hexanoate were then added, retention of the latter was strongly increased whereas retention of ethyl propionate decreased (**Figure 1.10.**).

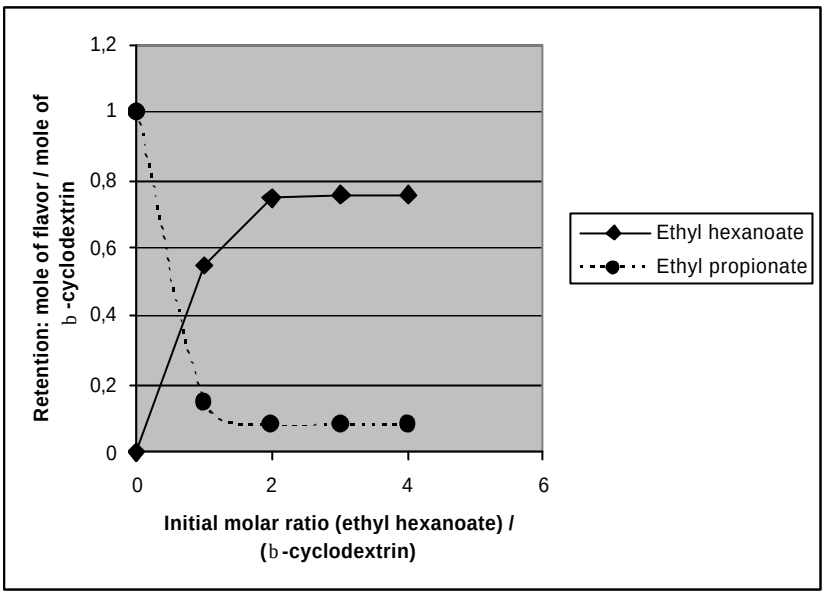


Fig.1.10*. Retention of esters after dehydration of mixtures initially containing 2 moles of ethyl propionate per mole of* **b-***cyclodextrin and increasing amounts of ethyl hexanoate*

When competition was done in the reverse order, retention of ethyl hexanoate decreased slowly as ethyl propionate retention increased. These results clearly show the preferential retention of ethyl hexanoate. The increasing affinity of the volatile compound for β-cyclodextrin with the increase of its hydrophobicity (log P), is in agreement with previous results (Tee et al., 1996) reported for homologous series of alcohols and ketones.

The compounds that were bound to the great extend to β-cyclodextrin were the most hydrophobic (Kant et al., 2004).

Wine

Ethyl esters, higher alcohols and aldehydes can be considered as representative aroma compounds of alcoholic beverages (Escalona et al., 1999).

Wine is one of the most complex alcoholic beverages, its aroma providing much of such complexity (Ortega-Heras et al., 2002).

The composition of a wine is affected by many factors, among them are: the varieties used in making it, the ripeness of the grape, the characteristics of the soil, the climatic conditions, the grapegrowing techniques, the winemaking methods, etc. (Arozarena et al., 2000).

The flavour of a wine is extremely complex, due to the great number of compounds present which have different polarities, volatilities and, moreover, are found in a wide range of

concentrations (Hernanz Vila et al., 1999). The authors specified that from this reason, sample preparation, especially extraction and concentration of aroma compounds remains one of the critical areas in aroma volatiles analysis.

More than 800 compounds have been identified in the volatile fraction of wine (Ortega-Heras et al., 2002; Guth, 1997). An important number of the volatile components in wine can only be found at very low concentration ($\mu g\ ml^{-1}$). The most studied and known compounds present in wines are the esters, alcohols, acids, terpenes, lactines, volatile phenols and aldehydes (Ortega-Heras et al., 2002; Hernanz Vila et al., 1999). Many of the aromatic components are unstable.

One of the main problems that appear when studying the compounds responsible for wine aroma is the choice of a suitable extraction procedure to qualitatively and quantitatively represent the wine original aroma. As Cies (1999) described, many aroma compounds of a wine are lost during processing of the wine, making it less pleasurable. Wine makers have been looking for ways to try and keep these compounds in the wine during periods such as fermentation, distillation, and processing. The same author remarked that wine makers have looked into many extraction processes to try and avoid loosing precious aroma compounds. Some include steam distillation, air stripping, and the spinning cone column.

The screening experiments by aroma extract dilution analysis (AEDA) and static headspace analysis-olfactometry (SHA-O), followed by quantification and calculation of odour activity values (OAV's) and reconstitution experiments are suitable tools to investigate wine flavour (Guth et al.; Escudero et al., 2000).

Another used method for the quantification of the volatiles in wines is stable isotope dilution assays (SIDA) (Schieberle et al., 1987; Guth et al., 1990; Sen et al., 1991; Guth, 1997).

Headspace solid phase microextraction (HS-SPME) was investigated as a solvent-free alternative method for the extraction and determination of some volatile compounds in red wine by capillary gas chromatography with flame ionization detector (FID) (Monje et al., 2002).

The static headspace (SHS) technique is a suitable tool for the analysis and quantification of most of the volatile compounds in wine because the preparation of the sample is very simple

and the extraction and analysis is completely automated (Ortega-Heras et al., 2002; Noble, 1978).

Concentrations of volatiles in the headspace influence the aroma character of alcoholic drinks. (Conner et al., 1998; Escalona et al., 1999).

The partition coefficients wine/air for the volatile compounds, using SHS, were determined (Clarke et al., 2004). From the experiments was observed that the volatile organic compounds with high boiling points, but low solubility in water (or wine) have very high partition coefficients. In contrast, compounds with low boiling points and high water solubility have low values. The authors pointed out that this phenomenon is a consequence of the inherent hydrophobicity of the volatile compound, reflected in the ratio of the number and size of non-polar to polar groups, and their positioning in the particular molecule.

They noticed also that in any homologous series, for example aliphatic esters, alcohols, ketones, etc., partition coefficients will be at their lowest for compounds at the bottom of the series, with the lowest molecular weights. Partition coefficients will rapidly increase with increasing molecular weight.

One of the advantages of the SHS method versus for example the liquid-liquid extraction is that the analytes are extracted from the sample matrix without the use of an organic solvent, so in the chromatogram the solvent peak does not appear. However, the method is only sensitive for detection of highly volatile components or medium volatile ones present in high concentrations, such as 2-phenyl-1-ethanol (Ortega-Heras et al., 2002).

The ability to link analytical and sensory information has been advanced through application of numerous multivariate statistical analyses.

The impact of these advances on the understanding of wine flavour has recently been reviewed (Ebeler, 1999; Ebeler, 2001; Ebeler et al., 2000; Noble et al., 2002).

Ebeler (2004) showed, that most food and beverage flavors are extremely complex and arise from the combination of a number of chemical components. For example, the distinctive varietal flavor of most wines is not due to a single impact compound but to the combination of several components, most of which are not unique to a single grape variety.

In similar studies, Fischer et. al., 1999 have shown that sensory properties of Riesling wines can vary significantly, even within the same vineyard designation. Heymann and Noble, 1987 and Guinard and Cliff, 1987 have shown that judges can distinguish among wines of different geographic origin on the basis of their aroma properties.

The quality of alcoholic beverages is significantly determined by the content of hundreds of volatile substances (Nykänen et al., 1983). Most of them are produced by fermentation processes.

Ethanol is the most abundant compound, affecting only moderately the smell and taste of all alcoholic beverages. Ethanol influences the volatility of aroma compounds in wines, it leads to modification in macromolecule conformation such as protein, which changes the binding capacity of the macromolecule (Voilley et al., 1999).

1.2.7. Molecular modelling studies on the prediction of solvation free energies of flavour compounds in different model systems

The solvation free energy is defined according to the following thermodynamic equation:

$$\Delta G = -RT \ln P \qquad \textbf{(1-15)}$$

where: ΔG – energy of solvation: kcal / mol;

R – universal gas constant: 8.314 $Jmol^{-1}K^{-1}$ = 1.98 10^{-3} kcal / mol K;

T – temperature: 298.15 K;

P – partition coefficient.

As it can be seen from the **Equation (1-15)**, the solvation free energy is correlated with partition coefficient.

Molecular modelling appears to be concerned with ways to mimic the behaviour of molecules and molecular systems (Leach, 2001).

Molecular modelling investigations are important for the consideration and prediction of complex chemical processes that take place at molecular level. From molecular modelling studies one can understand representation and treatment of real three-dimensional molecule structures and their physical-chemical properties.

Bakker et al. (1996) mentioned that using mathematical modelling to describe the various aspects of flavour release gives a fundamental understanding of the mechanism of flavour release.

Mathematical models are used to describe in physical terms the events leading to flavour release, and allow predictions regarding the factors of importance for flavour release from defined food structures.

As Bachs et al. (1994) presented, the theoretical simulation of chemical processes in solution is difficult due to the large number of solvent molecules to be considered. This impedes a pure quantum mechanical (QM) approach to the study of solvated systems and makes necessary the use of simplified methods. The authors said also that among these methods, the most popular are:

- classical (force-field-derived) models;
- the hybrid QM-classical models, and
- self-consistent reaction field (SCRF) methods.

Classical models (Jorgensen, 1991) represent both solute and solvent by means of classical Hamiltonians (force field), which permit a fast calculation of solvation free energies using molecular dynamics (MD) or Monte Carlo (MC) techniques.

QM- classical models (Singh et al., 1976; Weiner et al., 1989; Field et al., 1990; Luzhkov et al., 1992; Gao, 1992; Floris et al., 1997) describe the solute at the QM level (usually using a semi empirical Hamiltonian), whereas the solvent is treated at the classical level.

They observed that to build up the effective Hamiltonian and to solve the corresponding Schrödinger equation one has to introduce a cavity in the continuum solvent distribution, where the solute is accommodated. The need of using a cavity to solve the Schrödinger equation leads to a definition of the basic energetic quantity in a form also containing a contribution corresponding to the energy spent to form the cavity. This basic energy quantity has the status of a free energy.

Finally, SCRF methods (Tapia, 1992) use a QM description of the solute (either at the *ab initio* or semi empirical levels) and a "quasi" continuum representation of the solvent.

Bachs et al. (1994) explained that SCRF methods are based on the theory of electrostatic interactions in fluids.

SCRF methods provide a fast representation of solvent effects and allow one to consider explicitly polarization effects, which are neglected or only partially considered in classical and QM-classical calculations.

MST/SCRF Method

Miertus, Scrocco, and Tomasi developed a rigorous SCRF model (MST) (Tomasi et al., 1994; Miertus et al., 1981 ; Miertus et al., 1982). The MST model relies on the continuum model (known as polarizable continuum model).

This method makes a precise description of the perturbation operator in terms of the molecular electrostatic potential (MEP) (Scrocco et al., 1973), thus avoiding the use of truncated expansions of the solute charge distribution.

Bachs et al. (1994) described that the accuracy of the results obtained in MST calculations will depend in practice on several factors:

- the quality of the basis set in the SCF procedure;
- the cavity use to simulate the solute / solvent interface; and
- the reliability of the method used to represent steric effects (cavitations and van der Waals interactions).

Unfortunately, the criteria for the selection of the cavity size and for the calculation of steric contributions are not so clear. Particularly, the proper selection of the cavity size is crucial in MST-SCRF calculations: A cavity that is too large will underestimate the solvent effect, whereas a cavity that is too small will overestimate such an effect (Bachs et al., 1994).

The free energy of solvation in the MST model is expressed as the sum of three contributions (**Equation 1-16**): cavitations (ΔG_{cav}), van der Waals (ΔG_{vW}), and electrostatic (G_{ele}) (Curutchet et al., 2001):

$$\Delta G_{sol} = \Delta G_{ele} + \Delta G_{cav} + \Delta G_{vW} \qquad \textbf{(1-16)}$$

As Luque et al. (1996) presented, the transfer of a given solute from the gas phase into solution can be partitioned into three steps: (i) creation of the solute cavity inside bulk solvent; (ii) generation of the van der Waals particle inside the cavity, and (iii) generation of the solute charge distribution in solution. If changes in the internal degrees of freedom of the solute are neglected, ΔG_{sol} can be expressed as in **Equation (1-15)**.

Monte Carlo (MC) calculations were performed to help in determining the best solute / solvent interface for the electrostatic component of the free energy of solvation (Curutchet et al., 2001).

Also, Duffy and Jorgensen (2000) used Monte Carlo (MC) statistical mechanics to predict the free energies of solvation in hexadecane, octanol, and water for more than 200 organic solutes, including 125 drugs and related heterocycles. The study provided links between statistical mechanics simulations for solutes in solutions, traditional physical-organic analyses, quantitative structure-property relationships (QSPR), and linear response approaches for estimating free energies of solvation.

Luque et al., 1996 explained that the three main differences between the SCRF methods are: (i) the shape of the solute/solvent interface; (ii) the definition of the solvent reaction field, and (iii) the evaluation of nonelectrostatic contributions to the free energy of solvation, ΔG_{sol}.
A computational method to introduce solvent effects in the description of molecular systems in the ground state has been proposed few years ago (Pascual-Ahuir et al., 1987), and later on extended to systems subjected to a change of electronic state (Bonaccorsi et al., 1983).

Most chemistry and biochemistry occur in condensed media, in particular, aqueous solutions. Thus, the proper simulation of these processes has to take into account the solvent effects (Hernandes et al., 2002).
There are basically three models (Dillet et al., 1994; Cappelli et al., 2000) to describe the solvent, namely:

- the continuum or dielectric model;
- the discrete or super molecule model; and
- the discrete-continuum model, which attempts to combine the two previous ones.

The continuum model treats the solvent as a structureless dielectric medium and the solute is inserted in a cavity.
Hernandes et al. (2002) also specified that the continuum models are not able to describe specific solute-solvent interactions, in particular, hydrogen bonds. In addition, the definition of the solute cavity and the dielectric constant are arbitrary.
Cappelli et al. (2000) explained that continuum solvation models are generally focused on purely electrostatic effects; the solvent is modelled as a homogeneous continuous medium, usually isotropic, whose response is determined by its dielectric constant, ε . Electrostatic effects usually constitute the dominating part of the solute-solvent interaction but in some cases explicit solute-solvent interactions should be taken into account to reach a reliable and accurate estimate of the phenomenon.

The standard-state free energy of solvation is the free energy difference associated with the transfer of a solute X from the gas-phase to a given solvent Y (Ben-Naim, 1987), and it is a fundamental quantity that describes the interactions between a solute molecule and the solvent in which it is dissolved (Ben-Naim, 1987; Tomasi et al., 1994; Cramer et al., 1999).

The free energy of solvation in two solvents provides enough information to calculate the partition coefficient of a solute between the two solvents (Thompson et al., 2004).

The standard –state free energy of solvation, ΔG^0_S of a solute in a liquid solvent is written in SM5.43R continuum solvation model proposed by Thompson et al. (2004) as:

$$\Delta G^0{}_S = \Delta E + G_P + G_{CDS} + \Delta G^0{}_{conc} \qquad \textbf{(1-17)}$$

where: G_P is the electronic polarization energy from mutual polarization of the solute and the solvent; ΔE is the change in the solute's internal electronic energy when the solute is placed in the solvent; G_{CDS} is a semiempirical term that accounts for all interactions except bulk electrostatics, and ΔG^0_{conc} accounts for the concentration change between the gas-phase and the liquid-phase standard states.

The discrete model treats the solvent as individual molecules, which interact with the solute via a parametric potential (Allen et al., 1987) (classical models) or an instantaneous Coulombic interaction between the electrons and the nuclei of the solute and the solvent molecules (quantum models).

As Dillet et al. (1994) specified, in the case of a molecule interacting with a solvent, such an approach becomes inoperating because one would have to apply statistical mechanics to a system made of a large number of atoms or molecules and the computation of the electron properties of each of the many configurations to be considered is out of reach of the most powerful modern computers.

The same authors explained that this is the reason why one currently uses simplified models. One of simplest possible models consists of considering the solvent as a macroscopic continuum and the solute as filling a cavity created in this continuous medium.

1.3. Aims of the work

The effect of food matrix composition on flavour release and partition coefficient should be investigated and discussed through complementary studies carried out by thermodynamic or kinetic approaches.The basic research in this area should make a contribution to the optimization of economic processes in the industrial food production.

The present studies are part of a research project (COST Action 921) at EU level with the following title: "*Food matrices: structural organisation from nano to macro scale and impact on flavour release and perception*". Essential impulses and efficiency for the treatment of the research subject arise from the involvement and bundling of experiences and research methods on this task by the European institutions. **COST Action 921** is an international project in the framework of the European Union, whose main objective is to understand the impact of structural organisation of food matrices, and their changes during mastication, on perception and flavour release.
Further objectives are as follows:

- To understand the perception of flavour and texture as a function of composition, structure and physiology;
- To develop appropriate methods to follow the aroma release and perception during oral processing;
- To extrapolate results obtained with simple model systems to food-like models;
- To develop mathematical models which predict the relationship between the structural organisation of food matrices at molecular and meso-structure level, rheology and transport phenomena, flavour release and sensory perception.

An aim of the present research work should be the clarification of the complex relationships of the flavour release as a function of the composition of the food matrix, at molecular level, especially the clarification of the influence of matrix effects onto the partition coefficients, odour activity values and sensory properties of selected flavour compounds, in model and in real food systems.

Further aims of the research work are the determination of physico-chemical parameters of selected flavour compounds, such as vapour pressures and partition coefficients.
Different matrices should be investigated to measure their influence onto the partition coefficients of odorants: water, water-ethanol-mixtures, matrices containing lipids and more

complex samples, such as mixtures of water, oil, proteins and polysaccharides. The studies should be accompanied by olfactory measurements of the biological responses of these substances in the matrices. The influence of the various matrices on the human biological response of odorants will be investigated by an olfactometer (e.g. determination of the threshold values of odorants in air and in the presence of ethanol).

The vapour pressures and partition coefficients should be determined by using headspace gas chromatography (HS-GC) techniques.

Concerning COST Action 921 custard samples should be investigated as real food, and the aroma compounds should be quantified in the matrix and in the headspace above the food.

Molecular Modelling methods should be used for the prediction of solvation free energies of the flavour compounds studied in different model solutions, e.g. water and water-oil systems. As Software package WinMOPAC 97 will be used.

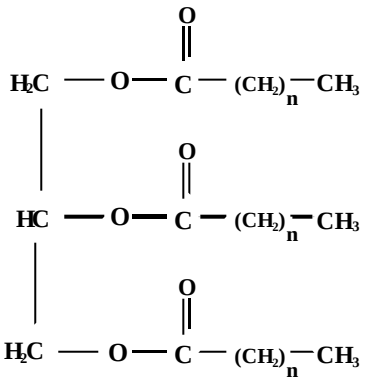
2. EXPERIMENTAL PART

2.1. Materials and Methods

2.1.1. Reference Substances

Aroma compounds: γ-decalactone, γ-nonalactone, γ-octalactone, δ-decalactone, δ-nonalactone, ethyl hexanoate were obtained from Sigma-Aldrich (Steinheim, Germany). δ-Octalactone, ethyl octanoate were obtained from Lancaster (Eastgate, White Lund, Morecambe, England), 2-phenylethanol was from Aldrich Chemical Co.Ltd. (Gillingham, Dorset-England), 3-methyl-1-butanol from Sigma-Aldrich (Steinheim, Germany), S(-)-limonene was obtained from Fluka Chemie AG (Steinheim, Germany).

- Ethanol from Roth GmbH (Karlsruhe, Germany)
- Miglyol 812, Charge 040112 from Sasol GmbH (Witten, Germany): fractionated coconut oil composed of saturated caprylic acid C_8 (50-65%) and capric acid C_{10} (30-45%) triglycerides, with the following general structure:



with n = 6, 8

- Emulsifier: Tween 85 (polyoxyethylene sorbitan trioleate) from Fluka Chemie GmbH, Germany, HLB (hydrophilic-lipophilic-balance) value: 11.0 ± 1.0
- β-Cyclodextrine: Cavamax® W7 Food from Wacker Chemie AG (Burghausen, Germany).
- Wine samples:

  Le Cadet-Sauvignon Blanc, Vintage 2000, Produce of France, wine A (cf. 2.4.1);
  Muscadet Sevre et Maine, Vintage 2000, Produce of France, wine B (cf. 2.4.1) ;
  Baden Trocken, Vintage 2000, Produce of Germany (Breisach), wine C (cf. 2.4.1).
- Model custard standard:

  Modified Tapioca starch E 1442 (Cerestar C*Creamtex 75720) from Swiss Federal Institute of Technology, Institute of Food Science and Nutrition (Zurich, Switzerland)

Full Fat Milk Powder (26% fat) from Friesland Coberco Dairy Foods (Corporate Research, Deventer, Netherland)

Strawberry aroma from Givaudan Schweiz AG (Dubendorf, Switzerland)

κ-Carrageenan (Meypro^{TM} Lact HMF, Gelymar Lot 114, Production January 2004) from Swiss Federal Institute of Technology, Institute of Food Science and Nutrition (Zurich, Switzerland)

Sucrose from Sigma-Aldrich Chemie GmbH (Steinheim, Germany)

The composition of the strawberry aroma is listed in **Table 2.1.**

Table 2.1. C*omposition of the strawberry aroma*

Aroma compound	Amount (mg / g)
Furaneol	5
Vanilin	5
Methyl cinnamate	24
Ethyl hexanoate	20
Ethyl butyrate	90
Benzyl acetate	2
Styrallyl acetate	1
Gamma-decalactone	20
Methyl anthranilate	1
Ethyl iso-pentanoate	10
Hexanal	1
cis-3-Hexenyl acetate	5
cis-3-Hexenol	15
Methyl dihydrojasmonate	5
Beta-ionone	1
Triacetin (solvent)	795

The custard was produced with the following ingredients: water, sugar (sucrose), milk powder, flavour, modified tapioca starch and carrageenan (thickener).

Model Custard Standard Recipe

Concentrations in g / 100 g custard:

4 g	modified tapioca starch E 1442 (Cerestar C* Creamtex 75720) (weight corrected for moisture content)
5 g	sucrose
0.01 g	κ-carrageenan
0.06 g	strawberry aroma
90 g	rehydrated full fat milk powder (3.5% fat)
water	weight to yield a total of 100 g (depending on moisture content of starch)

Preparation procedure of the custard sample (200 g)

Full fat milk powder (26% fat; 23.5 g) was mixed with water (45°C; 156.5 g) and left for 24 h in the refrigerator. κ-carrageenan (0.02 g) and sucrose (10 g) were mixed in the dry state in an Erlenmeyer flask, starch (8 g) was added to the mixture, and finally rehydrated milk powder at a temperature of 25°C was added. The total mixture in the flask was placed in a water bath at 97±0.5°C and stirred constantly with a propeller stirrer at 150 rpm. Water bath temperature was controlled using a thermostat and product temperature was measured. After 15 min the product temperature reached 94±1°C and heating was continued at this temperature for 15 min. After the heating process the evaporated water was replaced gravimetrically. Flavour mixture (0.12 g cf. **Table 2.1.**) was added to the mixture and the hot custard was stirred and cooled to 25°C in ice water within 15 min.

For the investigation of the flavour release of 3-methyl-1-butanol, ethyl octanoate and 2-phenylethanol the compounds were added (200 mg/200 g) to the custard sample. Before analysis the custard was stored two days in a refrigerator at 8°C.

Model mixtures:

- Water (tap water) (cf. 2.2.2.1.);
- Water (90%) + Ethanol (10%) (cf. 2.2.2.2.);
- Medium chain triglycerides (Miglyol 812, Charge 040112, Sasol GmbH (Witten, Germany) (cf. 2.2.2.3.);
- Emulsions - Model mixtures (cf. 2.2.2.4.):

  a) water (millipore), miglyol, emulsifier (polyoxyethylene sorbitan trioleate, Tween 85): 47.5 + 47.5 + 5 (w/w/w);

  b) water (millipore), miglyol, emulsifier (polyoxyethylene sorbitan trioleate, Tween 85): 85.5 + 9.5 + 5 (w/w/w);

c) water (millipore), miglyol, emulsifier (polyoxyethylene sorbitan trioleate, Tween 85): 90.25 + 4.75 + 5 (w/w/w).

