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Novel Analytical Tools
for Quality Control in Food Science

Christian W. Huck

Additional information is available at the end of the chapter

1. Introduction

Due to the fast technological and data treatment advancements new insights into food can be considered. The application of these novel analytical techniques belongs to the responsibility of food chemists and analysts. Thereby, an increase in efficiency is based on an improved lower limit of detection (LOD), selectivity to separate analytes of interest and speed of analysis.

High-performance liquid chromatography (HPLC) belongs to the traditional separation techniques applied to a broad range of hydrophilic and hydrophobic ingredients in both the reversed-phase (RP) [1] as well as normal-phase (NP) [2] mode. In a conventional HPLC system the inner diameter of the separation column, which is the core of the separation unit, is 4.6 mm. During the last decade miniaturization down to 20 μm allowed to increase on one side the sensitivity and on the other side speed of analysis could be enhanced dramatically. Therefore, novel stationary phases mainly based on polymers have been designed and brought to the market to enable both the separation of low and high-molecular weight analytes [3]. As an alternative separation technique capillary electrophoresis (CE), which separates analytes due to their different ion mobility based on charge and molecular weight in an electric field within a fused silica capillary having an inner diameter of approximately 200 μm can be applied [4]. Thereby, the appearance of the electroosmotic flow (EOF) can influence the separation efficiency by either speeding up the separation process or by improving the resolution. Capillary electrochromatography (CEC) is a hybrid technique of both HPLC and CE in which both pressure and an electrical field are applied and enables extreme high resolution. The drawback of this separation method is the fact, that real samples can hardly be analysed due to the disturbance by the matrix [5]. In many cases the analyte of interest is only available in very low con-

centrations. Therefore, selective enrichment and purification steps are the method of choice, which can be accomplished by solid-phase extraction (SPE). Therefore, a material designed for a special analytical question is filled into a cartridge or pipette tip and the sample of interest is put onto the material in liquid form [6]. In the following, analytes of interest can interact with the functional groups of the stationary phase and compounds being not of interest can simply be washed away. In the final elution step, only some micro liters of liquid are required to elute the analytes of interest from the stationary phase being available in relatively high concentrations for the following analytical steps. The following analytical procedure can be either a separation or spectroscopic method. Spectroscopic methods at this stage of the analytical procedure either include mass spectrometry (MS) and/or vibrational spectroscopy, respectively. In MS most of the samples are analysed applying electrospray ionization (ESI) as an interface with different types of mass detectors including e.g. time of flight (TOF), ion trap, ion cyclotron and quadrupoles. As an alternative, matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF/MS) can be applied for the determination of high molecular weight compounds including proteins, peptides and lipids. For the analysis of low molecular ingredients < 1000 Da the so called matrix-free laser desorption ionization (mf-LDI) MS technique must be applied [7].Vibrational spectroscopy in the field of food analysis is mainly applied in the mid (400 – 4000 cm^{-1}) as well as in the near infrared (4000 – 12000 cm^{-1}) of the electromagnetic spectrum. In combination with chemo metrical algorithms these methods can be used for the authentication of the material on one hand, on the other hand quantitative analysis allows to control selected quality parameters [8].

In the following a systematic analytical approach is introduced, which allows combining the different analytical techniques in a synergistic manner to get deeper insights into the composition and origin of food samples.
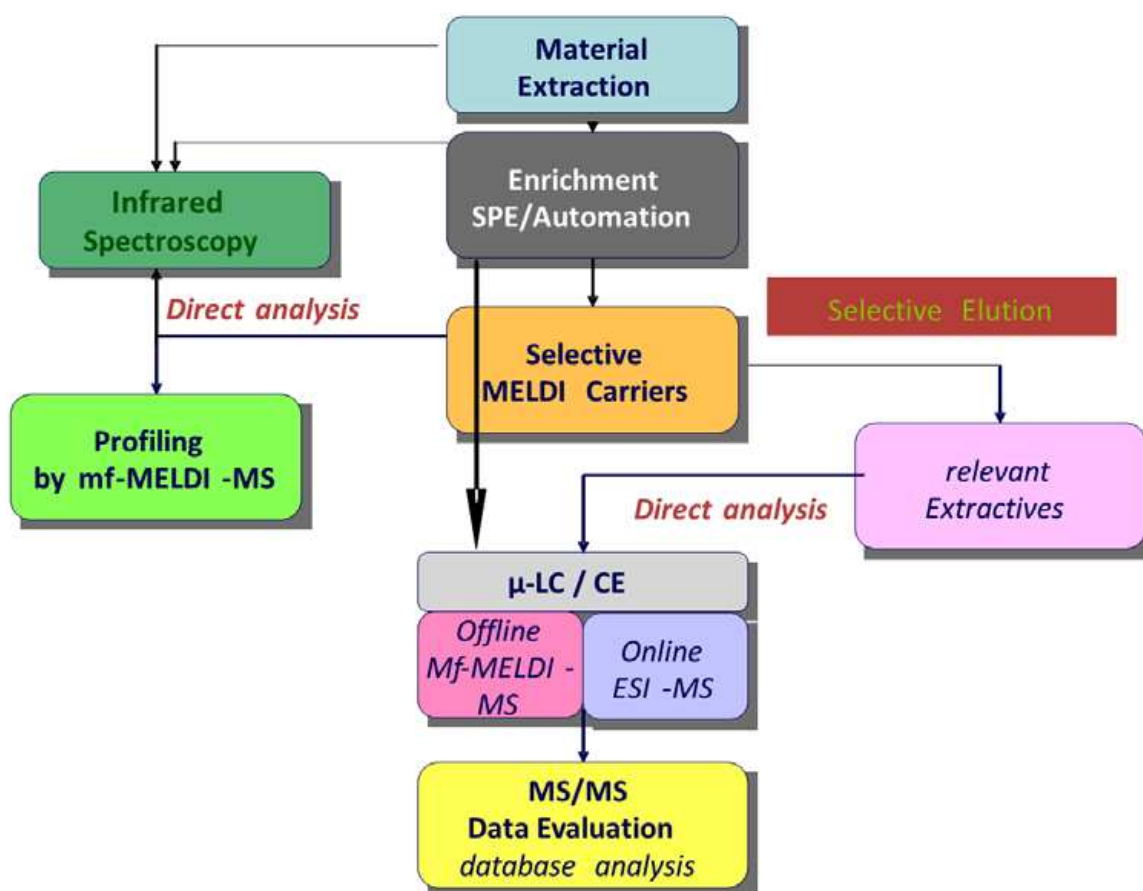
2. Systematic analytical approach

The key technologies described in the above chapter can be combined according to the scheme depicted in Figure 1. In this approach extraction of the material for the further analytical steps and individual procedures can be linked to sample enrichment/purification, separation, vibrational spectroscopy and mass spectroscopy followed by database analysis. The different parts are described in the following sub-chapters.