2.1.2. Instrumentation

2.1.2.1. Instrumentation for the preparation of emulsions

For the preparation of the emulsions the following two instruments were used:
a) Ultra-Turrax homogenizer: Typ T 18/10, Janke&Kunkel GmbH & Co. KG, Germany, IKA LABORTECHNIK, operating range (up to 200 mP s):10-500 ml; shaft tube-∅ mm:18; stator - ∅ mm: 18; rotor - ∅ mm: 12.7; maximum circumferential (m/s): 15.9; maximum depth of immersion (mm): 225.

b) Ultrasound – disintegrator (Cell disruptor) Branson sonifier B – 15: Branson Sonic Power Co. A SmithKline Com. Gerhard Henemann Labor-Ansrüstungen (Schwäbisch Gmünd, Germany).
Technical Data – SONIFIER B-15: *Generator, FTZ* –shielded; network connection: 220 V / 50 Hz; 2.5 A; output: 150 Watt; work frequency:20 kHz; dimensions: 360x365x135 mm (BxTxH). *Convertor*: oscillator: lead-zirconate-titanate; dimensions: 178x64 mm ϕ.

2.1.2.2. Static Headspace Gas Chromatography (SHS-GC)

Static headspace analysis (SHA) was performed at a Chrompack CP 4010 gas chromatograph connected to the TCT/PTI 4001 (Varian-Chrompack, Darmstadt; Germany) headspace injector (**Figure 2.1.**) (TCT– Thermal Desorption Cold Trap injector; PTI – Purge and Trap injector).
Pure substances and model mixtures (cf. 2.1.) were put into a thermostated vessel (250 ml) (30°C), sealed with a septum, and equilibrated for 3 hours. Headspace gas was drawn by a gas tight syringe (1-5 ml, velocity of injection: 10 ml/min) and then analysed by GC-FID (Hewlett-Packard 5890 Series II) (FID – Flame Ionization Detector).
The TCT/PTI 4001 system operated in the desorption mode for 15 min at a temperature of 200°C and a flow rate of 20 ml helium (desorption purge). The fused silica trap (30 cm x 0.53 mm, coated with CP-Sil5CB, film thickness 5 μm) was cooled with liquid nitrogen at

–110°C and after 15 min the trap was heated up to 200°C and this temperature was held for 1 min. The trapped compounds were flushed by the helium flow into the GC onto the capillaries detailed in conditions of SHS.

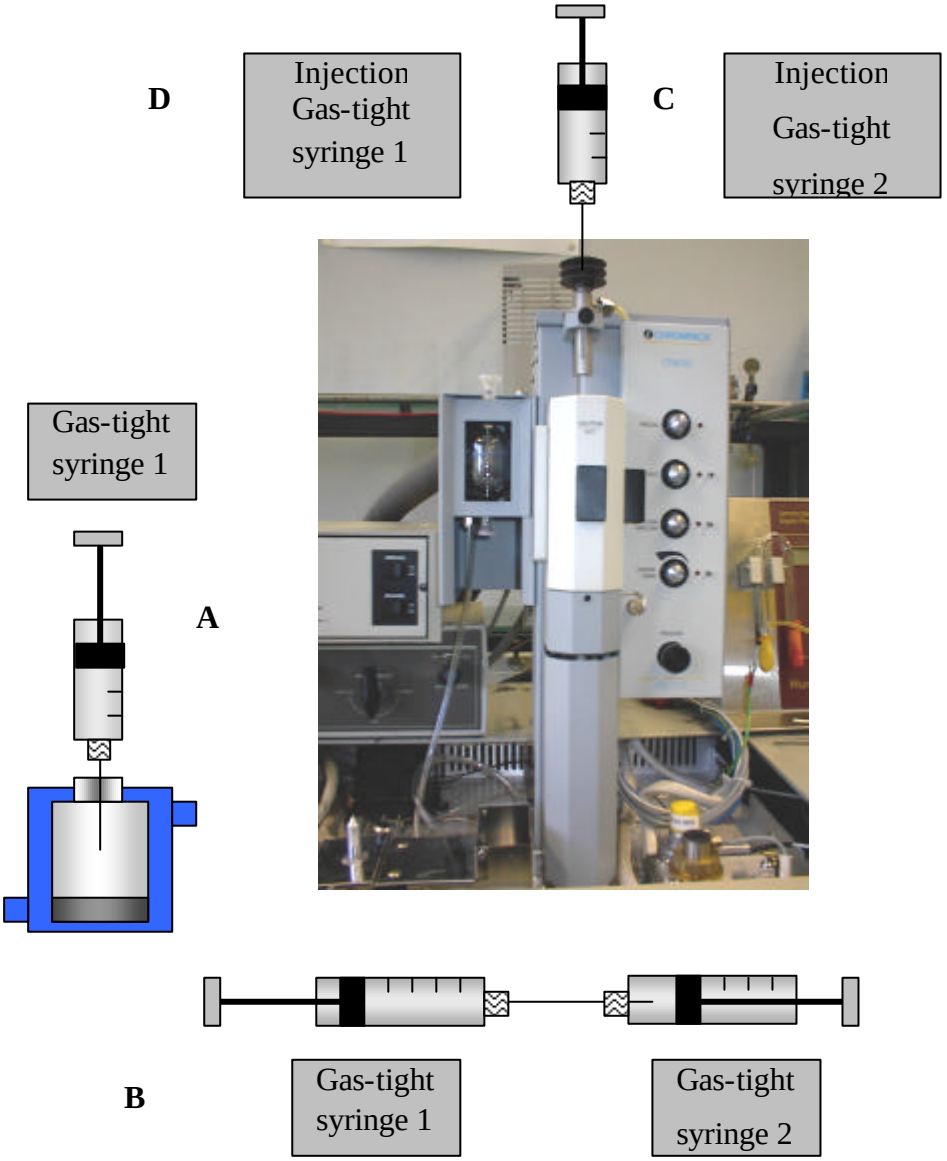


***Fig.2.1**: Schematic presentation of the method for the determination of partition coefficients (water/air, water-ethanol-mixtures/air, miglyol/air, emulsions/air) and odorant adsorptions to the gas-tight syringe. **A**: Headspace sampling. **B**: Coupling of syringe 1 and 2, transfer of a defined volume gas from syringe 1 to syringe 2. **C**: Injection with syringe 2. **D**: Direct injection with syringe 1.*

Conditions of SHS:

- Carrier gas: He, 30 kPa (20 ml/min);
- Backflush: 50 ml/min;
- Temperature program of the GC oven:

$$35°C\ (1\ min) \xrightarrow{40°C/\,min} 60°C\ (1\ min) \xrightarrow{8\ °C/min} 240°C\ (20\ min);$$

- Column: 30 m x 0.32 mm ; 0.25 µm film thickness DB-FFAP (Free Fatty Acid Phase), Phase: Nitroterephtalic acid modified polyethylene glycol, from J&W Scientific (Agilent Böblingen,Germany);
- Syringes used: 1ml and 5 ml syringes with valve (from SGE Germany);
- Equilibration time of samples: 3 h at 30°C;
- Vials volume: 250 ml.

Quantification of the odorants in the headspace was achieved by external calibration (concentration range of the standard solution:18-20 ng / 1 µl).

Adsorption measurements by static headspace gas chromatography

Adsorptions of the odorants at the gas-tight syringe were checked by the method detailed in ***Figure 2.1.*** (Guth and Sies, 2001). The calculation of the adsorption was made from five replicates (standard deviation: ± 10%).

$$\text{Adsorption (\%)} = 100 \times \frac{(odorant\ con\ syringe\ 1 - odorant\ con\ syringe\ 2)}{odorant\ con\ syringe\ 1} \qquad \textbf{(2-1)}$$

2.1.2.3. Instrumentation for the determination of odorant headspace concentrations in wine samples

For the determination of the headspace concentration of selected aroma compounds in wine samples the gas-chromatograph CP-3380 with Combi Pal SHS-Autosampler was used.

Gas-chromatograph CP-3380

- Varian GmbH (Darmstadt, Deutschland)
- Injector: Split / Splitless – Injector, Varian 1079

- Detector: FID
- Column: HP-1 (Crosslinked Methyl Silicone Gum) from Hewlett-Packard GmbH, Germany, 30 m x 0.53 mm x 2.65 µm film thickness
- Column: ZB-FFAP (Zebron Capillary GC Column), 30 m x 0.32 mm I.D. x 0.5 µm film thickness, phase: Nitroterephthalic acid modified polyethylene glycol, from Phenomenex (Aschaffenburg, Germany)

Combi Pal SHS – Autosampler (GC headspace system with liquid-autosampler)

- CTC Analytics AG (Zwingen, Zwitzerland)
- Varian GmbH (Darmstadt, Germany)
- HS-Syringe (Gastight) (volume 1 ml, Needle 23 Gauge), Axel Semran GmbH&Co KG (Germany)
- Vials for liquid injection inclusive cap and septum, 2 ml, from Supelco (Sigma-Aldrich, Germany), Screw top Vial with PTFE / Silicon Septum
- HS vials, volume 20 ml, from Supelco (Sigma-Aldrich, Germany), Headspace Clear Glass Vial,
  75.5 x 22.5 mm; long neck, HS Bottom
- Cap and Septum for HS vials: Microlitre Analytical (Sigma or Varian, Germany), Magnetic Cap, 8 mm opening, Teflon / Silicon-septum
- Liquid – Syringe (10 µl, Gauge 26S) from CS-Chromatographie Service GmbH (Langerwehe, Germany).

2.1.2.4. Mass-spectrometry (MS)

- GC – MS Instrument: GC- Hewlett-Packard -5890 Series II, MS - Hewlett-Packard 5989 A (MS 5 engine)
- Modus: Chemical ionization (CI) using CH_4 as reactant gas
- Temperature of ion source: 200°C
- Temperature of transfer line: 240 °C
- Column: 30 m x 0.25 mm I.D., 0.25 µm film thickness, DB-FFAP (Free Fatty Acid Phase), phase: Nitroterephtalic acid modified polyethylene glycol, from J&W Scientific (Agilent Technologies, Böblingen, Germany, High Resolution Gas Chromatography Column)
- Temperature of injector: 240°C

- Injection: on-column
- Detector: FID
- Carrier gas: Helium
- Temperature program of GC oven:

$$30°C\ (1\ min) \xrightarrow{40°C/min} 60°C\ (1\ min) \xrightarrow{8°C/min} 240°C\ (20\ min)$$

2.1.2.5. Olfactometer

The odour threshold values of selected odorants in air in the presence and absence of ethanol were determined with a LABC – Olfactometer (LABC-Labortechnik, Hennef, Germany), detailed in **Figure 2.2.**

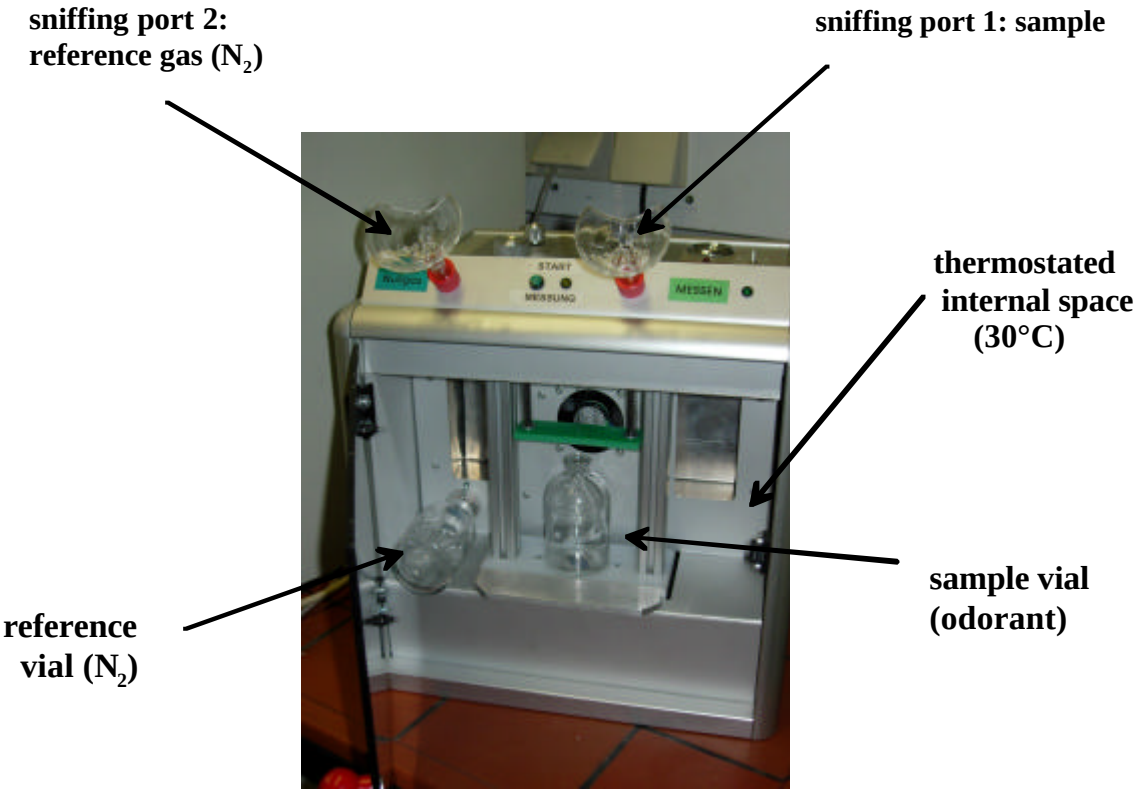


Fig.2.2*. The olfactometer*

The olfactometer has two sniffing ports, one for the reference gas (N_2) and one for the sample gas. The instrument was thermostated in the internal space at 30°C where the two vials (150 ml volume) are introduced.

2.1.2.6. Software packages

Molecular Modelling calculations were done with **WinMopac V2.0**, (Fujitsu and Chiba, Japan).

The mass transfer coefficients were calculated using the statistical program **TableCurve 2D v4** from SPSS Science (Erkrath, Germany).

Log P values were calculated using **Advanced Chemistry Development (ACD/Labs)** Software Solaris V4.67 and **Hyper Chem. 5.0.** (Hypercube, Florida, USA).

The particle size distribution of the emulsions was calculated using software package **ImageJ 1.33 u** (Wayne Rasband, National Institutes of Health, USA).

2.2. Determination of physico-chemical parameters of selected aroma compounds (lactones, esters and alcohols) with static headspace–gas chromatography (SHS-GC)

2.2.1. Determination of vapour pressure

The following procedure was used for the determination of vapour pressures of odorants: approximately 20 mg of odorant were put into a vial (headspace volume: 250 ml) and equilibrated for 3 hours, at 30°C. For lactones syringes with valve and a volume of 5 ml were used.

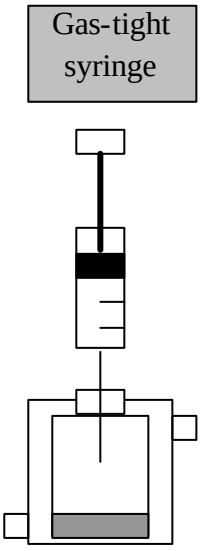


Fig.2.3. *Scheme of gas-tight syringe (Guth and Sies, 2001)*

For esters and alcohols, syringes with a volume of 5 ml and 1 ml, respectively, were used.

Adsorptions of odorants were measured according to the following procedure:

For the IInd syringe injection (cf. **Figure 2.1.**), the two syringes (I + II) were coupled together. A fixed amount of headspace sample (lactones: 1, 3, 5 ml, depending on lactone concentration; esters: 0.1 and 0.2 ml; alcohols: 1 and 0.1 ml) was taken from the vial, transferred into the syringe II and injected into the TCT-system. The syringes are equipped with a valve, which is closed after transferring of the vapour sample into the syringe, to prevent the loss of the volatile compound.

For the Ist syringe injection a fixed amount of gas sample was taken from the vial and injected directly into the TCT system.

Between two injections, a blank run was made, and before each injection the syringes were washed with hot water and dried.

From the chromatograms the areas of the compounds (Ist syringe and IInd syringe) were obtained. From these areas the adsorption effect on the syringe was calculated according to the **Equation (2-1)**. For the same odorant, between 2 and 5 injections have been done, and an adsorption average was calculated.

The concentration of the compound in the headspace was determined by comparison the area of headspace sample to the area obtained by injecting a standard solution of the compound in diethyl ether.The standard solution of specified compound (standard solution prepared in diethyl ether with a known concentration) was injected (1 µl) and the area was recorded. Also from all the standard injections an average was made (5 injections). The concentration average of the compound in the gas phase (ng/ml) was calculated according to the following equation:

$$c_a = \frac{a_{DI}}{a_{st} \times V_{gi}} \qquad \textbf{(2-2)}$$

c_a: concentration average (ng/ml); a_{DI}: average area of the direct injection; a_{st}: average area for 1 ng from the standard injection; V_{gi}: volume of gas injected (ml).

The vapour pressure for the selected pure compound, taking into account the adsorption effect was calculated according to the following equation:

$$pV = nRT \Rightarrow p = \frac{nRT}{V} \qquad \textbf{(2-3)}$$

p: vapour pressure (Pa); n: number of moles of odorant; R: gas constant (8.314 J K^{-1} mol^{-1}); T: absolute temperature (K); V: volume of gas (ml).

2.2.2. Determination of partition coefficients in different matrices

For the calculation of partition coefficients of selected aroma compounds in different matrices (water, water-ethanol mixtures, miglyol, emulsions) the procedure of work was similar as described for the vapour pressures. The syringes used for the injections were 1 ml and 5 ml syringes with valve. Partition coefficient (logP) for each compound was calculated as ratio of the odorant concentration in the matrix to the concentration in the headspace above the matrix.

2.2.2.1. Water / air partition coefficients of lactones, esters and alcohols

Solutions of selected lactones (γ-decalactone, γ-nonalactone, γ-octalactone and δ-decalactone, δ-nonalactone, δ-octalactone), alcohols (3-methyl-1-butanol, 2-phenylethanol) and esters (ethyl hexanoate and ethyl octanoate) in water were prepared with known concentrations (approximately 20 mg/100 ml and 100 mg/100 ml, respectively), depending on the solubility of selected compound in water. 10 ml of samples were put in vials (headspace volume: 250 ml) for equilibration. After equilibration (3 hours, 30°C) a defined headspace volume was injected into the TCT system according to the procedure described in 2.2.1.

The standard solution of each compound (standard solution prepared in diethyl ether with a known concentration) was injected (1 µl) and the area of the compound was obtained.

An adsorption and concentration average of specified odorant in the headspace (ng/ml) was calculated from five replicates (standard deviation: ± 10%).

2.2.2.2. Water-ethanol mixtures / air partition coefficients of lactones, esters and alcohols

Solutions of selected lactones (γ-decalactone), alcohols (3-methyl-1-butanol, 2-phenylethanol) and esters (ethyl hexanoate and ethyl octanoate) in water (90%) + ethanol (10%), v/v, were prepared with known concentrations (approximately 20 mg/50 ml, 20 mg/100 ml and 100 mg/100 ml, respectively).

The conditions of work and the injection procedure were similar as for the selected compounds in water solution (cf. 2.2.2.1.). Quantification of the odorants in the headspace was achieved by external calibration.

2.2.2.3. *Miglyol / air partition coefficients of lactones, esters and alcohols*

Solutions of selected lactones (γ-decalactone, γ-nonalactone, γ-octalactone and δ-decalactone, δ-nonalactone, δ-octalactone), alcohols (3-methyl-1-butanol, 2-phenylethanol) and esters (ethyl hexanoate and ethyl octanoate) in miglyol were prepared with known concentrations (approximately 1 g/50 ml, 10 mg/50 ml, 50 mg/50 ml and 100 mg/50 ml, respectively), depending on the solubility of selected compound in miglyol.

The conditions of work and the injection procedure was conforming those described in 2.2.2.1. Quantification of the odorants in the headspace was achieved by external calibration.

2.2.2.4. *Emulsions (Miglyol-Water-Emulsifier) / air partition coefficients of lactones, esters and alcohols*

The emulsions were prepared in glasses, by mixing calculated amounts of oil (miglyol) with water (Millipore water) and emulsifier, Tween 85 (polyoxyethylene sorbitan trioleate). An Ultra turrax homogenizer TP 18/10 (IKA-Labortechnik, Germany) was used (cf.2.1.2.1.), for homogenization 3 minutes at rotation speed indicator 4 (scale 1-10) and afterwards a Cell-disrupter disintegrator (continuous modus, 4 minutes, output control 7, on a scale:1-10) (cf.2.1.2.1.) for obtaining a better homogenization and a stable emulsion.

Three types of emulsions were prepared. The proportions between water, miglyol and emulsifier were the following ones:

- Emulsion I: Water / Miglyol / Emulsifier Tween 85: (47.5 + 47.5 + 5, w/w/w)
- Emulsion II: Water / Miglyol / Emulsifier Tween 85: (85.5 + 9.5 + 5, w/w/w)
- Emulsion III: Water / Miglyol / Emulsifier Tween 85: (90.25 + 4.75 + 5, w/w/w)

The syringes used were 1 ml and 5 ml syringes with valve. The headspace injection volume: 1 ml, 3 ml and 5ml, respectively, depending of the compound.

The quantities of water, miglyol and emulsifier were weighted at the analytical balance. First, water and emulsifier and then, during mixing with the Ultra-turax, was added slowly the weighted quantity of miglyol. The mixture was homogenized with the Ultra-turax. To obtain a stable emulsion a cell-disruptor disintegrator was used for homogenizing.

The emulsion stayed then approximately 1 hour at room temperature to stabilize. A known quantity of odorant (approximately 200 mg/10 ml, 10 mg/50 ml, 20 mg/25 ml, 25 mg/25 ml, 40 mg/20 ml, respectively) was weighted, depending on the solubility of the compound in emulsion. 10 ml from the emulsion were taken and put in vials (250 ml) for equilibration. After equilibration (1 hour, 30°C) the samples were injected into the TCT system (cf. 2.2.1.).

The standard solution of specified odorant (standard solution prepared in diethyl ether with a known concentration) was injected (1 µl) and the area of the compound was obtained. An adsorption and concentration average of selected compound in the headspace (ng/ml) was calculated from five replicates (standard deviation: ± 5%).

2.3. Interaction of odorants with b-cyclodextrin

The flavour release of ethyl hexanoate in the presence of β–cyclodextrin, respectively S-(-)-limonene in the presence of β–cyclodextrin, at different dilution stages were studied.
For the determination of the reduction of the odour compounds ethyl hexanoate and S-(-)-limonene in presence of β-cyclodextrin, a standard solution of aroma compound (solvent: tap water, pH 7.6) was prepared. A defined aliquot was taken and transferred into a headspace vial and filled up (10 ml) with water. The concentration of oligosaccharides was 10 mg/ml and for every measurement was weighted directly in the headspace vials.
For the calculation of the reduction of the esters in the headspace, a solution was prepared, which contained only the aroma compound in water. The solutions were stirred at ambient temperature (22°C) for one hour before analysis.

2.3.1. Condition for the determination of flavour release of ethyl hexanoate in the presence of b-cyclodextrin

Materials:

ethyl hexanoate water solution: 24 mg / 100 ml;
cyclodextrin: approximately 10 mg (weighted) in headspace vials (20 ml);
1 ml syringes with valve;
volume of injection: from 1 to 0.1 ml, depending on the dilution;
50 ml flasks for dilutions;
standard solution: ethyl hexanoate in pentane: 22.6 ng/ 1 µl;
volume injected: 1 µl.

Conditions of work:

equilibration time for each vial: 1 hour (stirring) at room temperature;
10 ml solution in vial (cyclodextrin + ethyl hexanoate or only ethyl hexanoate).

2.3.2. Condition for the determination of flavour release of S-(-)-limonene in the presence of b-cyclodextrin

Materials:

S-(-)-Limonene water solution: 10.6 mg / 1 L;
cyclodextrin: approximately 10 mg (weighted) in headspace vials (20 ml);
1 ml syringes with valve;
volume of injection: from 1 to 0.5 ml depending on the dilution;
50 ml flasks for dilutions;
standard solution: S-(-)-limonene in pentane: 12.1 ng/1 µl;
volume injected: 1 µl.

Conditions of work:

equilibration time for each vial: 1 hour (stirring) at room temperature;
10 ml solution in vial (cyclodextrin + S-(-)-limonene or only S-(-)-limonene).

2.4. Determination of partition coefficients of selected flavour compounds (alcohols and esters) in real food matrix

2.4.1. Wine matrix

Determination of the concentration of alcohols and esters in white wine samples

The wine samples used for the measurements were as follows: Le Cadet Sauvignon, Baden Trocken and Muscadet Sevre (cf.2.1.1.).

Standard addition method

The concentrations of 3-methyl-1-butanol, ethyl hexanoate and ethyl octanoate were determinate by standard addition method, using headspace-gas chromatography (HS-GC).
The standard addition method is used to prepare a calibration plot in cases where the composition of the sample matrix is variable or unknown so that a reagent / sample matrix blank response cannot be reliably subtracted from each standard to arrive at the analyte

response alone as with calibration plots. In these cases, the sample is spiked with increasing amounts of analyte.

The solutions were prepared according to the following procedure:

3-methyl-1-butanol: c = 11 mg/100 ml; ethyl hexanoate: c = 0,04 mg/100 ml; ethyl octanoate: c = 0,042 mg/100 ml.

For the determination of the concentration through addition procedure, method HS-GC with autosampler was used.

- HS – Autosampler (HP 1 column)
- Temperature program :

  35°C (1 min) $\xrightarrow{40°C / \text{min}}$ 60°C (0 min) $\xrightarrow{8°C / \text{min}}$ 240°C (10 min)

- Injection: 500 µl
- Temperature of incubation : 30°C
- Equilibration time: 1 h
- 9.5 ml sample in headspace vials (20 ml)
- Sample delivery : splitless

Isotope dilution analysis (IDA)

In the case of 2-phenylethanol, the concentration in wines could not be determined through standard addition method. The concentration of 2-phenylethanol was determined by isotope dilution analysis. As standard, 2-phenylethanol labelled compound ($^2[H]_2$ –phenylethanol) was used. $^2H_2$-Phenylethanol was synthesized according to:

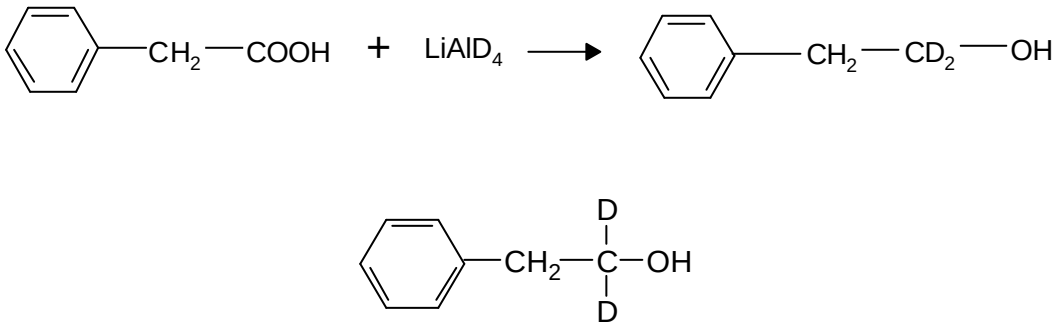


Fig.2.4*. Structure of $^2[H]_2$ –phenylethanol*

where: D = deuterium

The instrument used for the determination of the concentration of 2-phenylethanol in wine samples was GC – MS (cf. 2.1.2.4.).

Determination of the concentration of $^2[H]_2$ –phenylethanol

Methyl octanoate in diethyl ether of known concentration was mixed together with a solution of $^2[H]_2$ –phenylethanol standard (~ 40 mg/100 ml), each 1 µl of the obtained mixture was injected into the GC. The peak areas were recorded by FID detection. The concentration of $^2[H]_2$ –phenylethanol was calculated from five replicates.

Determination of mass spectrometer correction factor of $^2[H]_2$ –phenylethanol

For the determination of the correction factor (**f**), 2-phenylethanol and $^2[H]_2$ –phenylethanol with known concentrations were mixed together and 1 µl from the mixture was injected into the GC-MS (CI modus). The correction factor (**f**) was calculated according to the following equation:

$$f = \frac{\text{Area } ^2[H]_2\text{–phenylethanol} \cdot \text{Concentration of 2-phenylethanol}}{\text{Area 2-phenylethanol}} \times \text{Concentration of } ^2[H]_2\text{–phenylethanol} \qquad \textbf{(2-4)}$$

The correction value was calculated from three replicates (**f = 1.3**). The GC correction factor of $^2[H]_2$ –phenylethanol was taken from Guth H. (Habilitation work, 1997) and the value was **1.02**.

Determination of the concentration of 2-Phenylethanol in wine samples

Three samples, one for each wine, were prepared conform the following schematic procedure:

1ml wine + 100 µl $^2[H]_2$ –phenylethanol

⇓

Addition of sodium chloride (100 mg)

⇓

Addition of Pentane / Diethyl ether (1:1, v/v)

⇓

Extraction

⇓

Addition of sodium sulphate to the organic phase

⇓

Injection of 1µl organic phase into the mass spectrometer

1 µl from solvent extract was injected in GC-MS running in CI modus with CH_4 as reactant gas (cf.2.1.2.3.). The m/z for the two compounds, 2-phenylethanol and $^2H_2$-phenylethanol are: 105; 107, respectively.

Determination of the concentration of alcohols and esters in headspace above wines

The concentrations of alcohols and esters in headspace above wines were determined using headspace-gas chromatography method, with GC Varian with autosampler Combi Pal.
GC-HS conditions:

- GC Varian with HS Autosampler (HP 1 column)
- Temperature program :

  35°C (1 min) $\xrightarrow{40°C\ /\ min}$ 60°C (0 min) $\xrightarrow{8\ °C/\ min}$ 240°C (10 min)
- Injection: 500 µl
- Temperature of incubation : 30°C
- Equilibration time: 1 h
- 5 ml wine in headspace vials (20 ml)
- Sample injection : splitless
- Standard injection: 1 µl, with 5 µl syringe, manual injection after finishing the headspace injection of the 3 pure samples of wines. The sample delivery was: splitless, after 2 min with split. The standard solution contains all the compounds studied solved in diethyl ether at a known concentration. The areas from the standard injection were recorded.

The concentrations of each compound in the headspace above wine could be calculated from the areas of the compounds from the sample injection and the areas of the compounds from standard injection.

The partition coefficients of all compounds (esters and alcohols) in wine samples was calculated as ratio between the concentrations of the compounds in wines and the concentrations of the compounds in the headspace above wines.

2.4.2. Custard sample

Determination of odorant partition coefficients in custard samples

For the headspace injection custard sample (5 g) were weighted in the headspace vials (20 ml) and 0.5 ml air above the custard was drawn by a gas-tight syringe and injected into the GC instrument (GC-Varian CP-3380, cf. 2.1.2.3.).

Conditions of work:

- Injection volume: 500 µl, headspace
- Syringe temperature: 30°C
- Incubation temperature : 30°C
- Program temperature:

$$35°C\ (2\ min) \xrightarrow{40°C\ /\ min} 60°C\ (1\ min) \xrightarrow{8°C\ /\ min} 240°C\ (10\text{-}20\ min)$$

From the analysis the chromatograms were obtained and the peak areas for each compound were recorded.

The standard was injected (1 µl) and the peak areas for each compound in the standard mixture were determined.

The concentration of each aroma compound in 0.5 ml air was calculated knowing the concentration of each aroma compound in the standard, the peak areas from the standard injection and the peak areas from the headspace injection.

To calculate the concentration of each compound in custard the standard addition method was used.

Quantity of aroma compound added in 5 g custard is given in the following table:

Table 2.2. *Aroma compound added using standard addition method*

Aroma compound	mg / 5g custard 10% addition	mg / 5g custard 20% addition	mg / 5g custard 30% addition
3-Methyl-1-butanol	0.62	1.23	1.85
Ethyl hexanoate	0.06	0.11	0.17
2-Phenylethanol	0.54	1.09	1.63
Ethyl octanoate	0.54	1.08	1.62

Then the concentration of each aroma compound added function of peak area was graphically represented and the results obtained are summarized in the part Results and Discussion. From

the graphics the concentration of aroma compounds in custard were determined. After that, the partition coefficients custard / air of odorants were calculated.

Investigation of the aroma release as a function of matrix components

The following custards were prepared, under similar conditions and using the same concentration of the aroma compounds added:

1. Original Custard: κ-Carrageenan, Milk, modified Starch and Sucrose
2. Modified Custard: κ-Carrageenan, modified Starch and Sucrose
3. Modified Custard: κ-Carrageenan, Milk, native Starch and Sucrose
4. Modified Custard: Milk, modified Starch and Sucrose
5. Modified Custard: only with Milk and Water
6. Modified Custard: only with modified Starch and Water
7. Modified Custard: only with native Starch and Water

Then the aroma release as a function of matrix components for each compound was studied. The aroma compounds were analyzed and graphically represented (cf. Chapter 3).

Investigation of the time influence to the aroma release

With this attempt, the length of the incubation time of the original custard and milk was varied, to investigate their influence. The concentration and the conditions remained the same. The analyses for each compound have been done. To investigate the dependence, the data were graphically represented.

Calculation of LogP-value (octanol / water)

LogP values were calculated using **Advanced Chemistry Development (ACD/Labs**) Software Solaris V4.67 and **HyperChem. 5.0**.

Viscosity determination

The viscosity of the custard was experimentally measured using falling ball viscosimetry and the value was compared with the viscosity value for glycerine, taken as a model. For milk, the viscosity value was taken from the literature.

Custard (400 g custard prepared according to 2.1.1.)

The viscosity determination has been done in the following way:

ball (m = 2.09 g). Conditions of work: temperature = 22°C; ball diameter = 8 mm, r = 0.4 cm; berzelius glass: 400 ml, diameter = 90 mm, R = 4.5 cm; L = 7 cm (the length of the way the ball is falling); t_{med} = 4 s (the time the ball falls); $v = L / t_{med} = 7/4 = 1.75$ cm/s

$\rho_{ball} = m / V = m / (4\pi r^3/3) = 7.8$ g/cm^3

$\rho_{custard} = 1.07$ g/cm^3

The value for the custard was compared with the value for glycerine (as model) (cf. 3.5.2.1).

Glycerine

ball (m = 2.09 g). Conditions of work: temperature = 23°C; ball diameter = 8 mm, r = 0.4 cm; cylinder: 500 ml, diameter = 52 mm, R = 2.6 cm; L = 17 cm (the length of the way the ball is falling); t_{med} = 1.5 s (the time the ball falls); $v = L / t_{med} = 17/1.5 = 11.33$ cm/s

$\rho_{ball} = m / V = m / (4\pi r^3/3) = 7.8$ g/cm^3

$\rho_{glycerine} = 1.262$ g/cm^3

2.5. Determination of odorant threshold values (esters and alcohols) in air and in presence of ethanol using an Olfactometer

The threshold values in air and in presence of ethanol for three esters (ethyl butanoate, ethyl hexanoate and ethyl octanoate) and for an alcohol: 3- methyl-1-butanol were determined.

The instrument used for these determinations was a LABC – Olfactometer (LABC-Labortechnik, Hennef, Germany). The vials used: 150 ml vials.

The operation mode: automatically, the reference gas was nitrogen and the steps of dilution were recorded at the instrument.

Before the headspace vials (volume 150 ml) were introduced in the LABC-Olfactometer, a sample (2 µl of a water solution of the aroma compound, approximately 10-200 mg / L) by means of a micro litre syringe was injected inside the vial and 1 h at room temperature equilibrated. Subsequent, the pressure in the headspace vials was raised with nitrogen at 1 bar and after 1 minute, the aroma compound was released in a nose mask. The process was repeated (dilution steps) until no odour could be detected at the nose mask.

For the determination of the thresholds values in presence of ethanol, the sample volume

(2 µl) and ethanol (4.2 µl, calculated from the concentration of ethanol determined in the headspace) were injected, using a micro litre syringe, into the headspace vial. The vial was left 1 h, to equilibrate, at room temperature. Then the pressure was increasing at 1 bar and after 1 minute the aroma compounds were released as shown before.

The determination of the threshold value of the substance took place through the dilution series (1+1, w/w) of the aroma compound in water, after introducing of the headspace vials (150 ml) in the olfactometer, in the same conditions described above.

This value was obtained dividing the concentration of aroma compound in the headspace vial by the value written at the dilution step where no more aroma compound was detected by the nose.

Determination of the headspace odour activity values (HOAV's)

Headspace odour activity values (HOAV's) were calculated from aroma concentration in the headspace with and without ethanol divided by the threshold values of the odorant.

3. RESULTS AND DISCUSSION

3.1. Determination of the adsorption effects of odorants at the gas-tight syringe

The vapour pressures and partition coefficients of esters (ethyl hexanoate and ethyl octanoate), alcohols (3-methyl-1-butanol and 2-phenylethanol) and lactones (γ-decalactone, γ-nonalactone, γ-octalactone and δ-decalactone, δ-nonalactone, δ-octalactone) in different model systems (water, water-ethanol, miglyol, emulsion) were determined by static headspace gas chromatography (SHS-GC). Adsorptions of the odorants at the gas-tight syringe were checked by the method detailed in **Figure 3.1.**

The influence of the model systems on the adsorptions of the odorants at the gas-tight syringes were taken into account. Adsorption values are summarized in **Table 3.1** and calculated from five replicates (standard deviation: ± 10%).

Table 3.1 *Adsorption of selected flavour compounds at the gas-tight syringe depending on model system*

Compound	Adsorption (%)						
	Pure compound	Water	Water-ethanol	Miglyol	Emulsion (miglyol-water)		
					I[a]	II[b]	III[c]
Ethyl hexanoate	38	59	59	56	55	60	50
Ethyl octanoate	38	29	31	43	66	47	41
3-Methyl-1-butanol	68	63	20	55	58	47	58
2-Phenylethanol	17	23	19	55	48	56	36
γ-Decalactone	32	19	72	46	68	77	58
γ-Nonalactone	24	34	nd	26	23	28	30
γ-Octalactone	26	5	nd	24	23	30	48
δ-Decalactone	22	83	nd	39	87	89	85
δ-Nonalactone	5	67	nd	30	11	38	61
δ-Octalactone	33	18	nd	18	18	22	47

nd: not determined

a) Emulsion I: Water / Miglyol / Emulsifier Tween 85: (47.5 + 47.5 + 5, w/w/w)
b) Emulsion II: Water / Miglyol / Emulsifier Tween 85: (85.5 + 9.5 + 5, w/w/w)
c) Emulsion III: Water / Miglyol / Emulsifier Tween 85: (90.25 + 4.75 + 5, w/w/w)

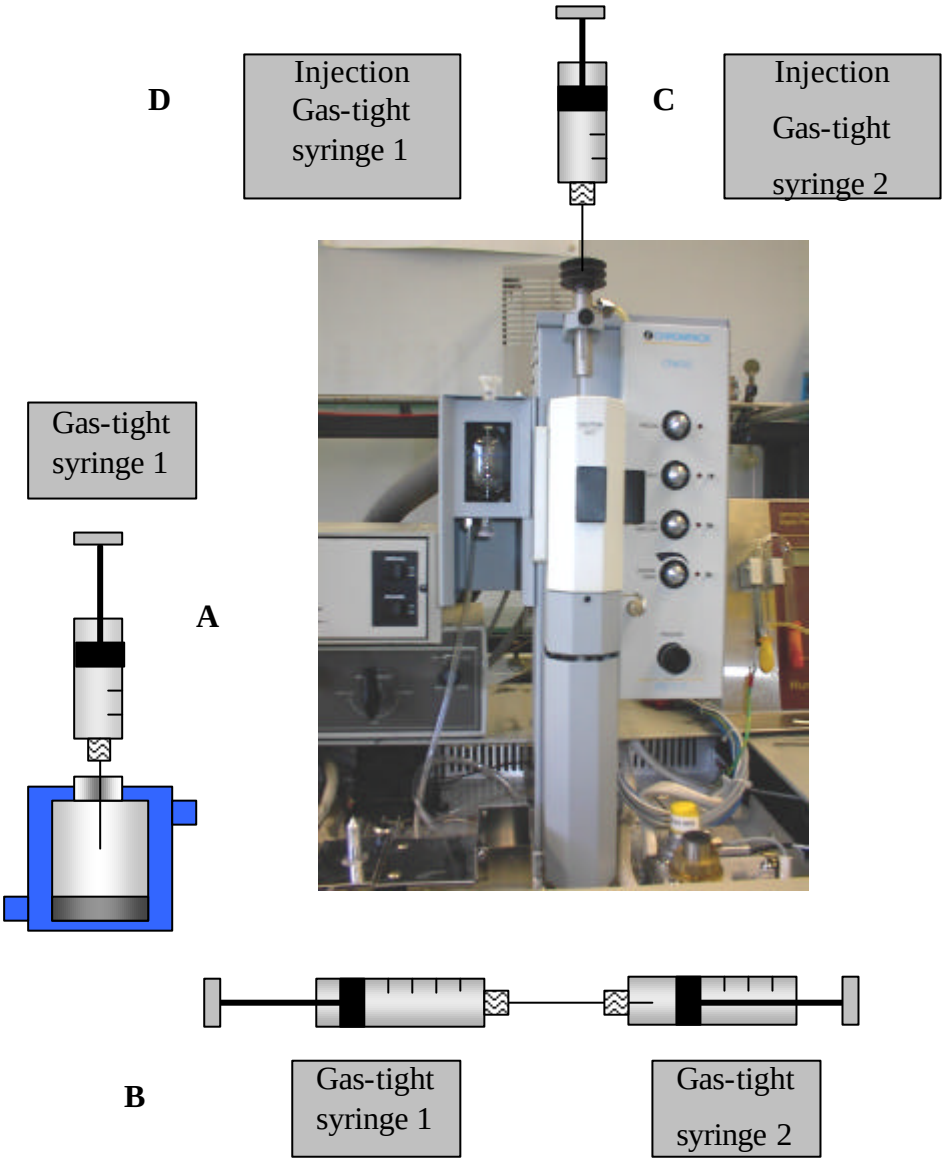


***Fig.3.1**: Schematic presentation of the method for the determination of partition coefficients (water/air, water-ethanol-mixtures/air, miglyol/air, emulsions/air) and odorant adsorptions to the gas-tight syringe. **A**: Headspace sampling. **B**: Coupling of syringe 1 and 2, transfer of a defined volume gas from syringe 1 to syringe 2. **C**: Injection with syringe 2. **D**: Direct injection with syringe 1.*

$$\text{Adsorption (\%)} = 100 \times \frac{(odorant\ con\ syringe\ 1 - odorant\ con\ syringe\ 2)}{odorant\ con\ syringe\ 1} \qquad \textbf{(3-1)}$$

Table 3.1 indicates that the adsorption values of the investigated esters and alcohols are higher in emulsion matrices than in water, water-ethanol and miglyol, respectively.
In the series of γ- and δ-lactones the compounds γ- and δ-decalactone have the highest adsorption at the gas-tight syringes in miglyol-water emulsions.

3.2. Determination of the vapour pressures of selected aroma compounds and comparison with literature data

The procedure of the determination of the vapour pressures of selected aroma compounds is described in the experimental part (cf. 2.2.1.). The values obtained in this study are summarized in **Table 3.2**, together with the values found in the literature.

Table 3.2 *Vapour pressures of selected aroma compounds*

	Present study	Literature
Compound	Vapour pressure (Pa)[a] (30°C)	Vapour pressure (Pa)[b]
Ethyl hexanoate	165	133 (92°C) [1], 878 (40°C) [2], 220 (25°C) [3], 238 (25°C) [4], 133.3 (25°C) [5]
Ethyl octanoate	35	133 (125°C) [1], 213 (40°C) [2], 29 (25°C) [3]
2-Phenylethanol	13	17 (30°C) [6], 9.42 (30°C) [7], 11.57 (25°C) [8]
3-Methyl-1-butanol	582	640 (30°C) [1], 616 (30°C) [6], 553 (25°C) [3]
γ-Decalactone	0.9	2 (40°C) [2]
γ-Nonalactone	1.1	7 (40°C) [2]
γ-Octalactone	7.0	14 (40°C) [2]
δ-Decalactone	0.5	2 (40°C) [2]
δ-Nonalactone	0.8	-
δ-Octalactone	2.4	10 (40°C) [2]

a) Adsorptions of pure compounds at the gas-tight syringe were taken into account (**Table 3.1**);

b) [1] Verschueren (1983), [2] Siek et al. (1970), [3] Meyer et al.(2004), [4] Goubet et al. (2001), [5] Roberts et al.(2000), [6] Guth (1997), [7] Vuilleumier et al.(1995), [8] Daubert et al. (1989).

Table 3.2 indicates that the vapour pressures of esters and alcohols are decreasing with the increasing of the carbon chain length. The values found in the present study are similar to the values found in the literature.

3.3. The influence of the matrix effects onto the partition coefficients of selected aroma compounds (model systems: water, water-ethanol, miglyol and miglyol-water emulsions)

For the selected aroma compounds the partition coefficients in different matrices were calculated. The results obtained were compared with those found in literature. The method used for the determinations of the partition coefficients was static headspace-gas chromatography (SHS-GC). The adsorption effect at the syringes walls was taken into account (cf. 3.1).

Partition coefficients (water /air) of esters, alcohols and lactones

The partition coefficients ($\log P_{W/A}$) determined experimentally by static headspace-gas chromatography (SHS-GC) for the selected aroma compounds are summarized in **Table 3.3**, together with the values found in the literature.

Table 3.3 indicates that in the series of esters and alcohols, the partition coefficients ($\log P_{W/A}$) are increasing with the carbon chain length. For lactones the partition coefficients increased with the molecular weight of the odorants.

Partition coefficients (water + ethanol / air) of esters, alcohols and lactones

The partition coefficients ($\log P_{W+Et/A}$) determined experimentally by static headspace-gas chromatography (SHS-GC) for the selected aroma compounds are summarized in **Table 3.4**.

The data found in this study were compared with literature data.

For esters, the partition coefficients water + ethanol / air are decreasing with the increasing of the carbon chain length. The values are higher in water + ethanol than in water, both for esters and alcohols.

For lactones, only one lactone was analysed (γ-decalactone) and the partition coefficient determined. The partition coefficient of γ-C_{10} in water + ethanol, obtained experimentally, is higher than the partition coefficient of γ-C_{10} in water. This means that the presence of ethanol in the matrix has an important influence on the partition coefficient.

The values found in the literature for some of the compounds are comparable with those found experimentally. The determination of the partition coefficients in water-ethanol models was important for further investigations of the partition coefficients in wines.