2.1. Sample enrichment/purification

In many cases interesting analytes are only available in extremely low concentrations and/or in very complex matrices, respectively. Therefore, pre-concentration steps based on solid-phase extraction (SPE) can be very helpful. Nano-materials such as nanotubes, fullerenes, diamond offer excellent physiochemical properties due to a high ratio of surface to size, which results in a high capacity and allows analyte detection with high sensitivity down to the femtomole range in the case when mass spectrometry is applied for

detection. Especially carbon nano materials can be easily further derivatised with a number of different functional groups including reversed-phase (RP), normal-phase (NP), ion exchange (IEX), immobilized affinity (IMAC) and so on depending on the specific demand. As an alternative they can be incorporated into a polymer matrix for highly selective extraction by certain compound characteristics. For the practical handling pipette tips have been tested to be most suitable and this special type of SPE is called "hollow monolithic incorporated tip" as it has an open flow channel in the middle enabling an easy pipetting procedure. For the highly efficient pre-concentration of phosphopeptides nano particular TiO_2, ZrO_2 and mixtures thereof are incorporated into a polymer matrix as depicted in Figure 2 [9]. By this technique hundreds of microliters can be flushed over the system and finally elution of the desired compounds to be analysed is carried out with only a few microliters causing a dramatic increase in concentration from which further analytical investigations can benefit due to the easier handling of the systematic investigation.


Figure 1. Multidimensional analytical approach
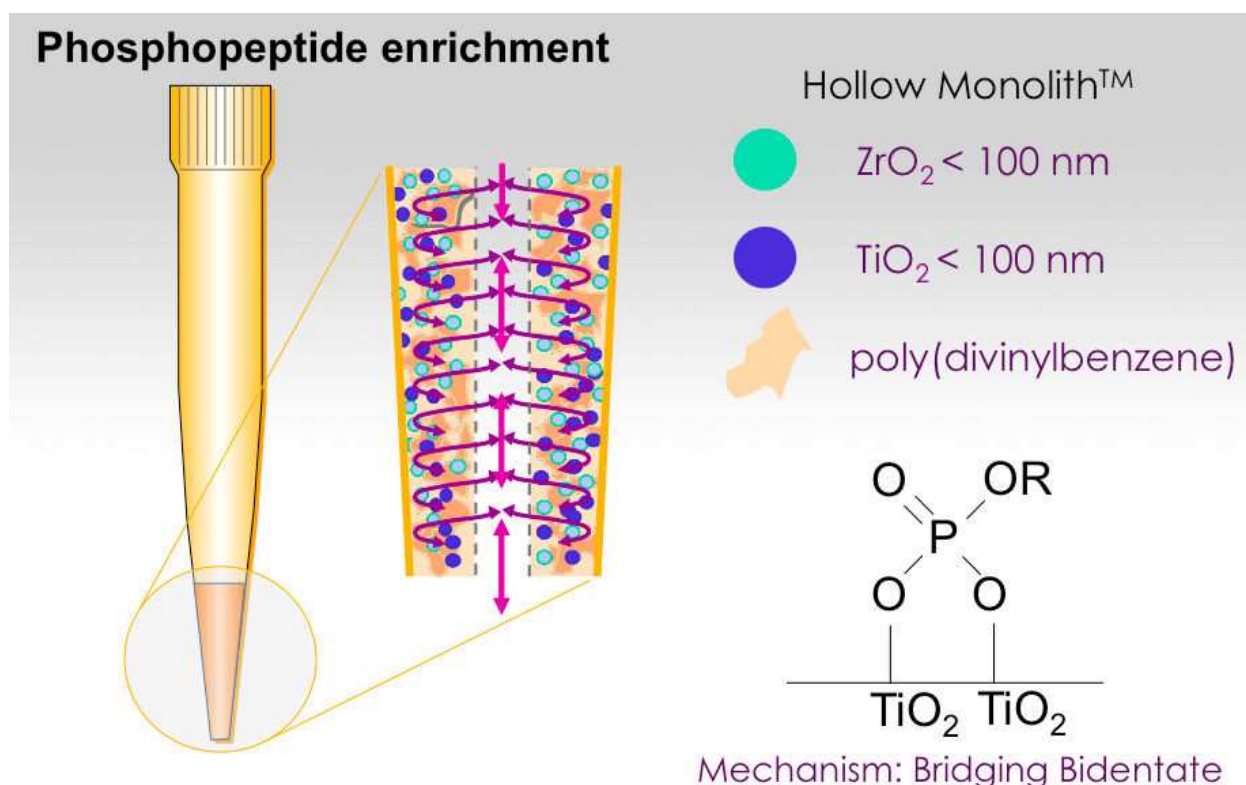
Figure 2. TiO$_2$, ZrO$_2$ incorporated into a polymer matrix for phosphopeptide enrichment