Table 3.3 *Partition coefficients (water/air) of selected aroma compounds*

Compound	Present study Partition coefficients water / air ($logP_{W/A}$)[a] (30°C)	ΔG^c (kcal/mol)	Literature Partition coefficients water / air ($logP_{W/A}$)[b]
Ethyl hexanoate	2.2	-3.1	1.2 [1]; 1.53 (25°C) [2]
Ethyl octanoate	2.56	-3.5	0.72 [1]
2-Phenylethanol	5.23	-3.4	4.63 [3]
3-Methyl-1-butanol	2.52	-7.2	2.88 [1]; 3.17 [3]
γ-Decalactone	3.9	-5.3	-
γ-Nonalactone	4.25	-5.8	-
γ-Octalactone	4.82	-6.6	-
δ-Decalactone	4.62	-6.3	-
δ-Nonalactone	5.25	-7.2	-
δ-Octalactone	5.68	-7.8	-

a) Adsorptions of pure compounds at the gas-tight syringe were taken into account (**Table 3.1**);

b) [1] Meyer et al. (2004), [2] Roberts et al. (2000) [3] Guth et al. (2001)

c) Solvation free energy: $\Delta G = - RT \ln P_{W/A}$

Table 3.4 *Partition coefficients (water + ethanol / air) of selected aroma compounds*

Compound	Present study Partition coefficients water + ethanol / air ($logP_{W+Et/A}$)[a] (30°C)	Literature values Partition coefficients water + ethanol / air ($logP_{W+Et/A}$)[b]
Ethyl hexanoate	2.9	-
Ethyl octanoate	2.53	-
2-Phenylethanol	5.37	4.65 [1]
3-Methyl-1-butanol	3	3.32 [1]
γ-Decalactone	4.16	-

a) Adsorptions of pure compounds at the gas-tight syringe: taken into account (**Table 3.1**);

b) [1] Guth et al. (2001)

Partition coefficients (miglyol / air) of esters, alcohols and lactones

The miglyol/air partition coefficients ($logP_{M/A}$) determined by static headspace-gas chromatography (SHS-GC) for the selected aroma compounds are summarized in **Table 3.5**.

Table 3.5 *Partition coefficients (miglyol / air) of selected aroma compounds*

Compound	Partition coefficients miglyol / air ($logP_{M/A}$)[a] (30°C)	ΔG[b] (kcal/mol)
Ethyl hexanoate	4.29	-5.9
Ethyl octanoate	5.34	-7.3
2-Phenylethanol	5.73	-4.6
3-Methyl-1-butanol	3.35	-7.9
γ-Decalactone	6.61	-9.1
γ-Nonalactone	6.28	-8.6
γ-Octalactone	6.19	-8.5
δ-Decalactone	6.89	-9.5
δ-Nonalactone	6.55	-9.0
δ-Octalactone	6.25	-8.6

a) Adsorptions of pure compounds at the gas-tight syringe were taken into account (**Table 3.1**).

b) Solvation free energy: $\Delta G = - RT \ln P_{M/A}$

In miglyol, the partition coefficients miglyol/air of esters and alcohols are increasing with the molecular weight of the odorants. The same tendency can be observed for γ-lactones and δ-lactones. For the same homologue of the serie, the values of the partition coefficients for the δ-lactones are higher than for the γ-lactones.

No literature data are available for the partition coefficients of selected odorants for miglyol/air models.

In the present study, the experimentally water-to-air partition coefficients ($logP_{W/A}$) for the selected aroma compounds have been compared with miglyol-to-air partition coefficients ($logP_{M/A}$). The data are summarized in **Table 3.6.** The solvation free energies (ΔG) of odorants in water were calculated and included in **Table 3.6**.

From the **Table 3.6** it can be seen for all compounds analysed, that the partition coefficients miglyol/air are higher than the partition coefficients values water/air.

Table 3.6 *Comparison of water-to-air and miglyol-to-air partition coefficients for selected aroma compounds (30°C)*

Compound	Partition coefficients water/air ($logP_{W/A}$)[a] (30°C)	Partition coefficients miglyol/air ($logP_{M/A}$)[a] (30°C)	Δ $logP_{M-A}$ ($logP_{M/A}$ - $logP_{W/A}$)
Ethyl hexanoate	2.22	4.29	2.07
Ethyl octanoate	2.56	5.34	2.78
2-Phenylethanol	5.23	5.73	0.50
3-Methyl-1-butanol	2.52	3.35	0.83
γ-Decalactone	3.9	6.61	2.71
γ-Nonalactone	4.25	6.28	2.03
γ-Octalactone	4.82	6.19	1.37
δ-Decalactone	4.62	6.89	2.27
δ-Nonalactone	5.25	6.55	1.30
δ-Octalactone	5.68	6.25	0.57

a) Adsorptions of pure compounds at the gas-tight syringe were taken into account (**Table 3.1**).

The differences, Δ $logP_{M-A}$, of the partition coefficients $logP_{M/A}$ and $logP_{W/A,}$ are presented in **Table 3.6.** For ethyl hexanoate the concentration is higher in the headspace above water than in the headspace above miglyol by a factor of 100. In the serie of alcohols, for 2-phenylethanol, the concentration in the headspace above water is higher in comparison to the concentration in the headspace above miglyol only by factor of 5.
In the case of γ-nonalactone, for example, the difference is by factor of 100. The reason of these differences is the hydrophobicity of the compounds.

For the water systems, the partition coefficients $logP_{W/A}$ determined experimentally were compared with the partition coefficients $logP_{W/A}$ calculated according to Guth (2002), from the Henry constant, taking into account the vapour pressure of the pure compounds and the solubility of the odorants in water. The data are summarized in **Table 3.7.**
For the miglyol matrix, an important comparison could be made with the work of Buttery et al. (1973) who determined experimentally the air to vegetable oil partition coefficients for a number of organic flavour compounds (aldehydes, ketones and alcohols) and developed a

model for the prediction of air/vegetable partition coefficients. The author proposed an equation for the determination of air/vegetable partition coefficients.

For our study, the equation given by Buttery for the partition coefficient can be re-written as follows:

P_{sa} = (solute concentration in solution) / (solute concentration in air) **(3-2)**

$$P_{sa} = \frac{1}{C \times (solvent\ conversion\ \ factor)} \quad \textbf{(3-3)}$$

The solvent conversion factor is a simple number which involves conversion of pressure units to mass units. This factor is dependent on the molecular weight of the solvent but is independent of the solute molecular weight which cancels out in the calculation of this factor.

As Buttery et al. (1971) outlined, from solution volatility theory:

$$C = p_0\ x\ \gamma \quad \textbf{(3-4)}$$

$$C = p_0\ x\ \frac{1}{N_s} \quad \textbf{(3-5)}$$

where p_0 is the vapour pressure of the pure compound, γ is the activity coefficient in that solvent and N_s is the solubility of the compound in that solvent in mole fraction terms. For the low solubility compounds $N_s = 1$, that means $C = p_0$. Then, **Equation (3-2)** becomes:

$$P_{sa} = \frac{1}{p_0\ \times \gamma \times (solvent\ conversion\ \ factor)} \quad \textbf{(3-6)}$$

For vegetable oil the conversion factor determined by Buttery et al. (1973) is 5.2×10^{-5}. Taking into account this value from Buttery and the activity coefficient generally approaches 1, the P_{sa} values (cf. **Equation 3-6**) for the compounds selected in the present study were determined. The data are summarized in **Table 3.7.**

Table 3.7 lists a comparison of experimentally determined water to air and oil (miglyol) to air partition coefficient ($logP_{W/A}$ and $logP_{M/A}$) with calculated values for the aroma compounds studied.

Table 3.7 show that the $logP_{W/A}$ of lactones, calculated according to Guth (2002) are quite close to the $logP_{W/A}$ determined experimentally.

For miglyol, the $logP_{M/A}$ calculated using the **Equation (3-6)** are in agreement with the experimentally values, for the investigated aroma compounds.

Table 3.7 *Comparison of experimentally determined water to air and oil (miglyol) to air partition coefficient (30°C) ($logP_{W/A}$ and $logP_{M/A}$) with predicted values*

Compound	LogP			
	Water[a]		Miglyol[b]	
	Experimental	Calculated	Experimental	Calculated
Ethyl hexanoate	2.2	nd	4.29	4.19
Ethyl octanoate	2.56	nd	5.34	4.86
2-Phenylethanol	5.23	nd	5.73	5.29
3-Methyl-1-butanol	2.52	nd	3.35	3.64
γ-Decalactone	3.9	4.15	6.61	6.45
γ-Nonalactone	4.25	4.53	6.28	6.36
γ-Octalactone	4.82	4.32	6.19	5.56
δ-Decalactone	4.62	3.82	6.89	6.70
δ-Nonalactone	5.25	5.24	6.55	6.50
δ-Octalactone	5.68	4.92	6.25	6.02

nd: not determined

a) Henry's constant (Pa m^3 mol^{-1}) = Vapour pressure (Pa) / S_{H2O} (mol/L) x 1000 (Guth, 2002), where S is the solubility of the compounds in water determined by Fritzler R. (Ph D work, 2003);

$LogP_{W/A}$ = R T / H, where R= 8.3144 Pa m^3 K^{-1}; T = 303 K (Guth, 2002);

b) The conversion factor of oil: 5.2 x 10^{-5} (Buttery et al., 1973).

Emulsion

Three model emulsions, in which water and miglyol were in different portions, were prepared. The emulsions were studied like a premise for further investigations of the complex matrices (custard sample) to estimate the influence of the fat content to the partition coefficients.

The emulsions were microscopically analyzed at the firm Sasol Germany GmbH. The analysis indicated that the three emulsions are from type oil in water (o/w). From the microscopic pictures (**Figures 3.2-3.4**) one can observe that, with decreasing of the concentration of the oil phase, the particle distribution becomes higher. Emulsion I (**Figure 3.2.**) showed very small particles in contrast to emulsion II (**Figure 3.3.**) and emulsion III (**Figure 3.4.**).

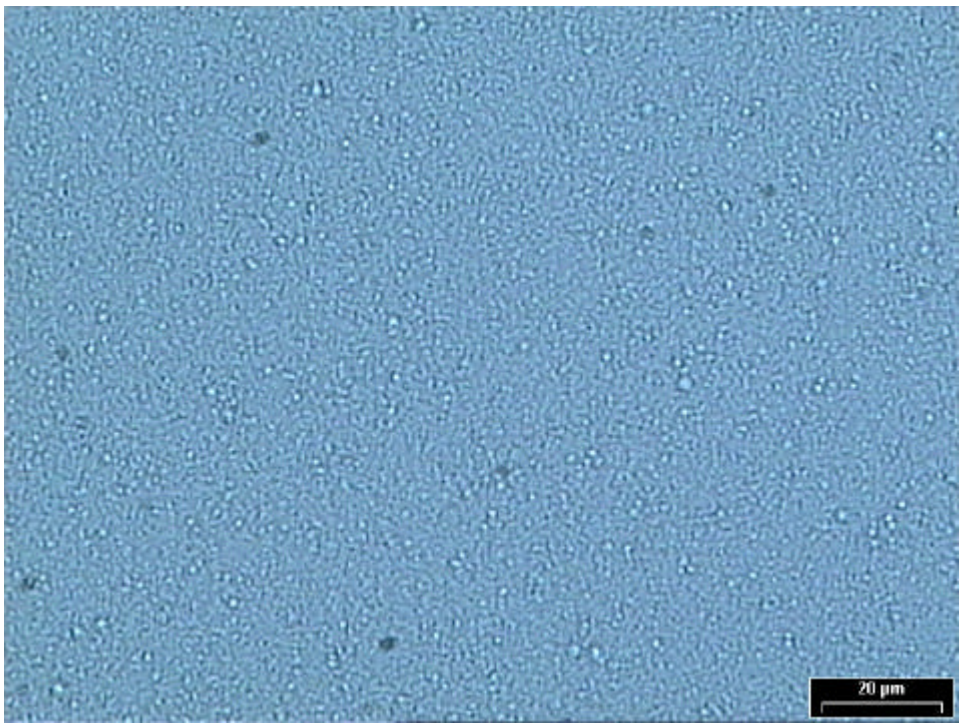


***Fig.3.2**. Emulsion I: Water / Miglyol / Emulsifier Tween 85: (47.5 + 47.5 + 5, w/w/w)*

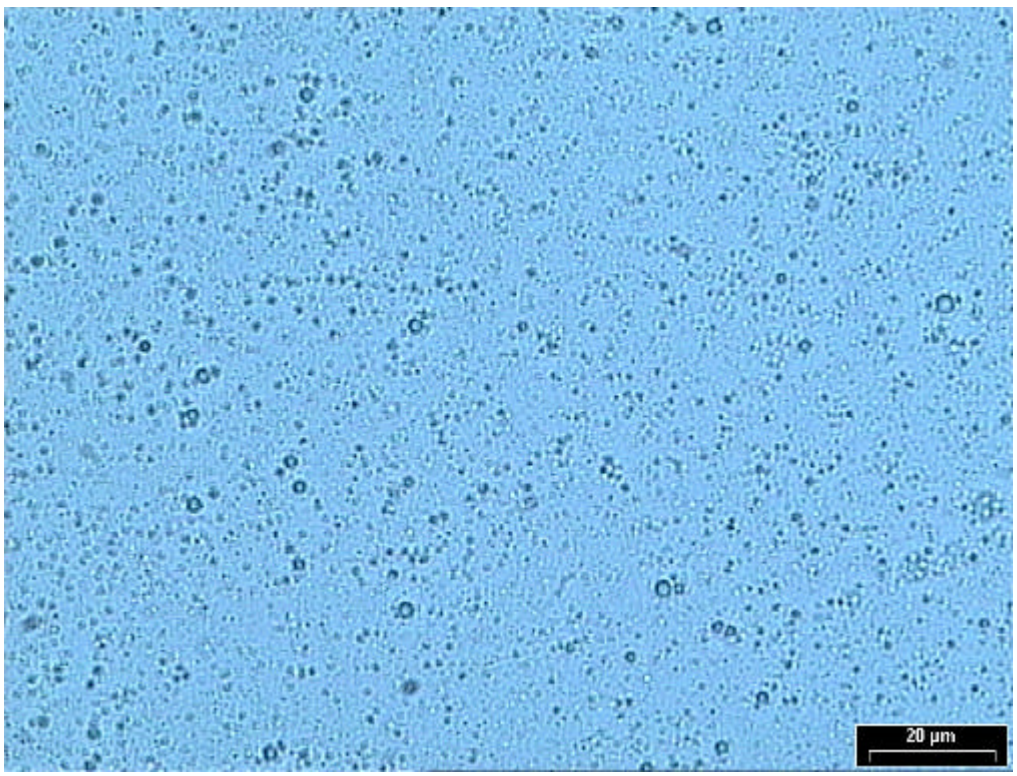


***Fig. 3.3**. Emulsion II: Water / Miglyol / Emulsifier Tween 85: (8.5 + 9.5 + 5, w/w/w)*

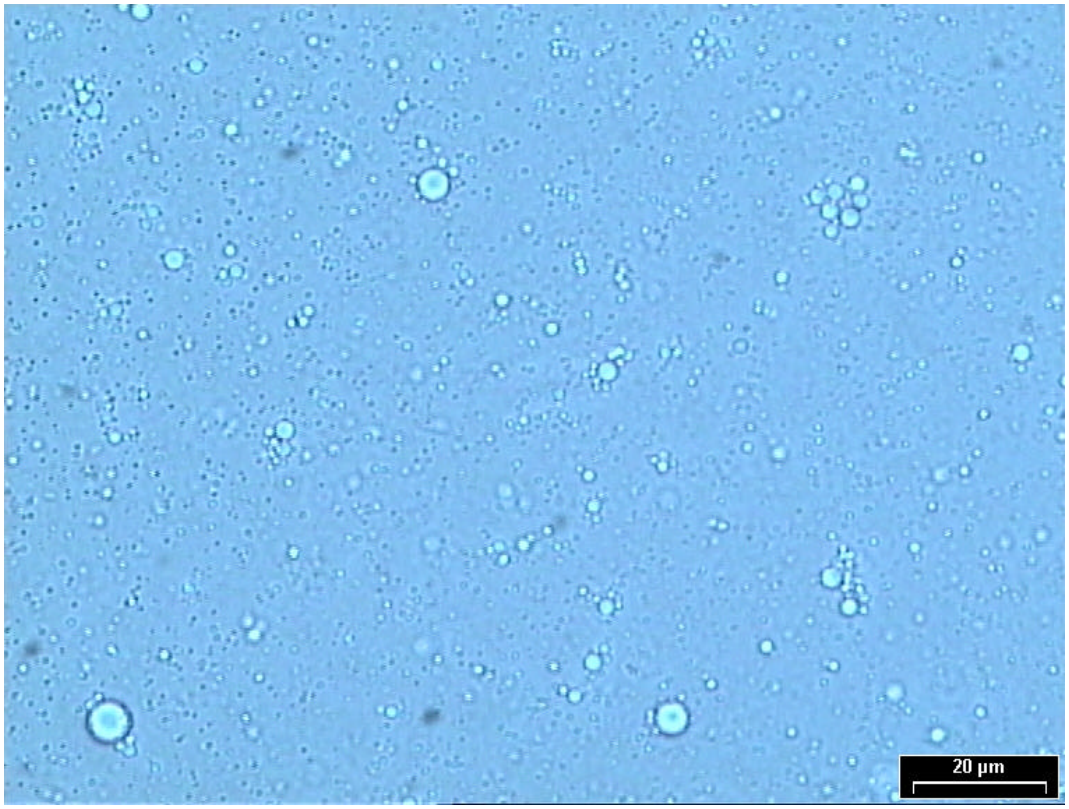


Fig. 3.4. *Emulsion III: Water / Miglyol / Emulsifier Tween 85: (90.25 + 4.75 + 5, w/w/w)*

For each emulsion (I-III), the particles were analyzed using software ImageJ. Determination of particle size distribution has been done, first by scaling the image (set scale, threshold images), and then by the command “analyze particles”. The software counts and measures objects in the binary or threshold images.

A portion of the threshold image for the emulsion I, used for the calculation, is given in **Figure 3.5**. The dark parts represent the oil particles.

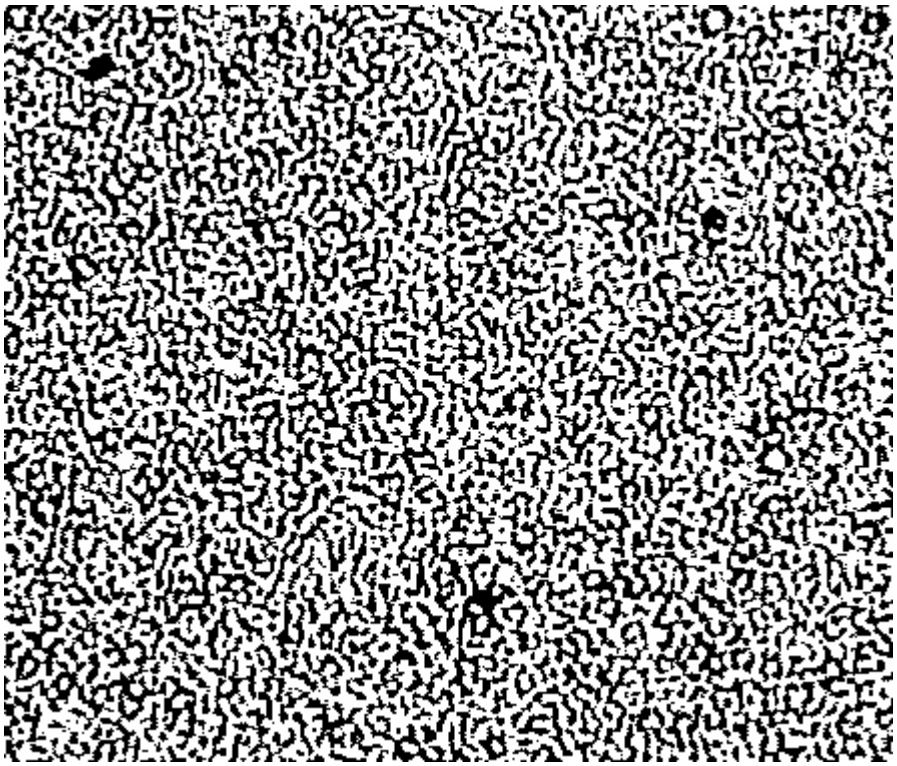


Fig.3.5 *Threshold image of emulsion I*

The results given by the software for emulsion I are: average particle size: 4.2 μm^2; area fraction: **46.6%.**

From the average size of the particles, the oil particle diameter has been calculated according to the following equation:

$$D = \sqrt{\frac{4\,A}{\pi}} \qquad \textbf{(3-7)}$$

D = particle diameter (μm); A = particle average size (μm^2)

For emulsion I, D = 2.313 μm (cf. **Equation 3-7**). The area fraction of miglyol calculated by the software is correlated very well with the portion of oil (miglyol) in emulsion I (47.5%).

For the emulsion II, the threshold image is shown in **Figure 3.6**.

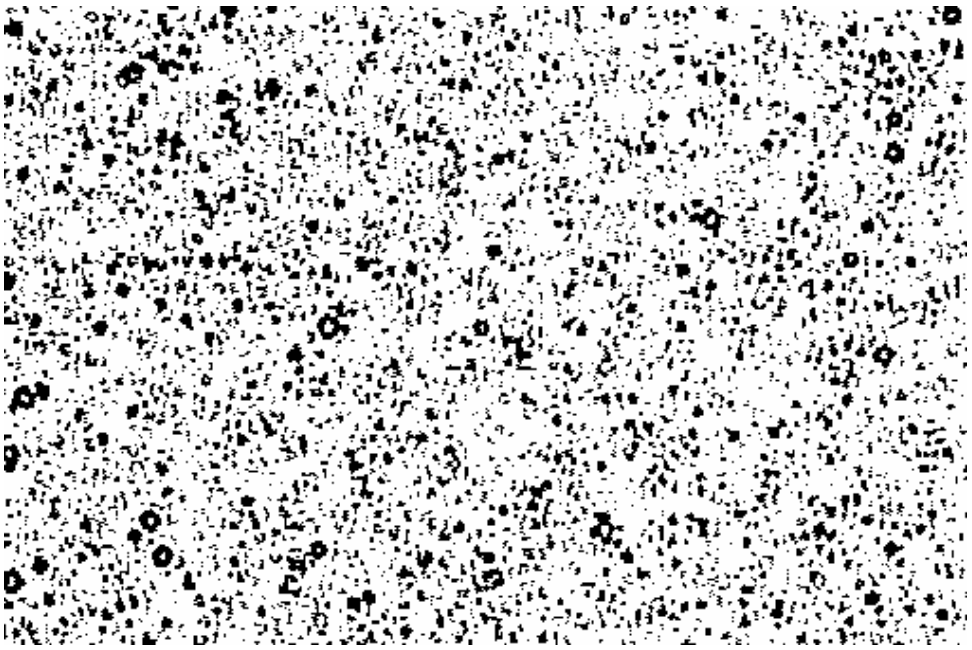


Fig.3.6 *Threshold image of emulsion II*

The results given by the software for emulsion II are: average particle size: 0.4 μm^2; area fraction corresponding to miglyol: **19.5%.**

Then D = 0.713 μm (cf. **Equation 3-7**). The area fraction of miglyol calculated by the software for emulsion II is a little higher than the portion of oil (miglyol) introduced at the preparation of the emulsion II (9.5%).

For the emulsion III, the threshold image is shown in **Figure 3.7**.

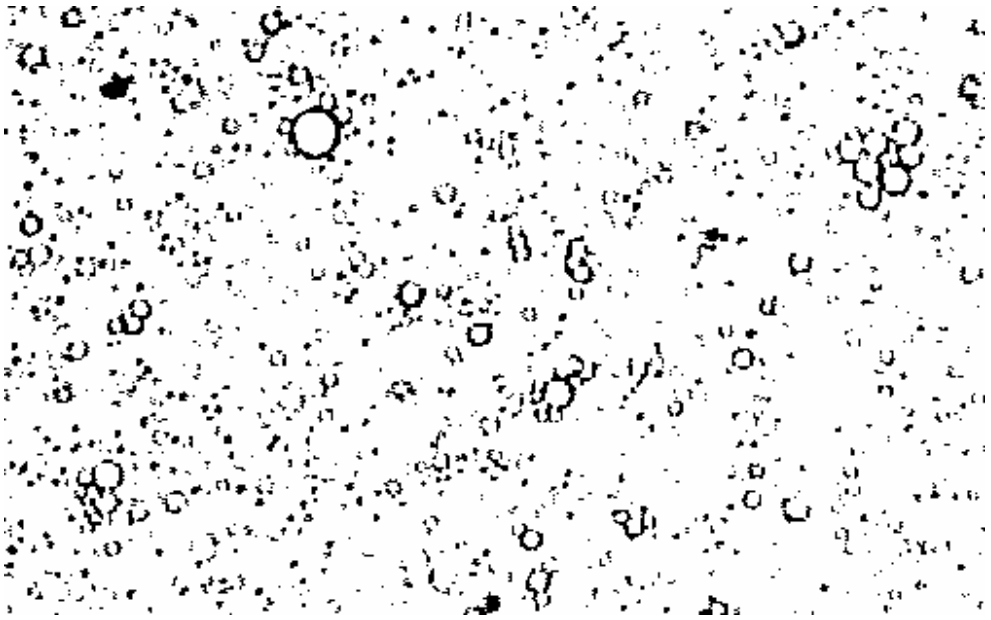


Fig. 3.7 *Threshold image of emulsion III*

The results given by the software for emulsion III are: average particle size: 0.4 μm^2; area fraction corresponding to miglyol: **8.6%.**

Then D = 0.713 µm (cf. **Equation 3-7**). The area fraction of miglyol calculated by the software for emulsion III almost corresponds to the portion of oil (miglyol) introduced at the preparation of the emulsion (4.75%).

Partition coefficients (emulsions/air) ($LogP_{E/A}$) of esters, alcohols and lactones

The partition coefficients emulsions/air for the selected aroma compounds were determined. The experimentally values obtained are summarized in **Table 3.8.**

Table 3.8 *Partition coefficients (emulsions/air) of selected aroma compounds*

Compound	Partition coefficients emulsion I / air ($logP_{EI/A}$)[a] (30°C)	Partition coefficients emulsion II / air ($logP_{EII/A}$)[a] (30°C)	Partition coefficients emulsion III / air ($logP_{EIII/A}$)[a] (30°C)
Ethyl hexanoate	4.25	3.39	3.34
Ethyl octanoate	5.06	4.75	4.59
2-Phenylethanol	5.75	5.48	5.44
3-Methyl-1-butanol	3.59	3.39	3.23
γ-Decalactone	6.50	5.95	5.91
γ-Nonalactone	6.33	5.91	5.88
γ-Octalactone	6	5.75	5.47
δ-Decalactone	6.56	5.87	6
δ-Nonalactone	6.83	6.35	6
δ-Octalactone	6.30	6.13	5.77

a) Adsorptions of pure compounds at the gas-tight syringe were taken into account (**Table 3.1**).

For the esters and alcohols in emulsion I, the partition coefficients emulsion I/air are increasing with the increasing of the carbon number. In the series of γ- and δ-lactones, it can be seen the same behaviour, exception is δ-nonalactone.

In emulsion II, the partition coefficients emulsion II/air for all aroma compounds selected are also increasing with the carbon number, except δ-decalactone.

In emulsion III, the partition coefficients values emulsion III/air is following the same behaviour as for emulsion I and II.

For the emulsions a comparison could be made with the work of Buttery et al. (1973) who determined experimentally vegetable oil-water mixtures/air partition coefficients for a number of organic flavour compounds (aldehydes, ketones and alcohols) and developed a model for the prediction of vegetable oil-water mixtures/air partition coefficients. The equation proposed by Buttery for the determination of oil-water mixtures/air partition coefficients can be re-written for our study as follows:

P_{ma} = (solute concentration in the mixture) / (solute concentration in the air) **(3-8)**

Equation (**3-8**) can be simplified to:

$$P_{ma} = F_w \times P_{wa} \times F_{oil} \times P_{oila} \quad \textbf{(3-9)}$$

where: P_{wa} = water/air partition coefficient;

P_{oila} = oil (miglyol)/air partition coefficient;

F_w = fraction of water in the mixture (%);

F_{oil} = fraction of oil (miglyol) in the mixture (%).

The total volume, $F_w + F_{oil}$ is equal to 1.

Using **Equation (3-9)**, it is possible to calculate miglyol-water/air partition coefficients for emulsion I, II and III. The results obtained are presented in **Table 3.9**, in comparison with the experimentally values.

Table 3.9 indicates that the logP values calculated according to **Equation (3-9.)** proposed by Buttery et al. (1973) are correlated with the experimentally determined values, in agreement with the results obtained by Buttery, where for the case of 1% and 10% vegetable oil-water mixtures, the experimentally and calculated vegetable oil-water mixture/air partition coefficients (logP) agree quite closely.

As example, the experimentally value of octanal, given by Buttery for the 10% vegetable oil-water mixture was $logP_{oil\text{-}water/air} = 3.46$ and the calculated value was $LogP_{oil\text{-}water/air} = 3.40$.

For 1% vegetable oil-water mixture the experimentally value was $logP_{oil\text{-}water/air}$ = 2.49 and the calculated value was $LogP_{oil\text{-}water/air}$ = 2.46.

Table 3.9 *Comparison of experimentally and calculated partition coefficients (logP) (30°C) of different mixtures of oil (miglyol) in water systems*

Compound	LogP					
	Emulsion I[a]		Emulsion II[b]		Emulsion III[c]	
	Exp.	Calculated	Exp.	Calculated	Exp.	Calculated
Ethyl hexanoate	4.25	3.99	3.39	3.33	3.34	3.06
Ethyl octanoate	5.06	5.03	4.75	4.35	4.59	4.05
2-Phenylethanol	5.75	5.55	5.48	5.32	5.44	5.28
3-Methyl-1-butanol	3.59	3.12	3.39	2.74	3.23	2.66
γ-Decalactone	6.50	6.31	5.95	5.62	5.91	5.33
γ-Nonalactone	6.33	5.98	5.91	5.31	5.88	5.05
γ-Octalactone	6	5.90	5.75	5.33	5.47	5.14
δ-Decalactone	6.56	6.59	5.87	5.91	6	5.63
δ-Nonalactone	6.83	6.27	6.35	5.71	6	5.54
δ-Octalactone	6.30	6.05	6.13	5.79	5.77	5.74

a) Emulsion I: Water / Miglyol / Emulsifier Tween 85: (47.5 + 47.5 + 5, w/w/w)
b) Emulsion II: Water / Miglyol / Emulsifier Tween 85: (85.5 + 9.5 + 5, w/w/w)
c) Emulsion III: Water / Miglyol / Emulsifier Tween 85: (90.25 + 4.75 + 5, w/w/w)

An interesting point concerning the partition coefficients of selected aroma compounds is to compare the experimentally values obtained in emulsions with the values in water and in miglyol. **Table 3.10** lists this comparison.

The listed data in **Table 3.10** show that the partition coefficients for the emulsions are situated between the values in water and those in miglyol, with some exceptions, alcohols and few lactones in emulsion I. The partition coefficients in emulsion I (where miglyol is present at ratio of 47.5% in the mixture) are closer to the values in miglyol, which means the oil has an important influence on the partition coefficients. In emulsion II and III (where miglyol is present in 9.5% and 4.75%, respectively) the experimentally data agree quite closely.

In general, the partition coefficients are decreasing from miglyol matrices to water matrices.

Table 3.10 *Comparison between water/air, miglyol/air and emulsion/air partition coefficients (30°C)*

Compound	Partition coefficients miglyol/air ($logP_{M/A}$) (30°C)	Partition coefficients emulsion I/air ($logP_{EI/A}$) (30°C)	Partition coefficients emulsion II/air ($logP_{EII/A}$) (30°C)	Partition coefficients emulsion III/air ($logP_{EIII/A}$) (30°C)	Partition coefficients water/air ($logP_{W/A}$) (30°C)
Ethyl hexanoate	4.29	4.25	3.39	3.34	2.22
Ethyl octanoate	5.34	5.06	4.75	4.59	2.56
2-Phenylethanol	5.73	5.75	5.48	5.44	5.23
3-Methyl-1-butanol	3.35	3.59	3.39	3.23	2.52
γ-Decalactone	6.61	6.50	5.95	5.91	3.9
γ-Nonalactone	6.28	6.33	5.91	5.88	4.25
γ-Octalactone	6.19	6	5.75	5.47	4.82
δ-Decalactone	6.89	nd	nd	nd	4.62
δ-Nonalactone	6.55	6.83	6.35	6	5.25
δ-Octalactone	6.25	6.30	6.13	5.77	5.68

a) Emulsion I: Water / Miglyol / Emulsifier Tween 85: (47.5 + 47.5 + 5, w/w/w)
b) Emulsion II: Water / Miglyol / Emulsifier Tween 85: (85.5 + 9.5 + 5, w/w/w)
c) Emulsion III: Water / Miglyol / Emulsifier Tween 85: (90.25 + 4.75 + 5, w/w/w)
nd: not determined

3.4. The influence of β-cyclodextrin onto the headspace concentration of aroma compounds selected (ethyl hexanoate and S-(-) limonene)

In the present study, the flavour release of different aroma compounds from carbohydrate-water solutions was examined. The static headspace method allows the measurement of the released odour components that interact with polysaccharides.

The headspace analyses of β-cyclodextrin as model oligo polysaccharide showed a reduction of the odour compound in presence of the carbohydrate.

β-Cyclodextrin is used in the food processing for the stabilization of the vitamins and flavouring materials as well as for the flavourful neutralization of bitter substances. Nevertheless, it must be taken into account that the used amounts of β-cyclodextrin are very high in the present case to achieve a visible reduction with the present analytical method.

a) Investigation of flavour release of ethyl hexanoate in the presence of β-cyclodextrin by means of static headspace (SHS) method

Ethyl hexanoate is a pleasant fruity smelling odorant which is used in many artificial fruit essence. It is the key aroma compounds in various fruits, for example, in the pineapple or strawberry.

The flavour release of ethyl hexanoate in the presence of β-cyclodextrin is presented in **Table 3.11.**

Table 3.11. *The flavour release of ethyl hexanoate in the presence of β-cyclodextrin*

Sample	Concentration ethyl hexanoate in solution (µg/ml)	Concentration in the headspace (ng/ml)		Reduction (%)
		(Cyclodextrin + ethyl hexanoate)-water	Ethyl hexanoate-water	
1	12	74	91	19.32
2	24	138	210	34.29
3	48	209	37.33	37.33

The listed data in **Table 3.11** show a reduction of the concentration of ethyl hexanoate in the headspace in the presence of β-cyclodextrin in comparison to the solution without β-cyclodextrin.

The reduction between ethyl hexanoate and β-cyclodextrin is presented in **Figure 3.8.**

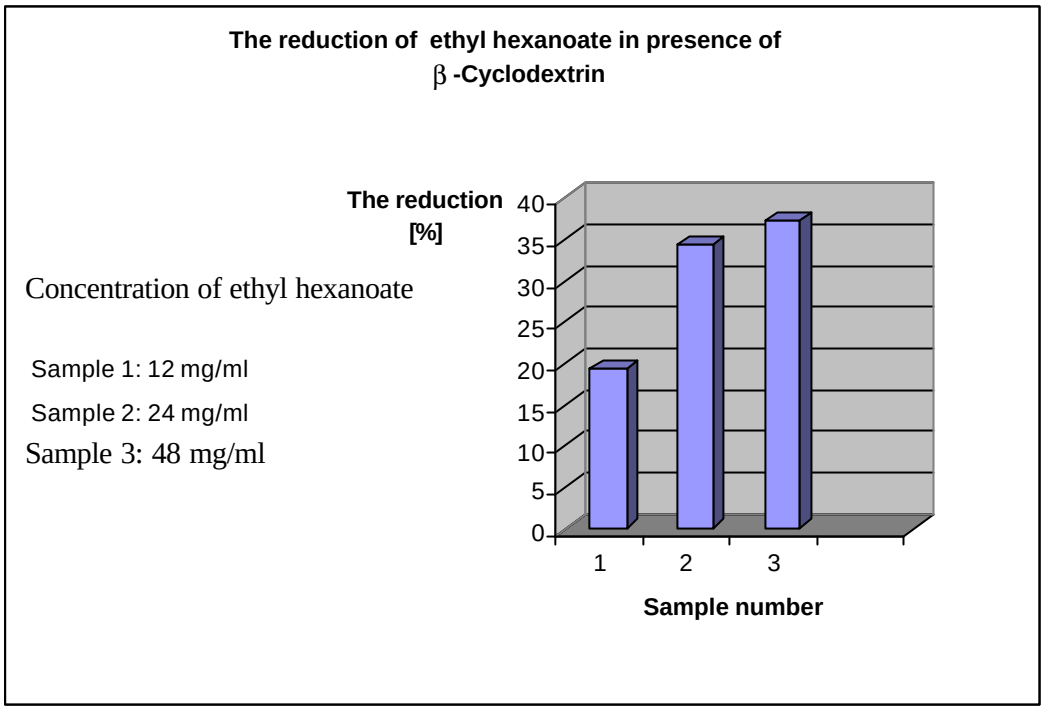
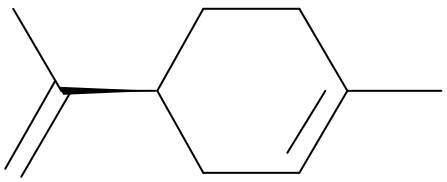


Fig.3.8 *The reduction of ethyl hexanoate in presence of β-cyclodextrin*

b) Investigation of flavour release of S-(-) limonene in the presence of β-cyclodextrin by means of static headspace (SHS) method

Limonene belongs chemically to the group of terpene. Most components of the oils of a lot of plants belong to the class of terpene. These ethereal oils can be obtained by steam distillation of the plants. The oils which are separated in the distillate have mostly characteristically smells which are typical for the used plants.



S-(-)-Limonene

Fig. 3.9 *The structure of S-(-)-limonene*

S-(-) limonene is a chirale molecule. Both enantiomers differ substantially in flavour.

The S-(-) limonene is found in american peppermint oil and owns a minty flavour.

D-(+) limonene is forming in the nature, 90% in sour orange oil, in the Caraway oil, or in the citrus oil, the perceived flavour is associated with oranges.

Furthermore, it is also known that (+/-) limonene is found, e.g., in the pine-needle oil, camphoric oil or nutmeg oil. Limonene is used in the dye and varnish industries.

The flavour release of S-(-) limonene in the presence of β-cyclodextrin is presented in **Table 3.12.**

Table 3.12 *The flavour release of S-(-) limonene in the presence of β-cyclodextrin*

Sample	Concentration S-(-) limonene in solution (μg/ml)	Concentration in the headspace (ng/ml)		Reduction (%)
		(Cyclodextrin + S-(-) limonene)-water	S-(-) limonene-water	
1	0.106	0.9	1	21
2	1.06	9	13	31
3	2.12	10	16	39

From the **Table 3.12** it can be seen a reduction of the concentration of S-(-)-limonene in the gase phase in the presence of β-cyclodextrin in comparison to the solution in absence of β-cyclodextrin. The reduction between S-(-)-limonene and β-cyclodextrin is presented in **Figure 3.10**.

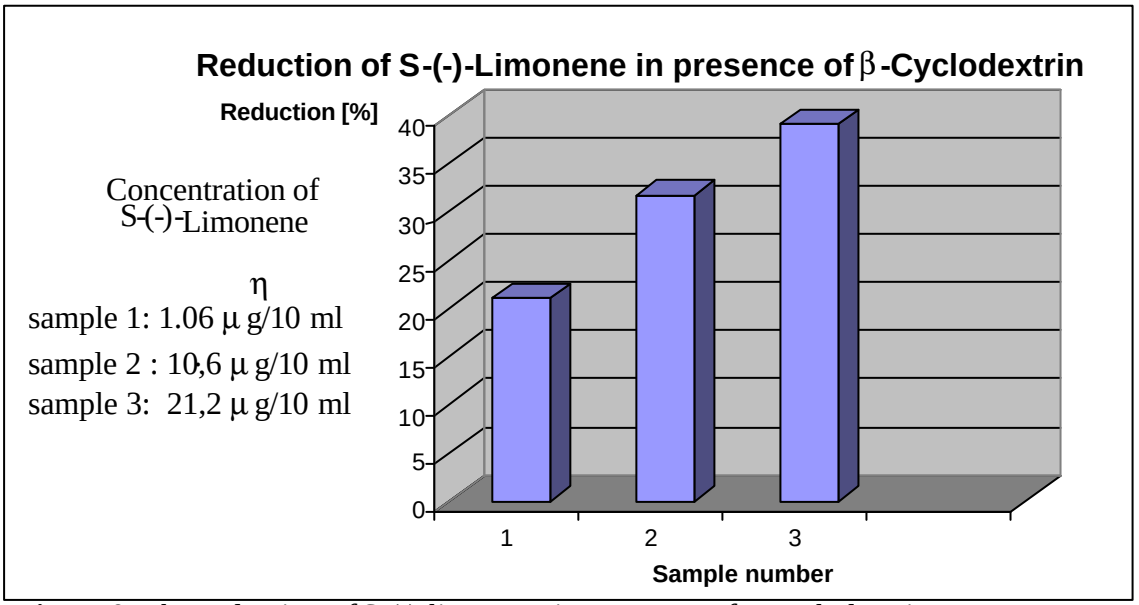


Fig.3.10 *The reduction of S-(-)-limonene in presence of β-cyclodextrin*

The present investigations by means of HS-GC showed that the concentrations of the carbohydrate were relatively high; however, the analytical results revealed that β-cyclodextrin with its hydrophobic hollow cavity can bind very well aroma compounds.

3.5. The influence of the food matrix onto the partition coefficients of selected flavour compounds

3.5.1. Wine matrix

To determine the partition coefficients in wines it is necessary to know the concentrations of alcohols and esters in wines (in white wines) and in the headspace above wines and then making a ratio between the odorant concentration in the wine matrix to the concentration in the headspace above wine, was possible to calculate the partition coefficients wines / air.
The concentrations of 3-methyl-1-butanol, ethyl hexanoate and ethyl octanoate in wines were determined by standard addition method, using headspace-gas chromatography (HS-GC), and by mass spectrometry, MS (CI method) for 2-phenylethanol (cf. 2.4.1.).

Standard addition method

The graphics for the determination of the concentration of 3-methyl-1-butanol, ethyl hexanoate and ethyl octanoate in wines are presented in **Figures 3.11-3.13**.

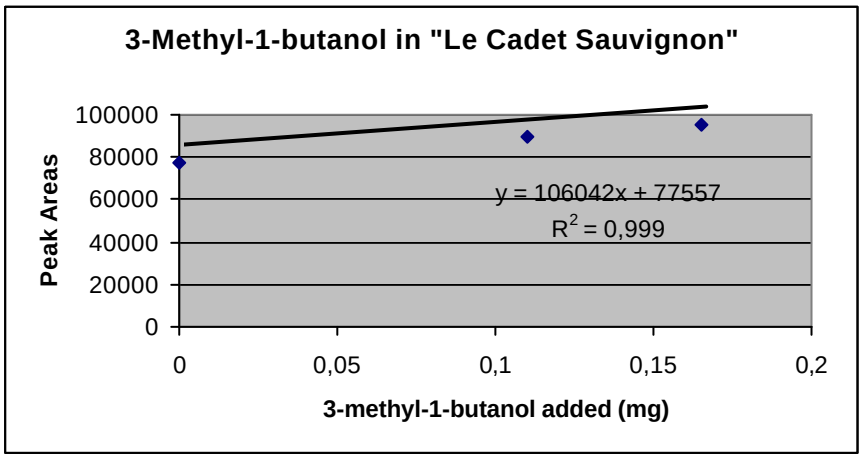


Figure 3.11 *Determination of the concentration of 3-methyl-1-butanol in "Le Cadet Sauvignon"*

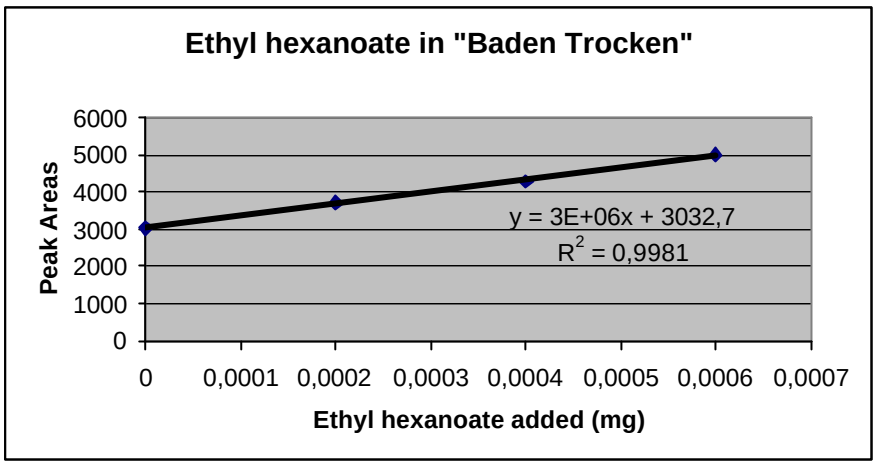


Figure 3.12 *Determination of the concentration of ethyl hexanoate in "Baden Trocken"*

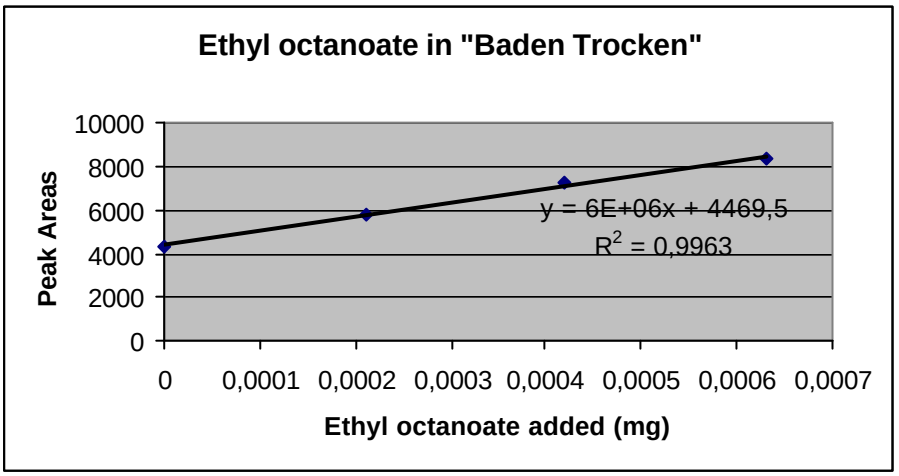


Figure 3.13 *Determination of the concentration of ethyl octanoate in "Baden Trocken"*

Isotope dilution analysis (IDA)

For 2-phenylethanol, the concentration in wines was determined by IDA using MS (CI) (cf.2.4.1.).

The wine sample contains $^{2}[H]_2$ –phenylethanol standard, with known concentration.