Immobilisation of such polymers into pipette tips with trypsin can be used for fast digestion of peptides and proteins within only a few minutes ensuring high capacity and sequence coverage (Figure 3) even in the high-throughput mode using robotic pipetting systems [10]. In comparison to this quite young approach the conventional digestion procedure lasts approximately 24 hours and doesn´t show in any case better results by higher sequence coverages. For this reason this approach is of high interest for the routine analysis and/or diagnostics, respectively. As a carrier glycidylmethacrylate-co-divinylbenzene (GMA/DVB) polymerized in pipette tips was chosen. The major advantages of in-tip digestion are easy handling and small sample amount required for analysis. Microwave-assisted digestion was applied for highly efficient and time saving proteolysis. Adaption to an automated robotic system allowed fast and reproducible sample treatment. Investigations with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS) and liquid chromatography coupled to electrospray-ionization mass spectrometry (LC-ESI/MS) attested high sequence coverages (SCs) for the three standard proteins, myoglobin (Myo, 89%), bovine serum albumin (BSA, 78%) and alpha-casein (α-Cas, 83%). Compared to commercially available trypsin tips clear predominance concerning the digestion performance was achieved. Storability was tested over a period of several weeks and results showed only less decrease (<5%) of protein sequence coverages. The application of microwave-assisted in-tip digestion (2 minutes) with full automation by a robotic system allows high-throughput analysis (96 samples within 80 minutes) and highly effective proteolysis.
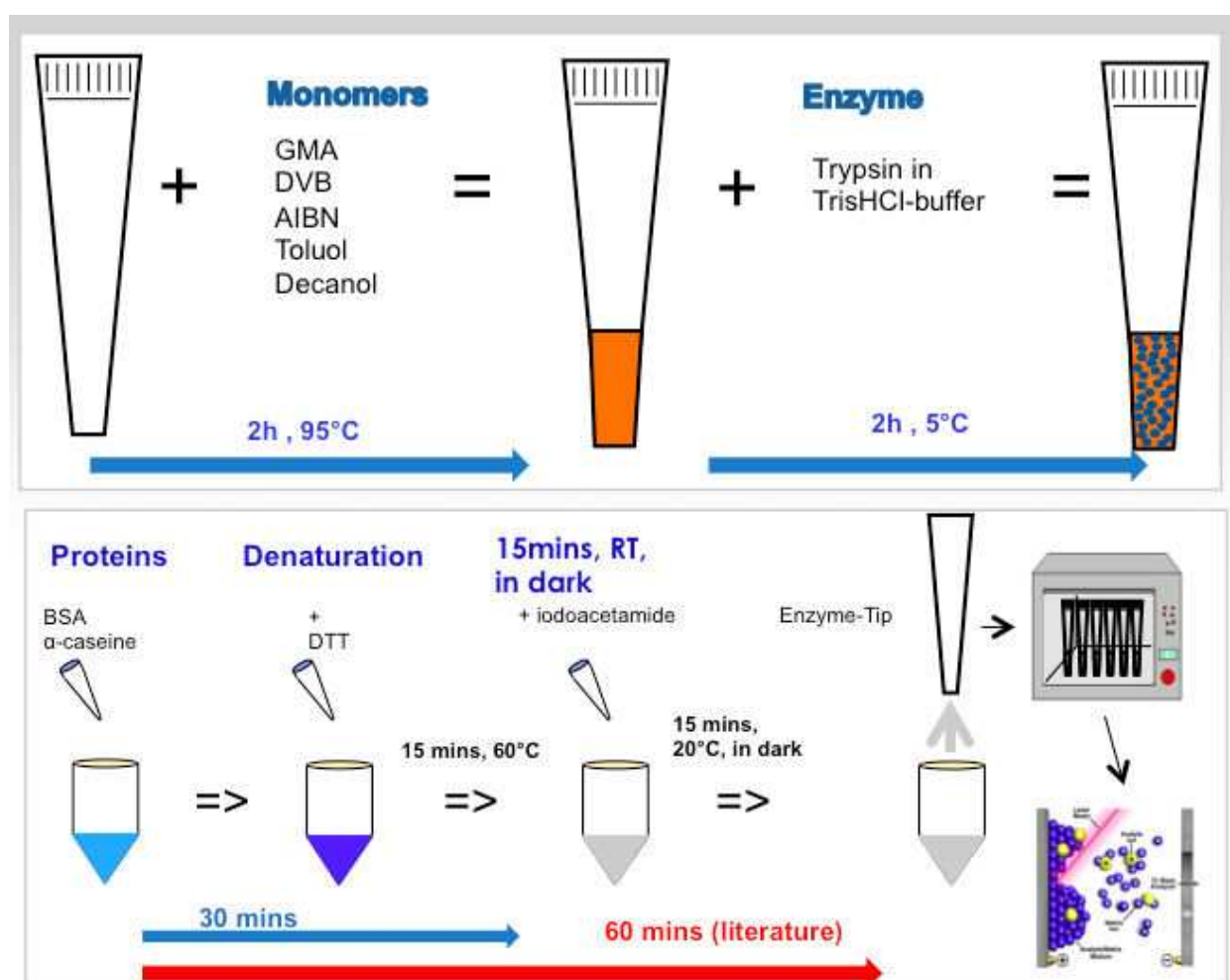
Figure 3. Trypsin immobilized pipette tips for high-throughput analysis of peptides

2.2. Selective MELDI-carriers

Material enhanced laser desorption ionisation (MELDI) is a method, which is based on the conventional matrix assisted laser desorption ionisation time of flight mass spectrometric (MALDI-TOF/MS) detection with the significant difference that before LDI MS step a selective enrichment procedure is carried out for the distinct analysis of a certain compound class. Compared to other similar techniques in this field, this approach benefits from the physical properties of the material itself (pore size, surface area, capacity, etc.) and its chemical derivatisation/functionalisation. In the past this technique was proven to be highly efficient for the analysis of biomarkers following an optimised strategy (Figure 4). In the first step a selected material including e.g., nanotubes, fullerenes, nano-crystalline diamond, polymers, cellulose, etc., which are derivatised with functional groups (C18, IMAC (immobilised metal affinity chromatography), IEX and others) is activated and the serum sample of interest is incubated. During this step, selective binding of molecules according to their functional group is achieved and finally undesired components can be washed away applying an optimised protocol. In the next step the incubated material is put onto a conventional

steel target used in MALDI-TOF/MS, a matrix substance is added (e.g., sinapinic acid) and finally the mass spectrum is generated by the laser desorption ionisation process. The result is a mass spectrum being characteristic for a patient and/or the nutrition profile. Multivariate analysis (MVA) can be applied for further data analysis and interpretation, a clustering into certain stages of an illness can be achieved, respectively. From the mass spectrum potent biomarker molecules can be selected and identified by further analytical steps. The biomarker itself and/or the profile of the corresponding mass spectrum can be used for the screening of certain diseases, stages therefrom, allergies, nutrition effects and so on [7].
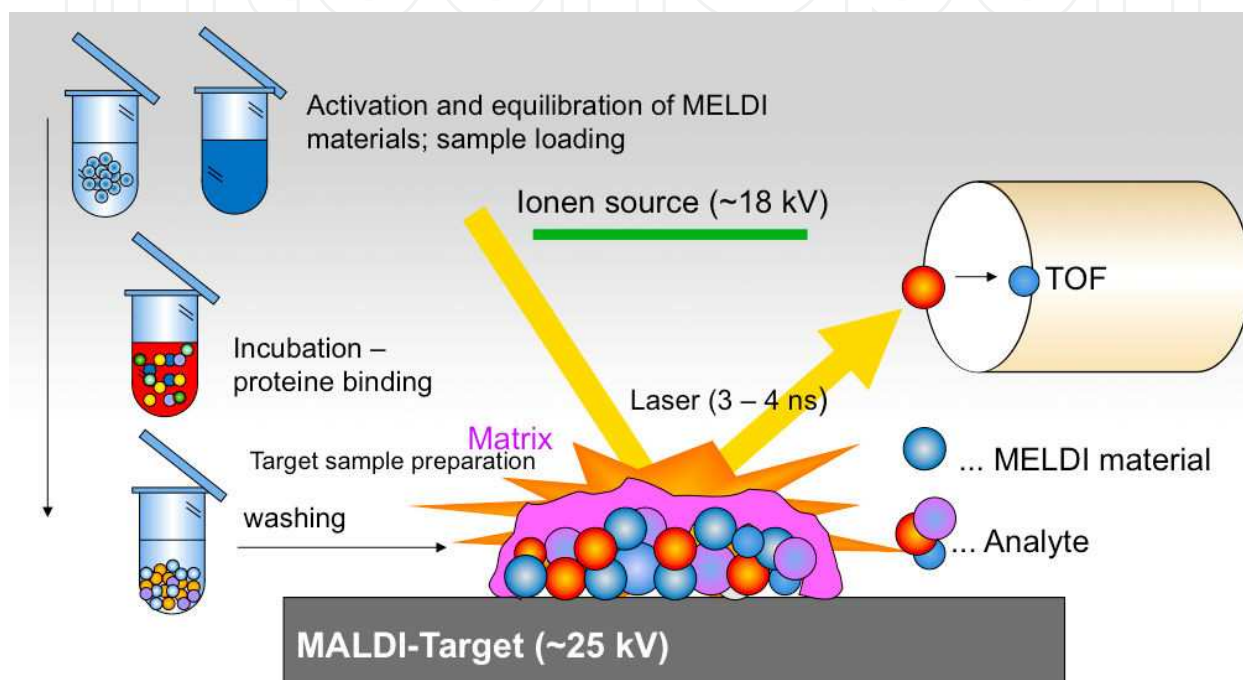
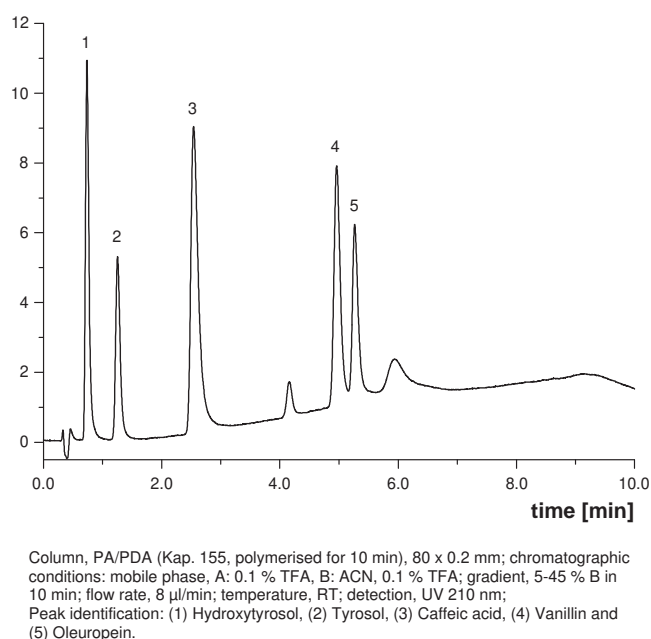**Figure 4.** Principle of material enhanced laser desorption ionization (MELDI)

For the analysis of low-molecular weight compounds (MW < 1000 Da) the conventional MELDI approach is replaced by the matrix-free (mf) MELDI approach for which the addition of a matrix substance is not required so that no disturbing peaks appear. In this approach a conventional steel target with a 50 nm thick titanium oxide layer can be applied fulfilling all requirements for a successful laser desorption ionization process [11].

As an alternative the incubated analytes of interest can be selectively eluted from the functinalised carrier material and further analysed by liquid chromatography (LC) or capillary electrophoresis (CE).

2.3. Liquid chromatography, capillary electrophoresis and electrochromatography

Novel materials used in miniaturised liquid chromatography (μ-LC) are mainly polymer based, e.g. poly(1,2-bis(p-vinylphenyl)ethane). These polymers possess the huge advantage that chemical (composition of the polymer) and physical parameters including mainly po-

rosity can be adjusted [12]. Extensive investigations on polymerisation time and tempera-
ture have been carried out enabling a tailored design of micro-, meso- and macro-pore
distribution [13, 14]. This results in the applicability of such capillaries with an inner diame-
ter between 20 and 200 μm for even the separation of high- and low-molecular weight com-
pounds. These capillaries can be highly successfully applied analysing peptides, proteins,
oligonucleotides, DNA fragments as well as "small molecules" such as phenols, flavonoids,
catechins, acids etc. Figure 5 shows as an example the separation of olive oil ingredients.
This separation is characterised by a very high ratio of flow to back pressure, which is of
high interest to perform extremely rapid Coupling to mass spectrometry enables a highly ef-
ficient analysis even of crude samplesoffering all the possibilities of collision induced disso-
ciation (CID) and database search [15].