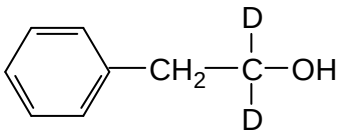


Fig.3.14 *The structure of $^{2}[H]_2$ –phenylethanol*

where: D = deuterium

1 µl from the wine sample was injected in MS by CI modus and the spectrum for both compounds in wine (2-phenylethanol and $^{2}[H]_{2}$ –phenylethanol standard) was obtained. The concentration of 2-phenylethanol in wine was calculated from the peak areas of both compounds and the known concentration of $^{2}[H]_{2}$ –phenylethanol, taking into account the correction factor, **f**.

The mass trace for the two compounds, the $^{2}[H]_{2}$ –phenylethanol standard and 2-phenylethanol are shown in **Figure 3.15 a and 3.15 b**.

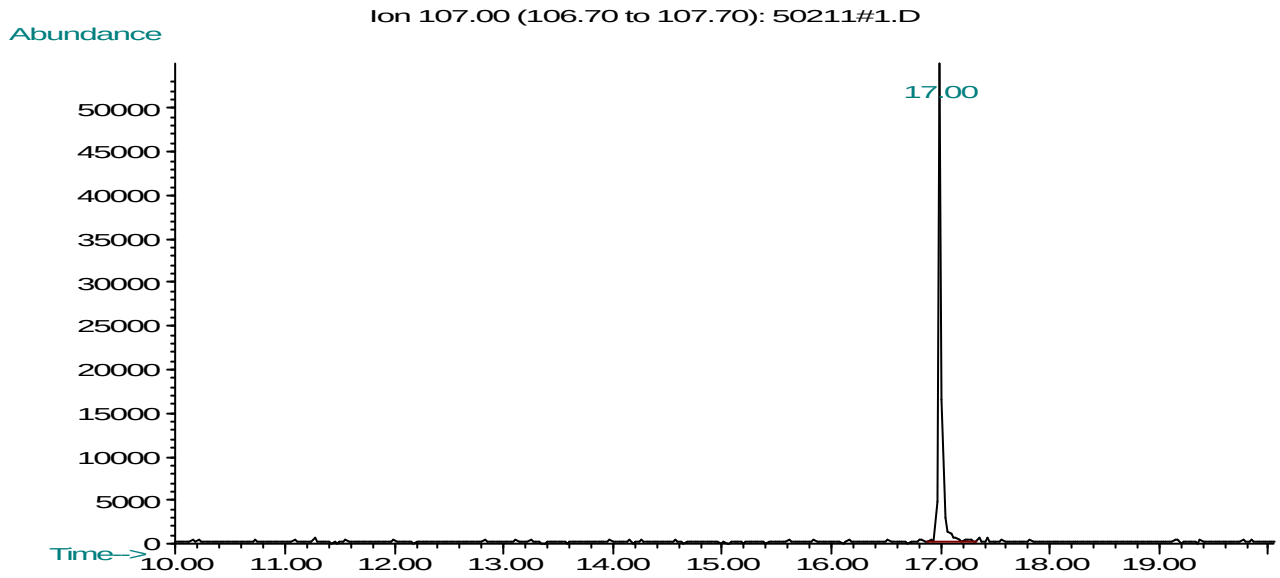


Fig. 3.15 a *Mass trace of $^{2}[H]_{2}$ –phenylethanol standard*

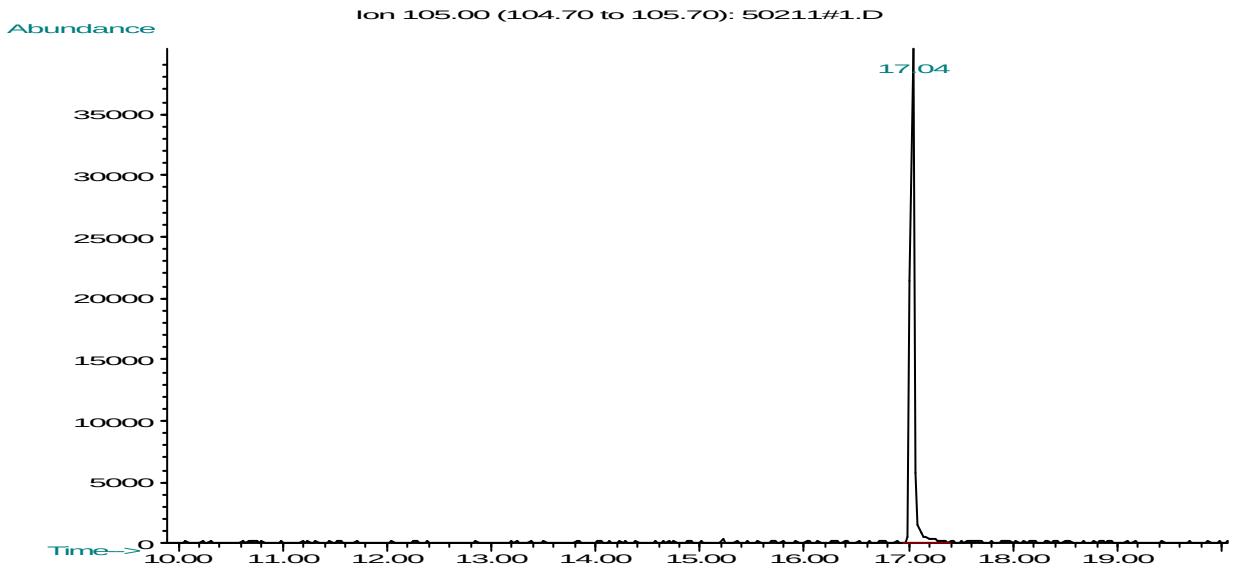


Fig. 3.15 b *Mass trace of 2-phenylethanol*

The concentration of the compounds in wines is given in **Table 3.13.** For each wine, the concentrations were calculated from four replicates (standard deviation: ± 10%).

Table 3.13 *Concentrations of selected odorants in white wines*

Compound	Concentration in wine A (µg / L)	Concentration in wine B (µg / L)	Concentration in wine C (µg / L)
3-Methyl-1-butanol [a)]	145000	131000	231000
Ethyl hexanoate [a)]	444	200	315
Ethyl octanoate [a)]	515	246	272
2-Phenylethanol [b)]	73602	63008	103370

a) The concentrations were determined through standard addition method using HS-GC (cf. 2.4.1.);
b) The concentration was determined by isotope dilution analysis (IDA) using MS (CI) (cf. 2.4.1).

For the determination of the concentration of alcohols and esters in headspace above wines, the headspace-gas chromatography (HS-GC) method was used.

The concentrations of the compounds in headspace above wines are given in the **Table 3.14** and calculated from two replicates (standard deviation: ± 5%).

Table 3.14 *The concentrations of selected aroma compounds in headspace above wines*

Compound	Concentration in wine A (ng / L)	Concentration in wine B (ng / L)	Concentration in wine C (ng / L)
3-Methyl-1-butanol	76835	104890	117775
Ethyl hexanoate	4870	2660	3295
2-Phenylethanol	1540	2590	1110
Ethyl octanoate	7925	7920	5245

After the determination of the concentration of each compound in wines and in the headspace above wines, the partition coefficient of compounds in wines was calculated.

The partition coefficients were calculated from 2-5 replicates (standard deviation: ± 3%). The results are summarized in **Table 3.15.**

Table 3.15 *Partition coefficients of selected aroma compounds in wines*

Compound	Partition coefficients wineA/air (logP)	Partition coefficients wineB/air (logP)	Partition coefficients wineC/air (logP)
3-Methyl-1-butanol	3.26	3.10	3.28
Ethyl hexanoate	1.82	1.7	1.85
Ethyl octanoate	1.62	1.22	1.43
2-Phenylethanol	4.68	4.43	4.97

Table 3.15 shows that the highest partition coefficients in wines have the two alcohols: 2-phenylethanol and 3-methyl-1-butanol. The partition coefficients for esters agree quite closely, but the values are lower in comparison with the values obtained for alcohols in wines.

3.5.1.1. Influence of ethanol on the partition coefficients

In **Table 3.16** the partition coefficients of wine odorants in water-ethanol mixtures in comparison with the values in water and wines are presented. The presence of ethanol in the matrices only slightly influences the partition coefficients of selected aroma compounds.

Table 3.16 *Partition coefficients of wine odorants in water, water-ethanol mixtures and wines A, B and C*

Compound	Partition coefficients water/air ($logP_{W/A}$)	Partition coefficients water + ethanol/air ($logP_{W+Et/A}$)	Partition coefficients wine/air ($logP_{wine/A}$)		
			wine A	wine B	wine C
3-Methyl-1-butanol	2.52	3	3.26	3.10	3.28
Ethyl hexanoate	2.22	2.9	1.82	1.7	1.85
Ethyl octanoate	2.56	2.53	1.62	1.22	1.43
2-Phenylethanol	5.23	5.37	4.68	4.43	4.97

The data in **Table 3.16** show no large differences between the partition coefficients in all investigated samples. This indicates that ethanol did not reduce the amounts of the odorants ethyl hexanoate, ethyl octanoate, 3-methyl-1-butanol and 2-phenylethanol in the headspace above the different liquids. The partition coefficients seem to be only slightly influenced in the presence of ethanol in the liquid.

For the wines studied, the partition coefficient wine/air for 3-methyl-1-butanol is slightly higher than the partition coefficient of the same alcohol in water and water-ethanol model solution. For 2-phenylethanol, the partition coefficients in wines are lower than the values in water and in water-ethanol. For esters, the presence of ethanol in wine matrix has not a large influence on the partition coefficients wine/air of esters; the values are lower than the values in water or in water-ethanol.

3.5.2. Custard sample

The knowledge about the binding behaviour of the odorant to the macromolecule in relation to their partition coefficients (K_{HF}, **Fig.3.16**), which is defined as ratio of the odorant concentration in the food matrix ($C_F(A)$, **Fig. 3.16**) to the concentration in the headspace above the food ($C_H(A)$, **Fig.3.16**), is of great importance for the science and for the flavour industry to product high-quality foodstuffs.

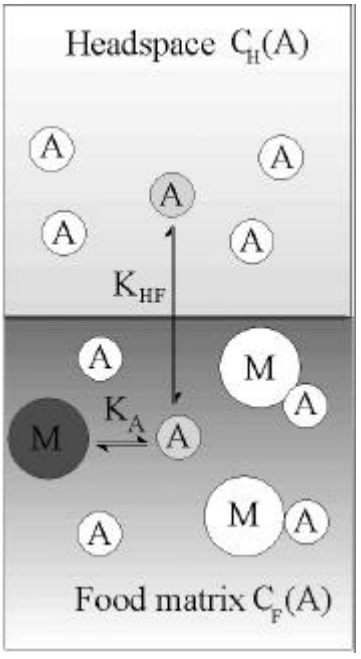


Fig.3.16 *Schematic presentation of the complex macromolecule–odorant interactions*

As outlined in the experimental part (cf.2.1.1), the "original" custard was produced with the following ingredients: water, sugar (saccharose), milk powder, flavour, modified tapioca starch and carrageenan (thickener). The model standard custard recipe and the preparation procedure were described in the part 2.1.1. The flavour release of the following odorants was

investigated in the custard samples: 3-methyl-1-butanol, ethyl octanoate, ethyl hexanoate, 2-phenylethanol.

The main goal was the determination of the following subjects in custard:

- Investigation of the aroma release as a function of matrix components;
- Investigation of the time influence to the aroma release;
- Comparison of custard/air – and octanol/water partition coefficients (logP);
- Comparison of custard/air -, water/air - and emulsion/air partition coefficients (LogP).

Determination of partition coefficients in custard sample

The results concerning the influence of the matrix onto the partition coefficients of selected aroma compounds are presented.

As described in the experimental part, 5 g custard was weighted in the headspace vials (volume: 20 ml) and 0.5 ml air above the custard was injected into the gas chromatograph.

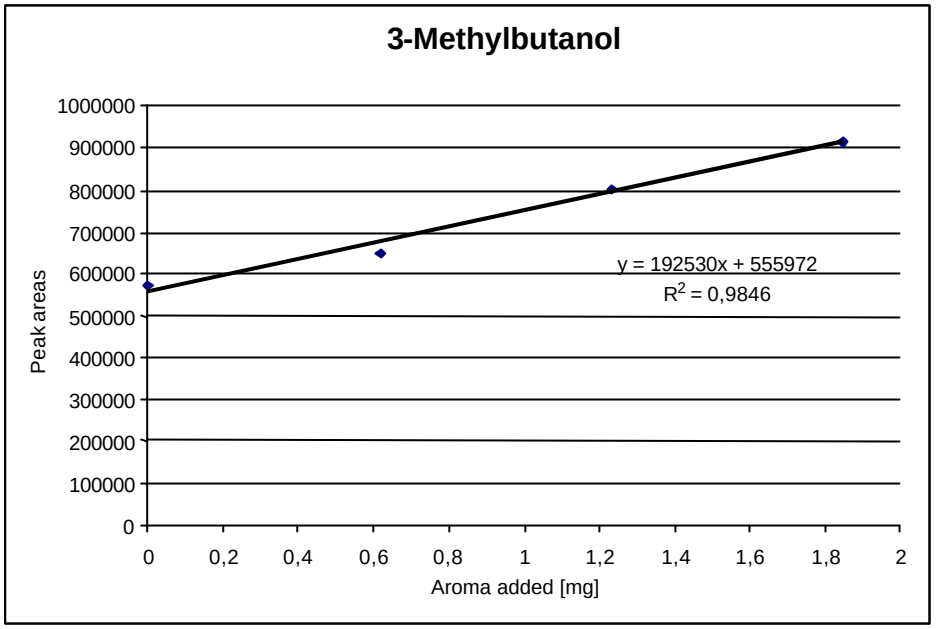
Table 3.17 lists the headspace concentrations above the custard obtained (ng/ml).

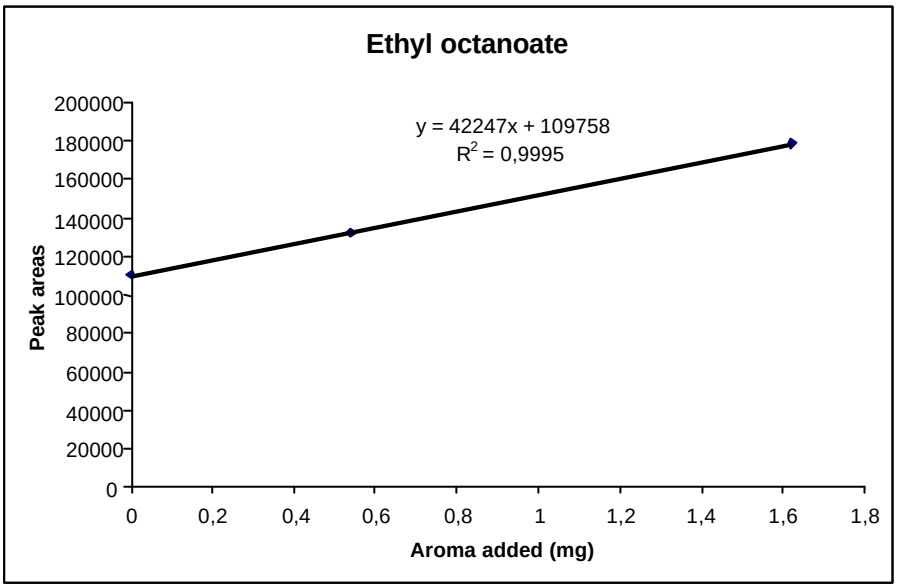
Table 3.17 *Headspace concentrations obtained for the selected flavour compounds above the original custard sample*

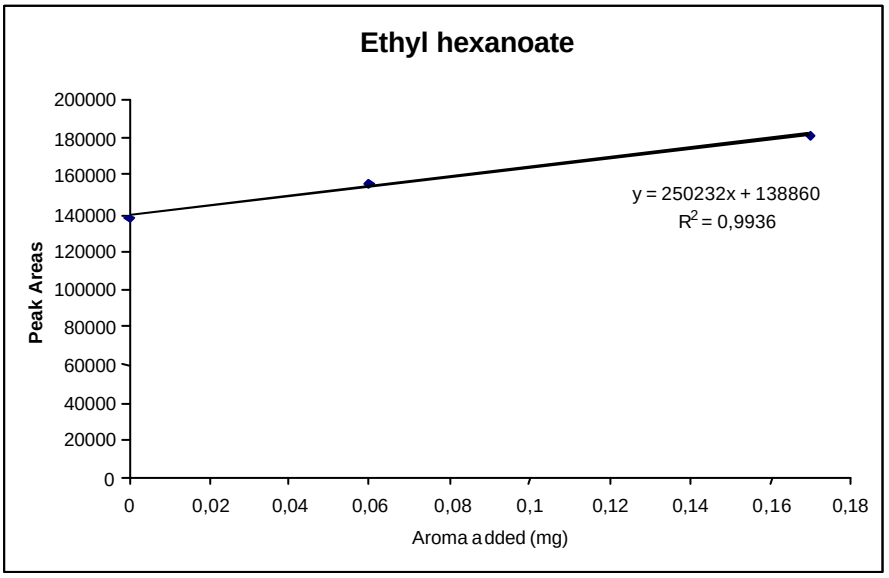
Aroma compound	Concentration (ng/ml air)
3-Methyl-1-butanol	264
Ethyl hexanoate	58.3
2-Phenylethanol	5.4
Ethyl octanoate	39.4

Determination of the concentrations of odorants in the custard samples

For the determination of the losses of odorants during the preparation of the custard the standard addition method was used (**Figure 3.17**).


3-Methylbutanol
Peak areas
1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
0
0,2
0,4
0,6
0,8
1
1,2
1,4
1,6
1,8
2
Aroma added [mg]
y = 192530x + 555972
R2 = 0,9846


Ethyl octanoate
Peak areas
200000
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
0
0,2
0,4
0,6
0,8
1
1,2
1,4
1,6
1,8
Aroma added (mg)
y = 42247x + 109758
R2 = 0,9995


Ethyl hexanoate
Peak Areas
200000
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
0
0,02
0,04
0,06
0,08
0,1
0,12
0,14
0,16
0,18
Aroma added (mg)
y = 250232x + 138860
R2 = 0,9936

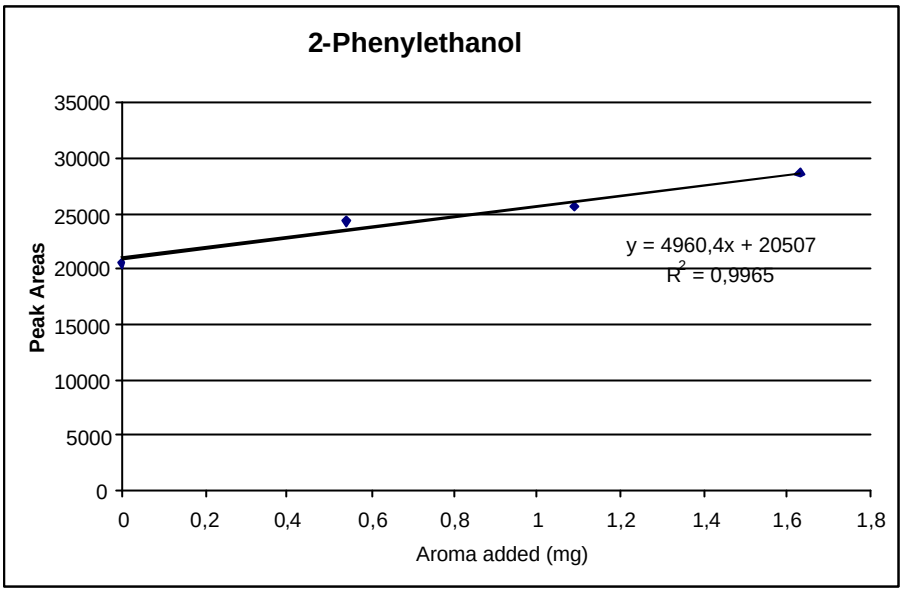


Fig. 3.17 *Standard addition method of 3-methyl-1-butanol, ethyl octanoate, ethyl hexanoate and 2-phenylethanol*

Amount of flavour compounds in 200 g custard sample

The results are summarized in **Table 3.18.**

Table 3.18 *Recovery of odorants in custard sample*

Aroma compounds	µg odorant added /g custard	µg odorant found /g custard	Recovery (%)
Ethyl hexanoate	120	112	93.3
2-Phenylethanol	1023	827	80.8
Ethyl octanoate	878	520	59.2
3-Methyl-1-butanol	809	575	71

The partition coefficients custard/air were calculated according to the following equation:

$$\log P_{c/a} = \frac{C_c}{C_a} \qquad \textbf{(3-10)}$$

where:

$P_{c/a}$= partition coefficient custard/air
Cc = concentration in custard sample
C_a = concentration in air

The partition coefficients custard/air ($logP_{c/a}$) for the selected aroma compounds calculated (cf. **Equation 3-10**) are summarized in **Table 3.19.**.

Table 3.19 *Partition coefficients custard/air of aroma compounds*

Aroma compounds	ng odorant /ml custard[a]	ng odorant /ml air	Partition coefficient, custard/air ($logP_{c/a}$)
Ethyl hexanoate	120000	58.38	3.31
2-Phenylethanol	885000	5.4	5.21
Ethyl octanoate	556000	39.4	4.15
3-Methyl-1-butanol	615000	280	3.34

a) Density of the custard: 1.07 g/ml. The density was gravimetrically determined.

Analysis of the aroma release as a function of matrix components

The influence of the matrix components (κ-carrageenan, modified- and native tapioca starch and milk powder, used for the preparation of the custard samples) on the headspace concentrations of odorants were investigated.

For this purpose, model mixtures containing one of the before mentioned high molecular matrix component (sample 1-3), water and selected odorants were prepared. Furthermore, the original custard sample (sample 4) was investigated by leaving out one of the above mentioned components (5-7).

For 3-methyl-1-butanol (**Figure 3.18**), the lowest concentration in the headspace was found in the model mixture containing modified tapioca starch and water (sample 2, 222 ng/ml) and the highest concentration was found in the model mixture containing milk powder and water (sample 1, 390 ng/ml).

In model mixtures containing neither native tapioca starch nor modified tapioca starch there are small differences in the concentration of 3-methyl-1-butanol in the headspace. This means that native and modified tapioca starch have similar effect on the flavour release.