Column, PA/PDA (Kap. 155, polymerised for 10 min), 80 x 0.2 mm; chromatographic
conditions: mobile phase, A: 0.1 % TFA, B: ACN, 0.1 % TFA; gradient, 5-45 % B in
10 min; flow rate, 8 µl/min; temperature, RT; detection, UV 210 nm;
Peak identification: (1) Hydroxytyrosol, (2) Tyrosol, (3) Caffeic acid, (4) Vanillin and
(5) Oleuropein.

Figure 5. Separation of olive oil ingredients using a monolithic capillary column. Conditions: capillary 80 x 0.2 mm;
mobile phase, A: 0.1% TFA; B: CAN; gradient, 5-45%B in 10 min; Flow rate 8 µl/min; temperature, RT; detection, UV
210 nm. Peak assignment, (1) hydroxytyrosol, (2) tyrosol, (3) caffeic acid, (4) vanillin, (5) oleuropein.

As an alternative separation method capillary electrophoresis (CE) and /or electrochroma-
tography (CEC) can be applied. In CE separation of analytes is achieved due to their differ-
ent ion mobility based on charge and molecular weight in an electric field within a fused
silica capillary having an inner diameter of approximately 200 μm [4]. As has already been
remarked the electroosmotic flow (EOF) has a main influence on the separation and can be
used for speeding up. In CEC both an electrical field and high pressure are applied resulting
in high resolution. This technique can be applied to check the identification and purity of
standards compounds with very high efficiency. For the reproducible separation and analy-
sis of food ingredients such as phenols, acids, peptides, lipids, coating of the capillary's in-
ner wall was shown being advantageous as irreversible analyte adsorption by free
hydroxyl-groups from the silanole of the fused silica capillary can be avoided. Latex-diol

and fullerene coated capillaries were successfully introduced and as a detection system on-line hyphenation to MALDI-TOF/MS was shown to be highly efficient not only for the investigation of flavonoids but also for peptides, especially phosphorylated (Figure 6) [16, 17]. This system can be used for the investigation of the casein profile in milk offering the advantage over all other more classical analysis tools that in this case also higher phosphorylated species can be separated and detected. From the ratio of different phosphorylation degrees several interpretations concerning the quality but also the origin of the milk can be carried out.
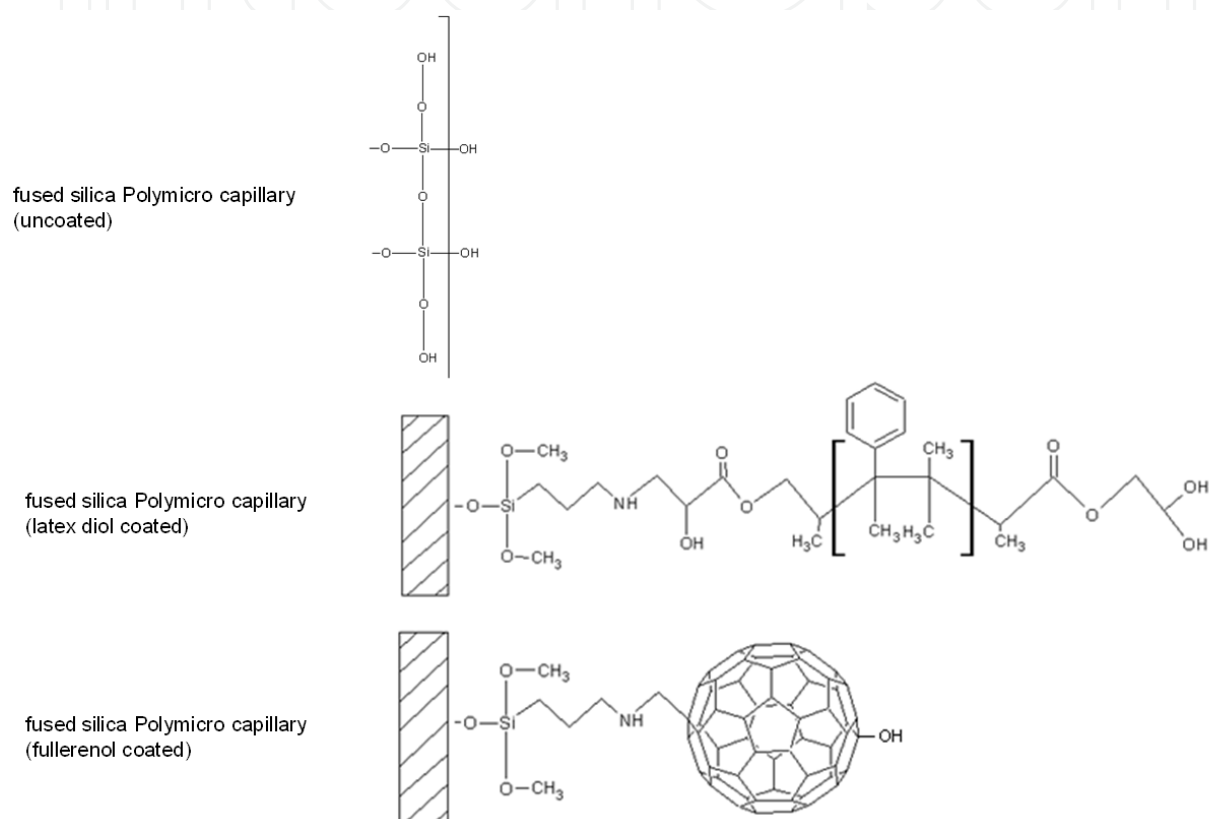

Figure 6. Inner capillary wall coatings applied in CE and CEC

2.4. Vibrational spectroscopy

For quality control both mid- (MIR, 400 – 4000 cm^{-1}) and near-infrared (NIR, 4000 – 12000 cm^{-1}) can be conducted. In MIR fundamental stretching and bending vibrations occur, in NIR the corresponding overtones and combination vibrations are detected. This means that NIR-spectra can contain a lot of more vibrational information, which is an advantage for the analysis of highly complex samples. Therefore, during the last decade several applications in the field of food analysis were developed in the NIR region. Samples can be analysed either in transmission, reflectance and interactance mode (Figure 7) so that liquid as well as solid samples can be investigated. Due to the quite broad bands compared to MIR, chemometrical spectra treatment is required for establishing adequate calibration models and to

analyse data. These are mainly multivariate (MVA) methods allowing to correct baseline, atmospheric noise etc. For qualitative analysis in most cased principal component analysis (PCA), for quantitative partial least square regression (PLSR) are applied [18].
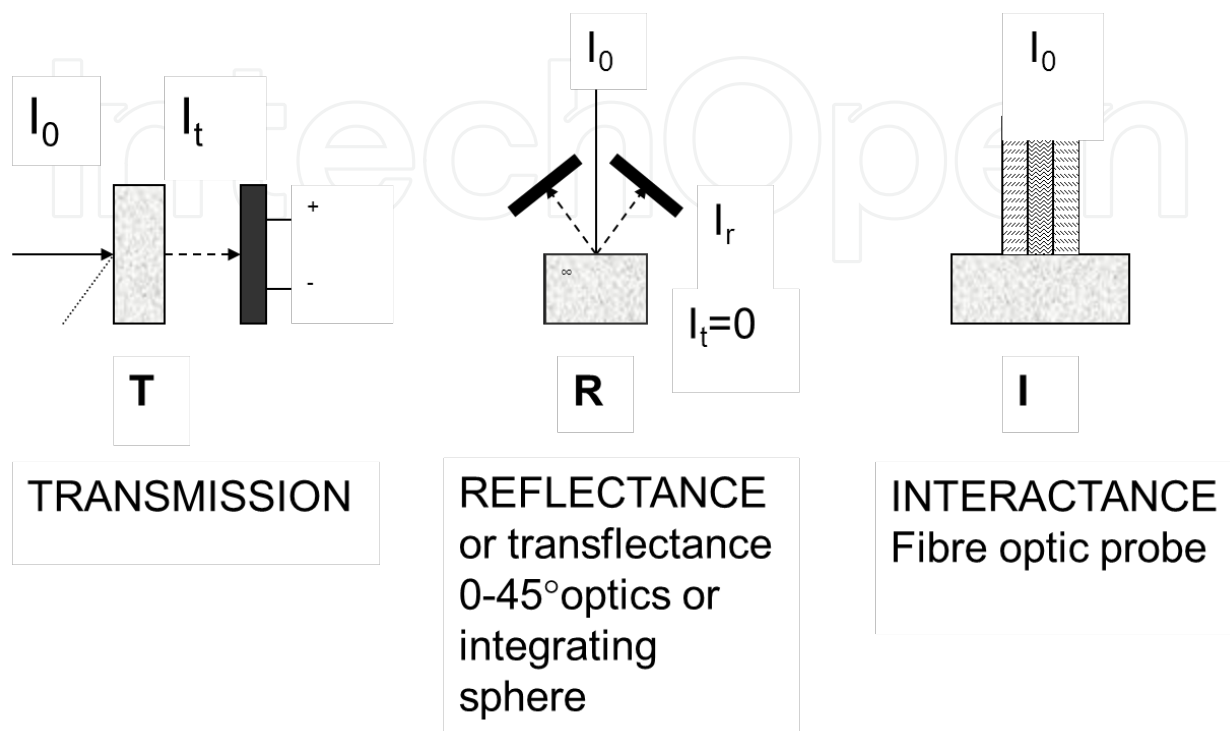

Figure 7. Sample measurement modes in NIR

An impressive example for the successful implementation in the food related production is the quality control of wine. It has been shown that NIR can be used to identify grapes, vines, age by qualitative (Figure 8) and its ingredients (acids, carbohydrates, pH etc.) simultaneously, non-invasively within a few seconds by quantitative analysis [19]. Another big advantage of this method can be found by the fact that the sample is not destroyed and can therefore be used for further purposes including following analytical steps.

Quantitative NIRS methods, which allow determining the carbohydrate, total acid, tartaric acid, malic acid, pH in grape variety and the polyphenol content in grapes were established [19]. The method can control the quality already at a very early stage during the wine production and allows improvement of its quality by this. Grapes of 12 different vines (*Weißburgunder, Chardonnay, Ruländer, Silvaner, Müller Thurgau, Gewürztraminer, Sauvignon, Lagrein, Grossvernatsch, Blauburgunder, Cabernet, Merlot*) were harvested in autumn 2000 and squeezed. The obtained grape variety was thermo stated at 23°C and analyzed quantitatively by NIRS in the transflection mode using an optical thin layer thickness of 1 mm. In order to establish a calibration model 252 spectra of samples with lower and upper concentration as a reference were recorded. 76 % of all spectra were randomly used for calibration, 24% for validation. Data preparation was carried out in order to minimize technical influences,

which mainly cause a drift in baseline. Quantitative analysis was carried out by partial least square regression (PLSR).
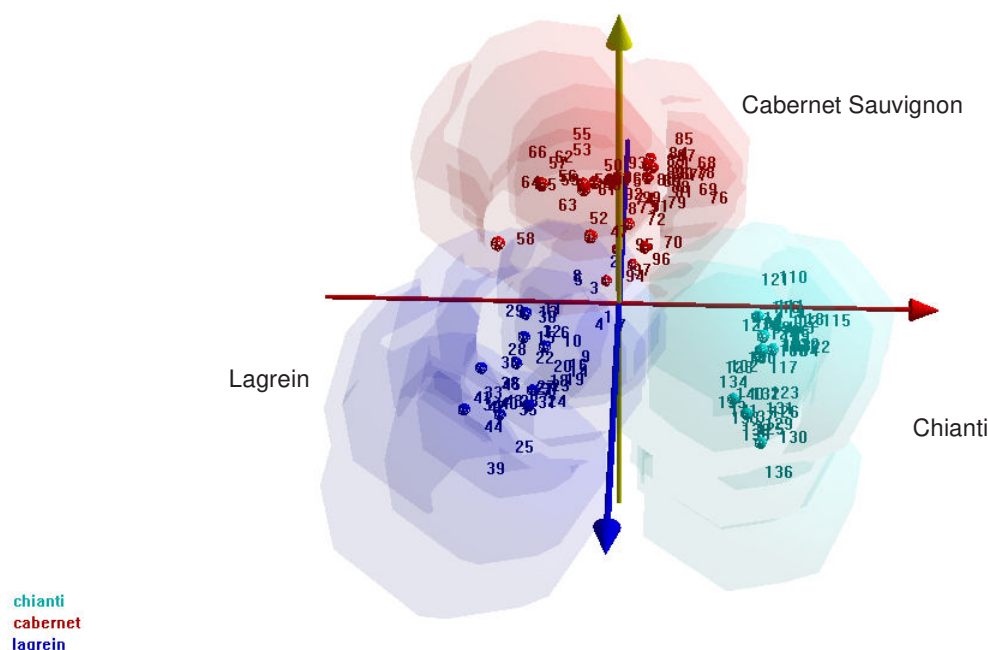
Figure 8. Factor plot of 141 specta of different wines (Lagrein, Chianti, Cabernet Sauvignon). Conditions: Normalisation, 1. derivative; wavenumber range, 4500 - 10000 cm^{-1}; thickness 3 mm; scans, 10; temperature, 23°C.