The highest headspace concentration of 3-methyl-1-butanol (103 ng/ml) was found in the model mixture containing only milk powder (sample 1). This means that milk has a large influence to the flavour release. 3-Methyl-1-butanol is a polar compound; the presence of milk (fat) has a positive effect to its release.

sample

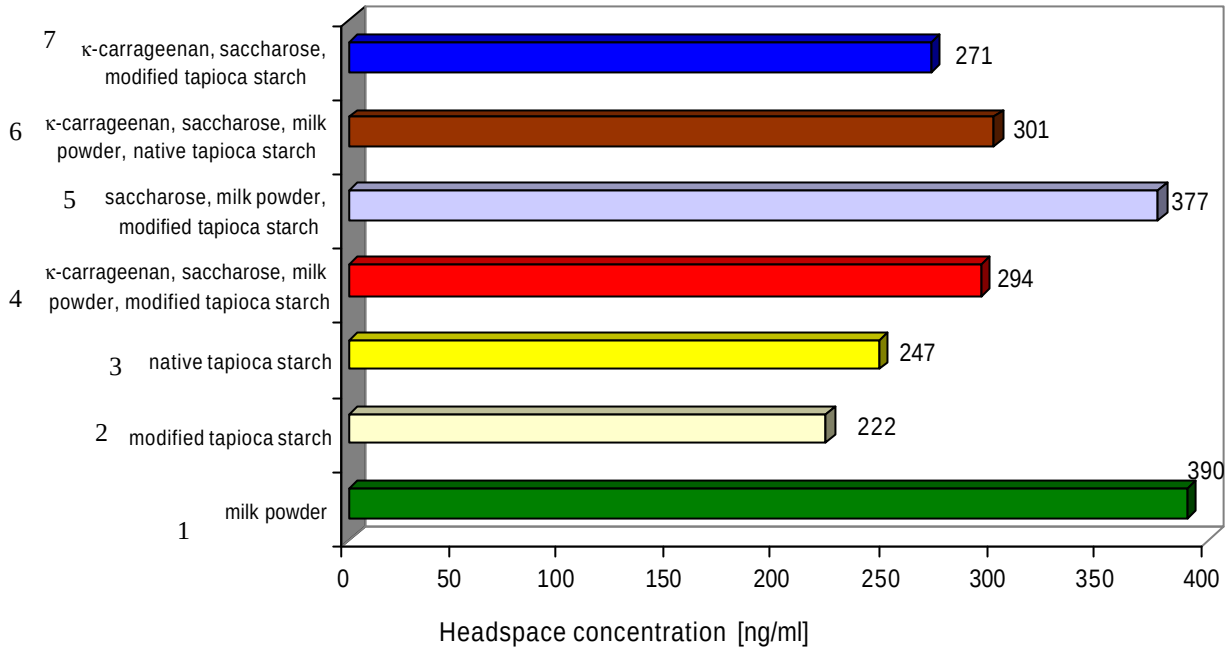


Fig.3.18 *Flavour release of 3-methyl-1-butanol from "original" and modified custard samples*

The influence of the matrix on the flavour release of ethyl hexanoate for strawberry custard is displayed in **Figure 3.19**.

sample

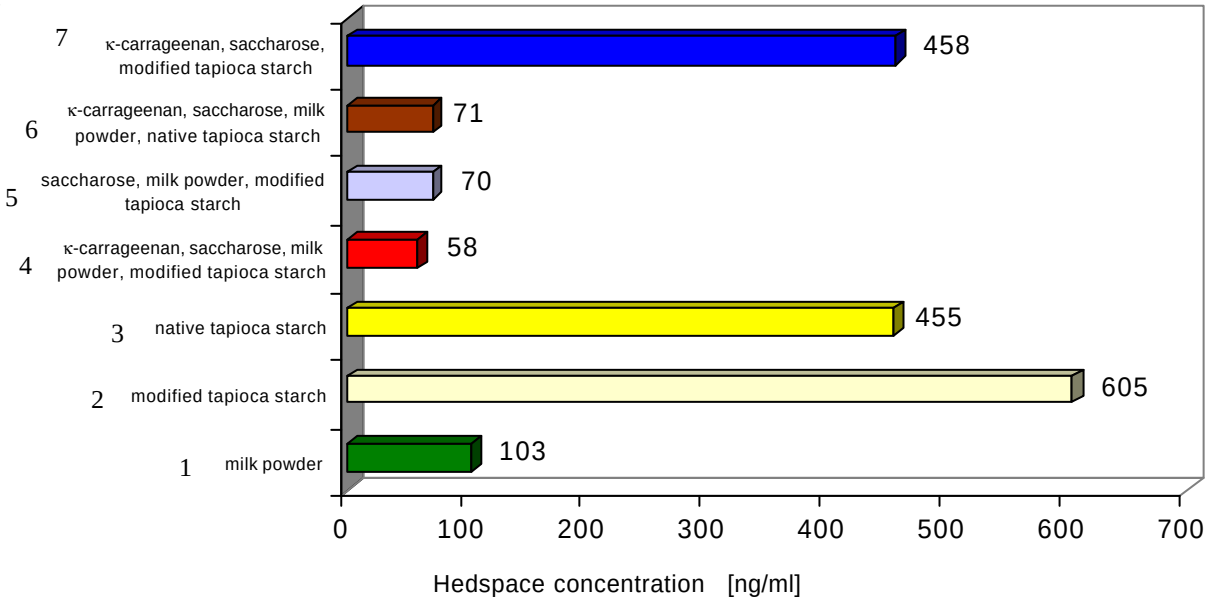


Fig. 3.19 *Flavour release of ethyl hexanoate from "original" and modified custard samples*

The highest headspace concentration of ethyl hexanoate (sample 2, 605 ng/ml air) was found above sample containing only water and modified starch. The lowest headspace concentration of the odorant (sample 4, 58 ng/ml air) was measured above the original custard sample. The headspace concentration of ethyl hexanoate increased slightly (70 ng/ml air) in the sample without κ-carrageenan (sample 5), compared to the original custard (sample 4, 58 ng/ml air). In contrast to the sample containing no milk powder a drastically effect on the headspace concentration of ethyl hexanoate was observed; the concentration was higher by a factor of eight (sample 3, 457 ng/ml air) compared to the original custard. These results showed that the constituents of the milk (proteins and fats) are responsible for the reduction of ethyl hexanoate in the headspace above the food. In most cases a change of the food matrix leads to a change in flavour release. Caused by these changes the consumer's acceptance can go back for a food or be promoted. The strength of the interaction of an odorant with a macromolecule should be considered closer for the proteins and lipids in milk.

- Milk has a large influence to the aroma release
- Native starches and modified starches have different influence to the aroma release

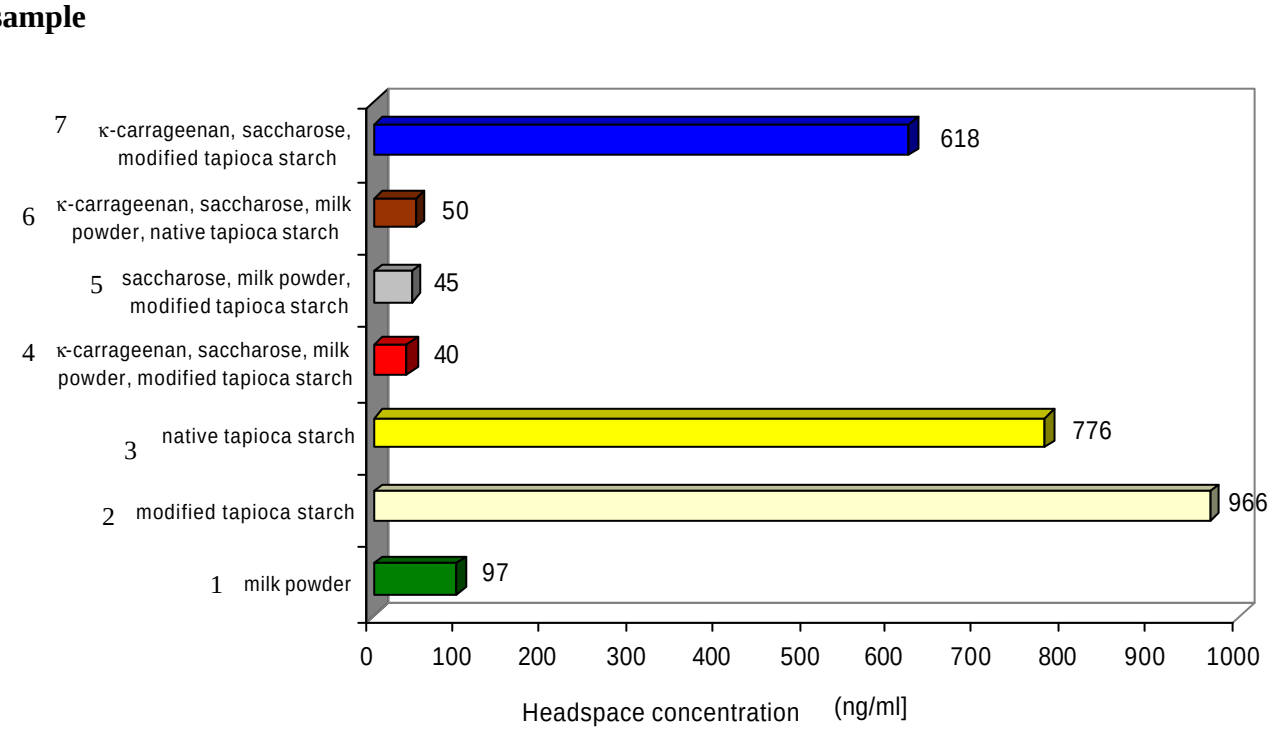


Fig.3.20 *Flavour release of ethyl octanoate from "original" and modified custard samples*

The highest headspace concentration of ethyl octanoate was found in the model mixture containing modified tapioca starch and water (sample 2, 966 ng/ml) and the lowest concentration in the original custard sample (sample 4, 40 ng/ml). Between modified samples without κ-carrageenan and the sample where modified tapioca starch was replaced by native tapioca starch there were not large differences in the concentrations of ethyl octanoate found in the headspace to the original custard sample (sample 4). In contrast, in the sample containing no milk powder, a drastically effect on the headspace concentration of ethyl octanoate was observed. The concentration was higher by a factor of 15 compared to original custard. The concentration of ethyl octanoate found in the headspace in model mixtures containing native tapioca starch and water (sample 3) and modified tapioca starch and water (sample 2), respectively, was higher but almost similar between both of them, which mean the effect on aroma release is similar.

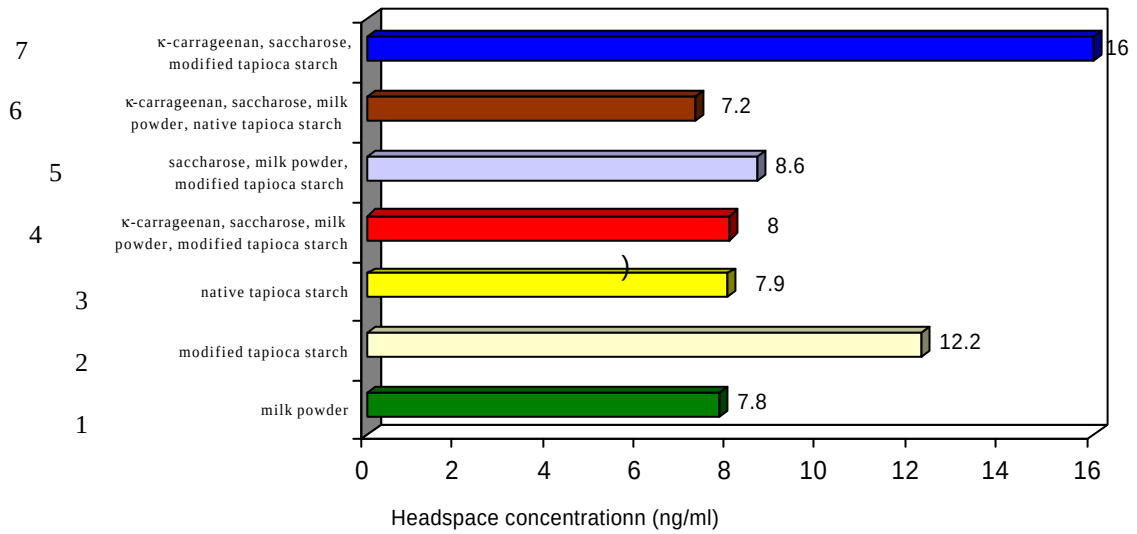


***Fig.3.21**. Flavour release of 2-phenylethanol from "original" and modified custard samples*

The highest headspace concentration of 2-phenylethanol was found in the modified sample where no milk powder was present (sample 7, 16 ng/ml), and the lowest in the modified sample where modified tapioca starch was replaced by native tapioca starch (sample 6, 7.2 ng/ml). Making a comparison between the concentrations of 2-phenylethanol in the headspace, in original custard (sample 4) and the modified custard (sample 5, without κ-carrageenan), or the modified custard (sample 6, where instead of modified tapioca starch was native tapioca starch), it can be observed that the concentrations are almost similar. Only in the modified custard without milk powder (sample 7) the headspace concentration increased by factor of 2. In the two model mixtures containing only native tapioca starch and water (sample 3) and modified tapioca starch and water (sample 2), respectively, the concentration in the headspace of 2-phenylethanol is almost similar. That means both starches have similar effect on aroma release.

Comparison of custard/air – and octanol/water partition coefficients (logP)

The data are summarized in **Table 3.24**.

Table 3.24 *Comparison of logP octanol/water and logP custard/air of selected aroma compounds*

Aroma compounds	LogP octanol/water[a]	LogP custard/air
3-Methyl-1-butanol	1.22	3.34
Ethyl hexanoate	2.82	3.31
Ethyl octanoate	3.9	4.15
2-Phenylethanol	1.36	5.21

a) $LogP_{o/w}$ were calculated using **Advanced Chemistry Development (ACD/Labs**) Software Solaris V4.67 and **Hyper Chem. 5.0**.

The data listed in **Table 3.24** show that the partition coefficients custard/air are higher than the partition coefficients octanol/water for each aroma compound selected. The partition coefficients are decreasing with the carbon chain length, both for esters and alcohols.
Table 3.28 shows that there is no correlation between $logP_{o/w}$ and $logP_{c/a}$.

Comparison of custard/air -, water/air - and emulsion/air partition coefficients (LogP)

The data are summarized in **Table 3.25.**

Table 3.25 shows that the LogP custard/air is situated between logP water/air and LogP emulsion/air for the aroma compounds selected, but closer to the logP emulsion/air. The LogP values octanol/water for esters is between logP water/air and LogP emulsion/air. For alcohols, the LogP octanol/water is lower than the logP water/air and LogP emulsion/air.

Table 3.25 *Comparison of custard/air -, water/air - and emulsion/air partition coefficients of selected aroma compounds*

Aroma compounds	LogP custard/air	LogP water/air	LogP emulsion (miglyol + water, 90,25 + 4,75 w/w)/air	LogP octanol/water
Ethyl hexanoate	3.31	2.22	3.34	2.82
3-Methyl-1-butanol	3.34	2.52	3.23	1.22
Ethyl octanoate	4.15	2.56	4.59	3.9
δ-Decalactone	4.90	3.9	5.91	-
2-Phenylethanol	5.21	5.23	5.44	1.36

3.5.2.1. Determination of mass transfer coefficients of some flavour compounds studied, in custard- and milk powder / water samples

In physical terms, the mass transfer of flavour compounds between two phases is the main mechanism of flavour release (Marin et al., 2000).

The mass transfer coefficients between the liquid phase (custard and milk, respectively) and the headspace were determined for some flavour compounds studied and the viscosity measurements of the custard and milk samples were correlated with mass transfer data.

Viscosity determination

The viscosity of the custard was experimentally measured (cf. 2.4.2) using falling ball viscosimetry and the value was compared with the viscosity value for glycerine, taken as a model. For milk, the viscosity values were taken from the literature.

Custard

The viscosity determination for custard and glycerine (as model) has been done (cf. 2.4.2.) and the results obtained after the experiments were the following ones:

$\eta_2 = 2\ r^2\ g\ (\rho_{ball} - \rho_{custard}) / 9\ v\ (1 + 2.4\ r/R) = 11057$ mPas

This value obtained for the custard was compared with the value obtained for glycerine (as model).

Glycerine

$\eta_2 = 2\ r^2\ g\ (\rho_{ball} - \rho_{glycerine}) / 9\ v\ (1 + 2.4\ r/R) = 1470$ mPas

The value obtained for glycerine (1470 mPas) is comparable with the literature value for glycerine at 20°C (1480 mPas).

The results obtained show that the viscosity of the custard is higher than the viscosity of glycerine.

The mass transfer coefficients were calculated using a statistical program, **TableCurve 2D v4** from SPSS Science (Erkrath, Germany). The time influence to the aroma release was analysed and the data are shown in **Figures 3.22-3.25.** The data points from the graphics were fitted according to the following equation:

$$\int_0^{c_g} \frac{dc_g}{K \bullet c_l - c_g} = \int_0^{t} \frac{k \bullet A}{V_R} \bullet dt \quad \rightarrow \quad c_g(t) = K \bullet c_{l(t=0)} \bullet \left(1 - e^{\frac{-k \bullet A}{V_R} \bullet t}\right)$$

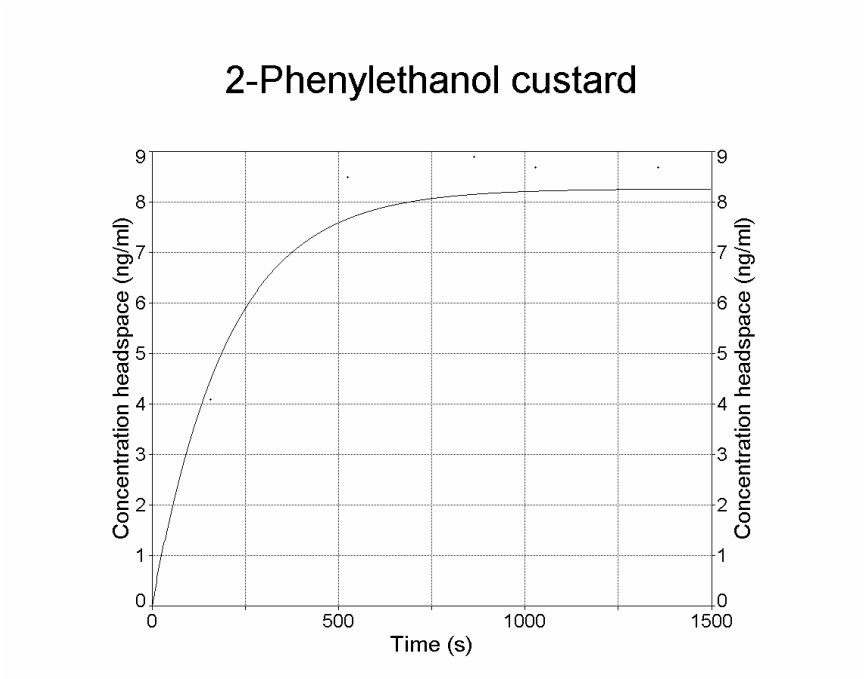
K: partition coefficient
k: mass transfer (m/s)
A: area
c_g: odorant concentration headspace
c_l: odorant concentration liquid

(3-11)

From the **Equation (3-11)** the mass transfer coefficients (***k***) for the selected flavour compounds, in custard- and milk powder/water samples were calculated.

The graphics for the calculation of the mass transfer rate of selected aroma compounds in model systems are the following ones:

2-Phenylethanol-custard and 2-phenylethanol milk powder/water samples



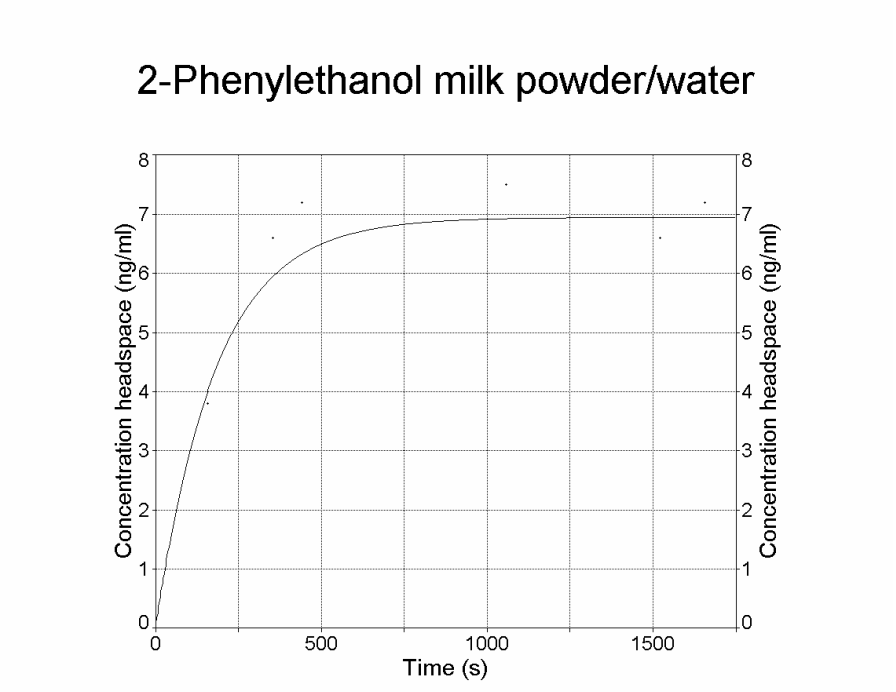


Fig. 3.22 *Time influence onto the headspace concentration of 2-phenylethanol in custard and milk powder/water*

3-Methyl-1-butanol custard and 3-methyl-1-butanol milk powder/water samples

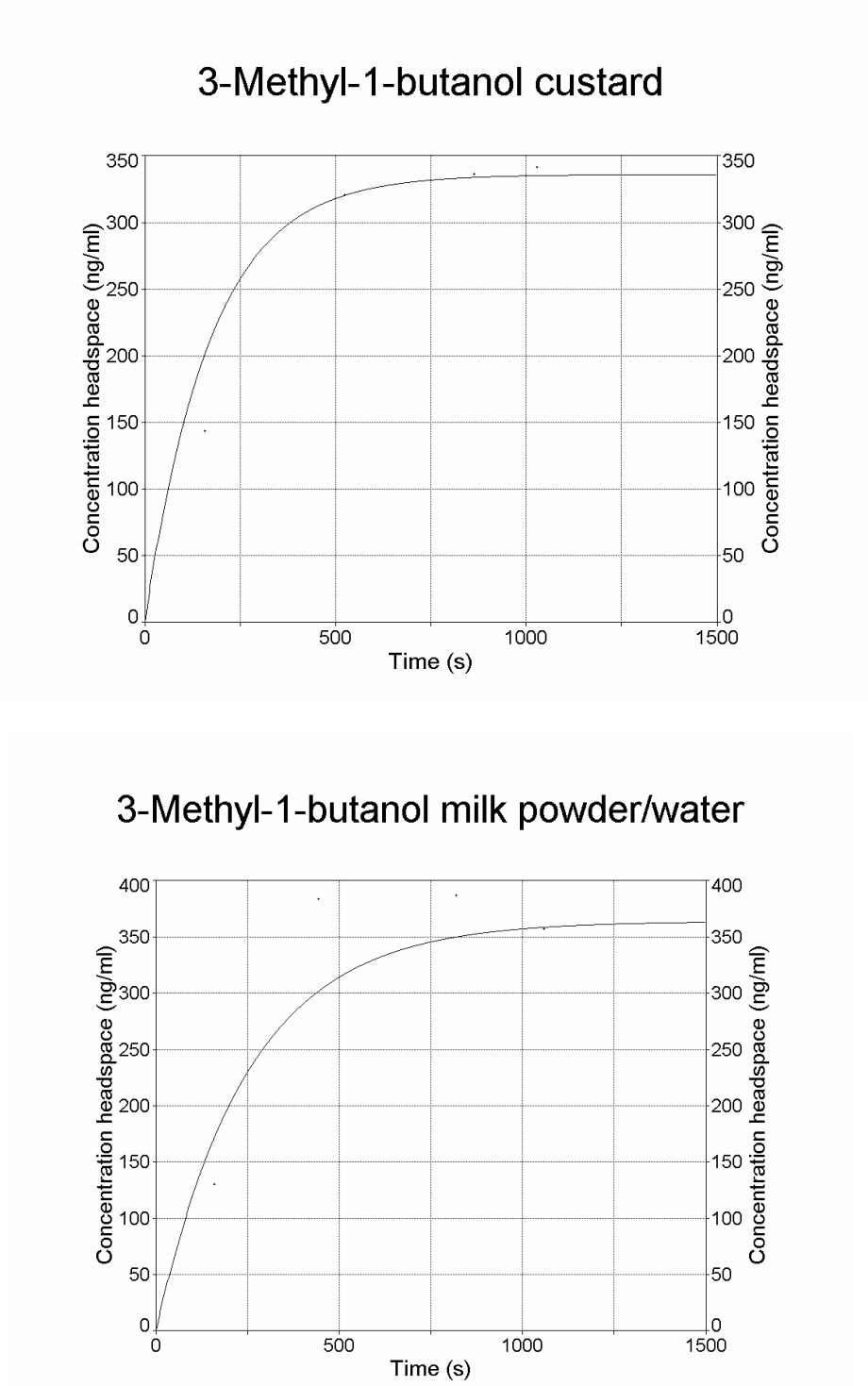


Fig. 3.23 *Time influence onto the headspace concentration of 3-methyl-1-butanol in custard and milk powder/water*