Carbohydrates. Data preparation comprised normalization between 0 and 1 and following calculation of the first derivative using a wavenumber range from 4500 - 7548 cm^{-1}. The PRESS function showed that 3 factors were needed for the calculation of the model. Calculation with 3 factors resulted in a good conformity between SEE and SEP. Linear regression between true and predicted values resulted in a value for the correlation coefficient of R^2=0.99 for calibration and R^2=0.99 for validation. Results for SEE and SEP: 0.13° KMW and 0.11 °KMW, the BIAS value is 2.30×10^{-15}.

Total acids. PLSR in a concentration range between 5 and 11 g/l included normalization between 0 and 1, full multiplicative scatter correction (MSC) and calculation of the 1st derivative (Taylor 3 points) between 4500 and 7548 cm^{-1}. 3 factors were necessary to obtain a minimum for PRESS and an agreement between SEE (0.60 g/l) and SEP (0.61 g/l) of nearly 100%.The highly linear model allows determining the total acid content with a prediction error of 0.61 g/l.

Tartaric acid. After normalization, performing of the 1st derivative over between 4500 and 7308 cm^{-1}, four factors were used for creation of the highly linear model depicted in Figure 6c with R^2=0.91 for calibration and R^2=0.87 for validation). Despite the small concentration range between 3.1 and 6.7 g/l used for calibration this system allows to determine the tarta-

ric acid content in grape variety with an absolute error of estimation of 0.40 g/l and prediction of 0.54 g/l.

Malic acid. Malic acid often shows 2-5 times higher values compared to tartaric acid. Calibration between 2.9 and 7.0 g/l after normalization between 0 and 1 and calculation of a second smoothened derivative was carried out using three factors, SEE and SEP showing acceptable agreement.Absolute values for SEE, and BIAS were 0.43 g/l and -4.25×10$^{-15.}$ Straight line for calibration showed a linearity of R^2=0.89 and allowed a prediction of the malic acid content with an absolute error of 0.55 g/l.

pH. Normalization and calculation of the smoothed 2nd derivative between 4500 and 7308 cm^{-1} showed an optimum for BIAS at five factors. Despite the narrow calibration range of pH 3.09 - 3.74 the calibration equation shows a R^2 of 0.82.

In order to enable the determination of these parameters with only one single measurement, simultaneous analysis of the carbohydrate, total acid, tartaric acid, malic acid content and pH was achieved by performing normalization (between 0 and 1) and calculating its 2nd derivative (Taylor 3 points). Four factors over a wavenumber range from 4500 to 7308 cm^{-1} showed 73-100% agreement between SEE and SEP. Linear regression showed high linearity for each investigated parameter with slightly lower values for R^2. Compared to the above-described single analysis this method allows a quantitative analysis of all parameters at once within a few seconds. Values for SEP are slightly increased (Table 1).

Polyphenols mainly influence taste, sensory properties and color of a wine. Therefore, a rapid method to analyze its quantity is important. The method according to Folin - Ciocalteu was used as a reference method (see Materials and Methods). Gallic acid-1-hydrate was used as reference standard in a concentration range from 0 to 4.93 µg/ml with equidistant steps. 24 gallic acid-1-hydrate solutions in a concentration range between 0.442 and 7.08 mg/ml were measured in the transmission mode threefold and in random order by NIRS. Evaluation using PLSR was achieved by dividing 72-recorded spectra randomly into a calibration (54 spectra) and validation (18 spectra) set. Data pretreatment comprised normalization between 0 and 1 and calculation of the 1st derivative (Savitzky-Golay) between 4008-7512 cm^{-1}. Using three factors, the PRESS function showed a minimum and a good agreement between SEE (0.45 mg/ml) and SEP (0.46 mg/ml). Linear regression between predicted and true values allowed to predict the gallic acid-1-hydrate concentration between 0 and 7 mg/ml with R^2=0.98.

In order to determine the total polyphenol concentration 30 must samples were measured in the transmission mode threefold and in random order. 90 spectra were divided into 72 calibration and 18 validation spectra. Normalization and performing of the 1st derivative allowed minimizing shifts in the baseline. 4 factors were necessary to obtain a minimum for the PRESS function and to get a maximum agreement of SEE and SEP. Linear regression allowed correlating true and predicted values with a R^2 of 0.97. Compared to the traditionally used Folin - Ciocalteu method in a winery, which is very time-consuming and expensive due to the usage of different chemicals, the NIRS method is very simple, precise and incomparably fast.