Ethyl hexanoate custard and ethyl hexanoate-milk powder/water samples

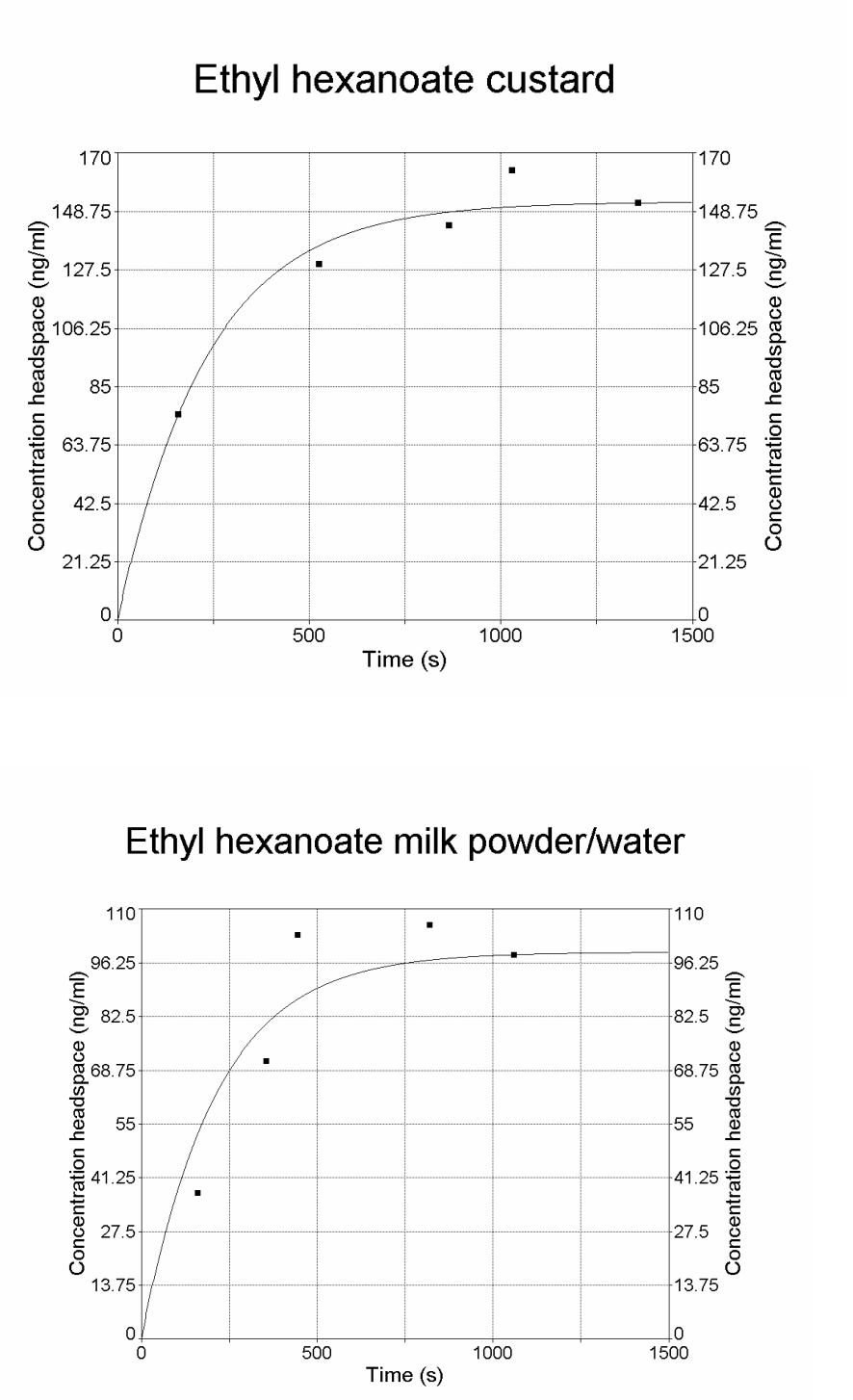


Fig. 3.24 *Time influence onto the headspace concentration of ethyl hexanoate in custard and milk powder/water*

Ethyl octanoate custard and ethyl octanoate-milk powder/water samples

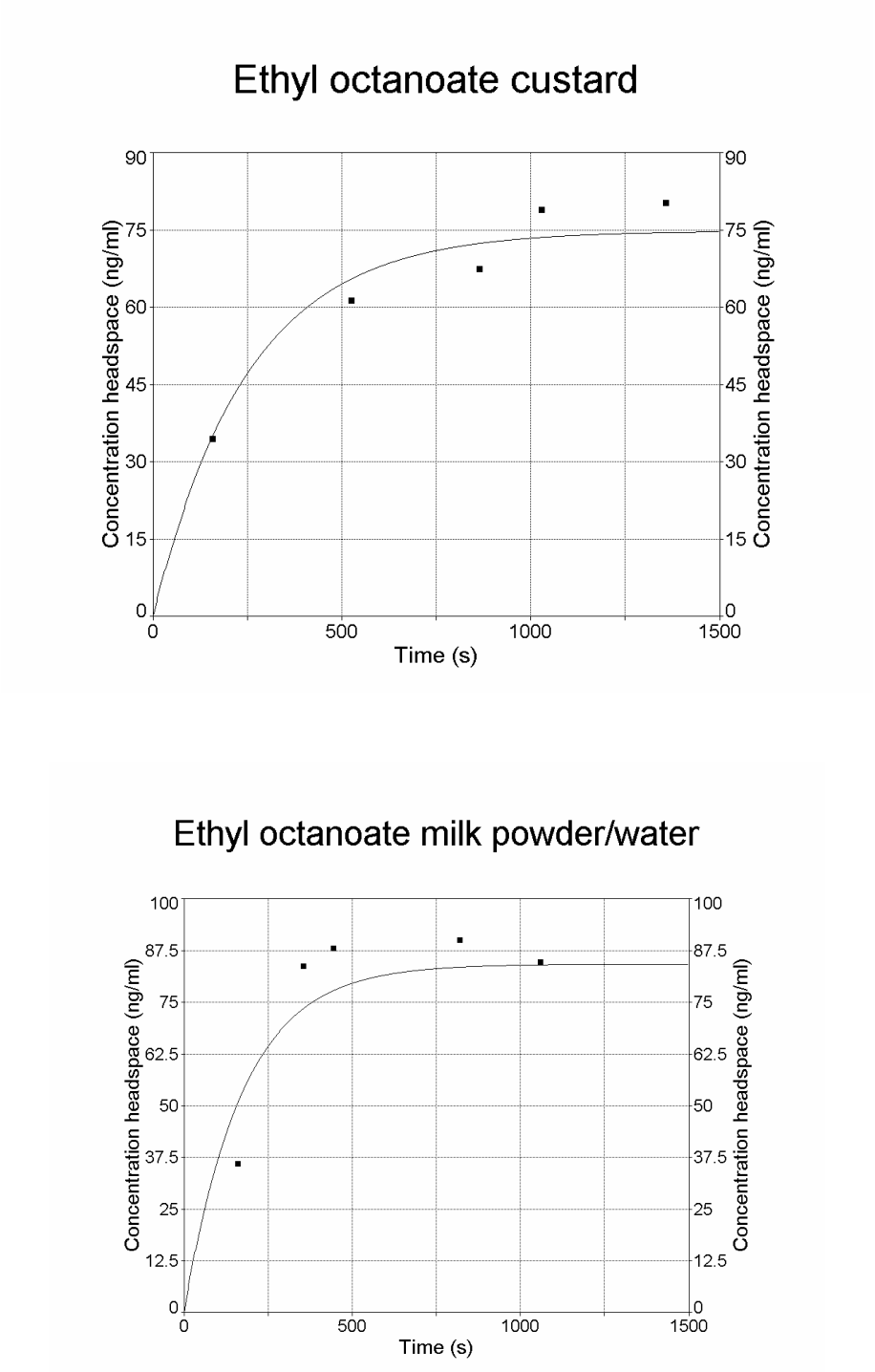


Fig. 3.25 *Time influence onto the headspace concentration of ethyl octanoate in custard and milk powder/water*

From the graphics the mass transfer coefficients were directly obtained. The values are summarized in **Table 3.26.**

Table 3.26 *Mass transfer coefficients of aroma compounds selected in model systems*

Model systems	Mass transfer coefficients (m/s)
2-Phenylethanol custard[a]	2.2×10^{-4}
2-Phenylethanol milk powder/water[b]	2.4×10^{-4}
3-Methyl-1-butanol custard	2.6×10^{-4}
3-Methyl-1-butanol milk powder/water	1.8×10^{-4}
Ethyl hexanoate custard	1.9×10^{-4}
Ethyl hexanoate milk powder/water	2.0×10^{-4}
Ethyl octanoate custard	1.7×10^{-4}
Ethyl octanoate milk powder/water	2.5×10^{-4}

a) The viscosity of the custard was experimentally determined and the value found was: **11057 mPas**;

b) Viscosity of the milk: **2.4 mPas**.

The data in **Table 3.26** indicated that the viscosity of the matrix did not significantly influence the values of mass transfer rate of selected aroma compounds.

From the **Table 3.26**, the values of the mass transfer rate are higher in milk powder/water systems than in custard model, for all the compounds investigated, except 3-methyl-1-butanol.

3.6. The influence of the matrix effects onto the odour activity values of selected flavour compounds

The matrix has an important contribution to the determination of the threshold values and odour activity values of selected aroma compounds.

The threshold values for three esters and an alcohol were determined in air in the presence and absence of ethanol, using a LABC – Olfactometer (cf. 2.1.2.5).

The procedure of work was in experimental part explained (cf. 2.5.).

The results are summarized in **Table 3.27** and the values obtained experimentally were compared with those found in the literature.

Table 3.27 *The odour threshold values of selected aroma compounds in air, in the presence and absence of ethanol, and comparison with literature data*

Compound	Threshold values (ng/L air)	Threshold values (ng/L air) [Reference] [a]	Threshold values (ng/L air in the presence of ethanol) [b]	Threshold values (ng/L air in the presence of ethanol) [Reference] [a]
Ethyl butanoate	2.9	2.5	11.7	200
Ethyl hexanoate	2.5	9	20	90
Ethyl octanoate	5.5	6	87	63
3-Methyl-1-butanol	200	125	802	6300

a) Reference: [1] Guth, H. (1997)

b) The ethanol concentration in the headspace was found 28 mg/L air.

From the **Table 3.27**, the values in air in absence of ethanol are lower than the values in presence of ethanol, which means the presence of ethanol in the matrix increases the threshold value of the component present in a known concentration in the matrix.
The threshold values in air, found experimentally are correlated with the threshold values in air given in the literature.

3.6.1. Calculation of the headspace odour activity values (HOAV's)

Headspace odour activity values (HOAV's) were calculated of aroma concentration in the headspace (ng/L) divided by the threshold value for the odorant (ng/L air) in absence and in presence of ethanol, respectively.

The results are presented in **Table 3.28.** The concentration of ethanol found in headspace was 28 mg / L air.
The data in **Table 3.28** indicates that the HOAV in the presence of ethanol are lower than the HOAV in air in absence of ethanol, for aroma compounds selected, that means ethanol has a significant influence to HOAV.

Table 3.28 *Headspace odour activity values (HOAV's) of selected odour compounds*

Compound	Headspace concentration (μ g/L)	Threshold values in air in absence of ethanol (ng/L)	HOAV in absence of ethanol	Threshold values in air in presence of ethanol (ng/L)	HOAV in presence of ethanol
Ethyl hexanoate	123.13	2.5	49252	20	6157
Ethyl octanoate	61.45	5.5	11173	87	706
3-Methyl-1-butanol	2753.91	200	13770	802	3434

3.7. Molecular modelling studies for the determination of the free energy of solvation in different model systems

The Molecular modelling methods have been used for the prediction of solvation free energies of the flavour compounds studied in different model solutions, e.g. water and water-oil systems. As Software package WinMOPAC 97 has been used.

Within MOPAC 97 there are two continuum models. One is the so-called Tomasi model and the other is COSMO.

For the water systems the Tomasi model is given and the free energy of solvation was possible to be calculated and then the experimentally data correlated with the model.

The solvation energy is defined accordance to the following thermodynamic equation:

$$\Delta G = - RT \ln P \quad , \qquad \textbf{(3-12)}$$

where: Δ G – energy of solvation: J mol^{-1}

R – universal gas constant: 8.314 J mol^{-1} K^{-1};

T – temperature: 298.15 K;

P – partition coefficient.

The free energy of solvation is expressed as the sum of three contributions: cavitation- (ΔG_{cav}), van der Waals- (ΔG_{vW}) and electrostatic energies (ΔG_{ele}):

$$\Delta Gsol = \Delta G_{ele} + \Delta G_{cav} + \Delta G_{vW} \qquad \textbf{(3-13)}$$

For the lipid system Miertus-Scrocco-Tomasi solvation model has been used for the calculation of the solvation free energy, with CCl_4 as the solvent.

3.7.1. In water systems

For the determination of the free energy of solvation in water systems for the aroma compounds selected, MOPAC 97 program was used, and the calculations with Miertus-Scrocco-Tomasi solvation model have been done, using water as the solvent.

The calculation final results are summarized in **Table 3.29.**

Table 3.29 *Final results calculated for aroma compounds selected, using MOPAC 97, Tomasi solvation model,* ***Water*** *as solvent*

Compound	Delta free energy (kcal/mol)	Free energy of cavitation (kcal/mol)	Van der Waals free energy (kcal/mol)	Van der Waals free energy (Tomasi) (kcal/mol)	Solvation free energy (kcal/mol)
Ethyl hexanoate	-8.8	21.9	-18.8	-17.2	-5.7
Ethyl octanoate	-7.8	27	-23.9	-21.7	-4.8
3-Methyl-1-butanol	-9.7	14.8	-12.4	-11.1	-7.4
2-Phenylethanol	-8.1	15.8	-14.3	-12	-6.7
γ-Decalactone	-8.7	24	-20.9	-19.1	-5.4
γ-Nonalactone	-8.2	21.4	-18.4	-16.9	-5.2
γ-Octalactone	-7.7	20.4	-17.3	-16	-4.7
δ-Decalactone	-8.7	23.9	-20.7	-19	-5.5
δ-Nonalactone	-8.4	22	-18.9	-17.3	-5.2
δ-Octalactone	-8.1	20	-17	-15.7	-5

The data in **Table 3.29** show that the solvation free energies values in water systems for esters and alcohols are increasing with the carbon chain length. For lactones, the decreasing of the solvation free energies is in contrast with the molecular weight of the odorants.

3.7.2. In water-oil systems

For the determination of the free energy of solvation in water-oil systems for the aroma compounds selected, MOPAC 97 program was also used, and the calculations with Miertus-Scrocco-Tomasi solvation model have been done, using CCl_4 as the solvent.

The calculation final results are summarized in **Table 3.30.**

Table 3.30 *Final results calculated for aroma compounds selected, using MOPAC 97, Tomasi solvation model,* ***CCl_4*** *as solvent*

Compound	Delta free energy (kcal/mol)	Free energy of cavitation (kcal/mol)	Van der Waals free energy (kcal/mol)	Van der Waals free energy (Tomasi) (kcal/mol)	Solvation free energy (kcal/mol)
Ethyl hexanoate	-0.9	19.8	-26.5	-24.2	-7.6
Ethyl octanoate	-0.5	23.6	-31.6	-29.4	-8.6
3-Methyl-1-butanol	0	14.1	-18.5	-16.5	-4.4
2-Phenylethanol	-0.7	15	-20.7	-17.6	-6.4
γ-Decalactone	-0.9	21.2	-28.7	-26.2	-8.3
γ-Nonalactone	-0.8	19.3	-26.1	-23.5	-7.6
γ-Octalactone	-0.7	18.4	-24.6	-22.3	-7
δ-Decalactone	-0.8	21.1	-28.3	-26	-8
δ-Nonalactone	-0.8	19.6	-26.1	-24	-7.4
δ-Octalactone	-0.8	18.1	-24	-22	-6.7

In water-oil systems **(Table 3.30)**, the increasing of the solvation free energies values for aroma compounds selected is in contrast with the carbon chain length. For lactones, the behaviour is similar as for the water systems.

The data in **Table 3.29 and Table 3.30** indicate that the solvation free energies are lower in water-oil systems than the values in water systems for the aroma compounds selected.

3.8. Comparison of the solvation free energy calculated by molecular modelling studies and experimentally values

The solvation free energy was experimentally calculated (in water and miglyol) (cf. 3.3) for all aroma compounds selected, according to the following thermodynamic equation:

$$\Delta G = - RT \ln P \qquad \textbf{(3-14)}$$

where: ΔG – energy of solvation: kcal mol^{-1}

R – universal gas constant: 8.314 J mol^{-1} K^{-1};

T – temperature: 303 K;

P – partition coefficients, experimentally values (in water and miglyol).

The correlations of the free energy of solvation of the model with the free energy of solvation calculated experimentally are given in **Table 3.31.**

Table 3.31 *Comparison between solvation free energies (in water and miglyol) calculated and solvation free energy found experimentally for aroma compounds selected*

Compound	Solvation free energy (kcal/mol)			
	Water		Miglyol	
	Calculated	*Experimental*	*Calculated*	*Experimental*
Ethyl hexanoate	-5.7	-3.1	-7.6	-5.9
Ethyl octanoate	-4.8	-3.5	-8.6	-7.3
3-Methyl-1-butanol	-7.4	-3.4	-4.4	-4.6
2-Phenylethanol	-6.7	-7.2	-6.4	-7.9
γ-Decalactone	-5.4	-5.3	-8.3	-9.1
γ-Nonalactone	-5.2	-5.8	-7.6	-8.6
γ-Octalactone	-4.7	-6.6	-7	-8.5
δ-Decalactone	-5.5	-6.3	-8	-9.5
δ-Nonalactone	-5.2	-7.2	-7.4	-9.0
δ-Octalactone	-5	-7.8	-6.7	-8.6

Table 3.31 shows that the increasing of the solvation free energies found experimentally for esters and alcohols in water systems is in contrast with the carbon chain length. For lactones, the solvation free energies found experimentally for the water systems are decreasing with the molecular weight of the odorants.

In water-oil systems, the experimentally values of esters and alcohols are following the same behaviour as for the water systems. But for lactones, in water-oil systems, the increasing of the solvation free energies found experimentally is in contrast with the carbon chain length.

Making a correlation between the experimentally values found for the solvation free energies in the two model systems studied: water and oil-water system, it can be observed that the experimentally values found for the water system are higher than the experimentally values found for the oil-water system for the aroma compounds selected., which means that the oil has a influence to the solvation free energy, reducing the value.

The experimentally values found for the solvation free energies in water systems are higher for esters and alcohols (except 2-phenylethanol) than the solvation free energies of the model. For lactones, the experimentally values found for the solvation free energies are lower (except γ-decalactone) than the solvation free energies of the model.

In water-oil systems, the experimentally values found for the solvation free energies are higher for esters than the solvation free energies of the model, and for alcohols and lactones, the experimentally values found for the solvation free energies are lower than the solvation free energies of the model.

4. CONCLUSIONS

Foods are complex multi-component systems which are composed of volatile and non-volatile substances. The flavour profile of a food is an important criterion for the selection of our foodstuffs. The structure of our food, in particular the presence of macromolecules as for example proteins, fats and polysaccharides, influence the mouth feeling and the extent of the flavour release. The effect of food matrix composition and structure on flavour release was presented and discussed through complementary studies carried out by thermodynamic or kinetic approaches.

The main objective of this study was the clarification of the complex relationships of the flavour release as a function of the composition of the food matrix at molecular level. Therefore the influence of matrix effects onto the partition coefficients, odour activity values and sensory properties of selected flavour compounds, in model and in real food systems were investigated. Different matrices were selected to measure their influence onto the partition coefficients of odorants: water, water-ethanol-mixtures, matrices containing lipids and more complex samples, such as mixtures of water, oil, proteins and polysaccharides. The studies included a series of lactones, esters and alcohols (γ-octalactone, γ-nonalactone, γ-decalactone, δ-octalactone, δ-nonalactone, δ-decalactone, ethyl hexanoate and ethyl octanoate, 3-methyl-1-butanol and 2-phenylethanol).

The vapour pressures and partition coefficients were determined using static headspace gas chromatography (HS-GC) techniques. The influence of the model systems on the adsorptions of the odorants at the gas-tight syringes were taken into account. The results obtained showed that the vapour pressures of the flavour compounds are decreasing with the increasing of the molecular weight of the compounds. The comparison of water/air partition coefficients ($\log P_{W/A}$) with miglyol/air partition coefficients of selected odorants ($\log P_{M/A}$) showed that for miglyol system, the $\log P_{M/A}$ are higher than the $\log P_{W/A}$ for all flavour compounds studied.
The measurement of the partition coefficients of selected aroma compounds in water-oil matrices (emulsions) revealed that the fat content of emulsions influence significantly the partition coefficients of odorants. The highest partition coefficients were obtained in emulsions where the portion between water/miglyol/emulsifier was: 47.5 + 47.5 + 5, w/w/w.

In the present study the flavour release of different aroma compounds (ethyl hexanoate and S-(-)-limonene) in carbohydrate-water solutions was examined. The static headspace method

allows the measurement of the released odour components that interact with β-cyclodextrin. The HS-GC analysis of β-cyclodextrin-water/odorant mixtures showed a reduction of the odorant in presence of the carbohydrate.

The influence of the various matrices on the human biological response of odorants was investigated by an olfactometer (e.g. determination of the threshold values of odorants in air and in the presence of ethanol) and the headspace odour activity values (HOAV's) were calculated. The results showed that the threshold values in air in absence of ethanol were lower than the values in presence of ethanol, which means the presence of ethanol in the matrix increase the threshold value of the odorant.
The studies also included the influence of wine matrix onto the partition coefficients of important wine flavour compounds. The quantification of the aroma compounds in white wine samples was achieved by isotope dilution analyses and standard addition method. Odorants in the headspace above wines were analysed by HS-GC techniques and the partition coefficients (wine/air) calculated. The results pointed out that the presence of ethanol in wine matrix does not influence the partition coefficients of selected aroma compounds. The highest partition coefficients in wines were found for the two alcohols: 2-phenylethanol and 3-methyl-1-butanol.

Concerning COST Action 921 custard samples were investigated as real foodstuff and the aroma compounds were quantified in the matrix and in the headspace above the food. The research data indicated that the partition coefficients custard/air are located between the partition coefficients water/air and partition coefficients miglyol/air, but closer to the miglyol/air values. Furthermore the mass transfer rates of selected odorants were investigated in custard- and milk powder/water samples. The values of the mass transfer rate were found higher in milk powder/water systems than in custard model. Nevertheless the results indicated that the viscosity of the matrix did not significantly influence the values of mass transfer rate of selected flavour compounds.

Molecular Modelling methods have been used for the prediction of solvation free energies of the flavour compounds studied in different model solutions, e.g. water and water-oil systems. The results showed that the predicted values (Mopac 97) for γ-decalactone, γ–nonalactone and 2-phenylethanol in water are in good agreement with experimentally solvation free energies.

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