Parameter	Unit	SEE		SEP		BIAS	
		a	b	a	b	a	b
Carbohydrates	KMW	0.13	0.21	0.11	0.19	2.30×10^{-15}	3.33×10^{-16}
Total acids	g/l	0.60	0.43	0.61	0.53	7.17×10^{-15}	-1.08×10^{-14}
Tartaric acid	g/l	0.40	0.41	0.54	0.55	-1.08×10^{-14}	-3.43×10^{-15}
Malic acid	g/l	0.43	0.49	0.55	0.65	-4.25×10^{-15}	-2.44×10^{-15}
pH		0.07	0.09	0.06	0.09	-1.26×10^{-15}	-7.15×10^{-15}

Note. a Single analysis; b Simultaneous analysis

Table 1. Prediction results for the determination of the carbohydrate, total acid, tartaric acid, malic acid content and pH

Quality control of coffee ingredients including caffeine, theobromine and theophylline [20] and of food additives deriving from the highly interesting field of Traditional Chinese Medicine (TCM) [21] can be carried out in a similar way. Thereby, emphasis must be put onto the calibration method for which the above mentioned techniques can be applied as a reference. A new analytical method based on near infrared spectroscopy (NIRS) for the quantitation of the three main alkaloids caffeine (Caf), theobromine (Tbr) and theophylline (Tph) in roasted coffee after discrimination of the rough green beans into Arabic and Robusta was established. This validated method was compared to the most commonly used liquid chromatography (LC) connected to UV and mass spectrometric (MS) detection. As analysis time plays an important role in choosing a reference method for the calibration of the NIR-spectrometer, the non-porous silica-C18 phase offers a very fast method. Coupling of the optimised LC method to a mass spectrometer (MS) via an electrospray ionisation (ESI) interface not only allowed to identify Caf, Tbr and Tph by their characteristic fragmentation pattern using collisionally induced dissociation (CID), but also to quantitate the content of the three analytes, which was found to be 6% higher compared to UV-detection. The validated LC–UV method was chosen as a reference method for the calibration of the NIRS system. Analysis of 83 liquid coffee extracts in random order resulted for Caf and Tbr in values for S.E.E. (standard error of estimation) of 0.34, 0.40 g/100 g, S.E.P. (standard error of prediction) of 0.07 and 0.10 g/100 g with correlation coefficients of 0.86 and 0.85 in a concentration range between 0.10 and 4.13 g/100 g. Compared to LC the lower limit of detection (LOD) of the NIRS-method is found at 0.05 g/100 g compared to 0.244–0.60 ng/100 g in LC, which makes it impossible to analyse Tph by NIRS.

The possibility to hyphenate a MIR/NIR spectrometer to a microscope unit allows determining the distribution of active ingredients within a tissue sample down to a resolution of 1.2 µm [22]. A "hyperspectral cube" is recorded with the dimensions of the sample on the x- and y-axis and the absorbance on the z-axis from which the image can be extracted (Figure 9).

Fourier Transform Infrared (FTIR) spectroscopic imaging and mapping techniques have become essential tools for the detection and characterization of the molecular components of

biological tissues and the modern analytical techniques enabling molecular imaging of complex samples. These techniques are based on the absorption of IR radiations by vibrational transitions in covalent bonds and their major advantage is the acquisition of local molecular expression profiles, while maintaining the topographic integrity of the tissue by avoiding time-consuming extraction, purification and separation steps. These new techniques enable global analysis of biological samples with high spatial resolution and provide unique chemical-morphological information about the tissue status. With these non-destructive examination methods it is possible to get qualitative and quantitative information of heterogeneous samples.
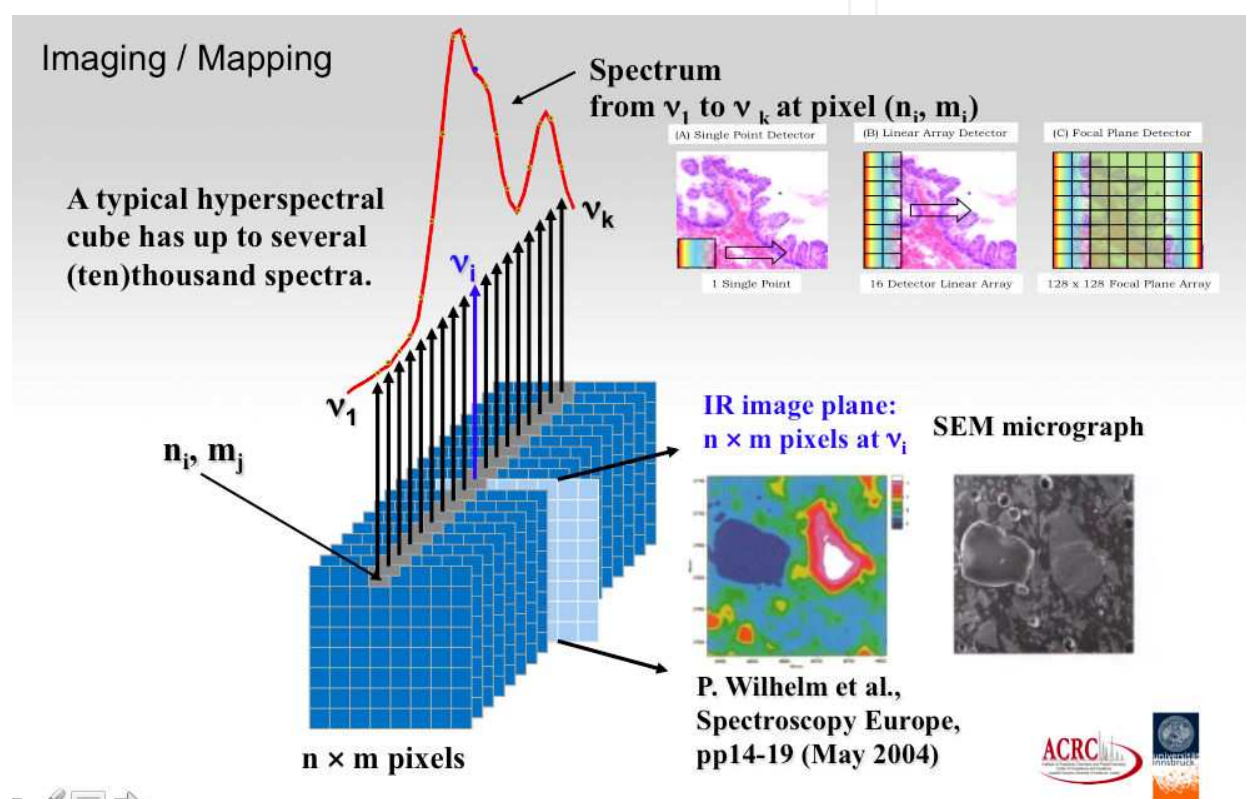
Figure 9. Principle of hyperspectral cube

Additionally, MALDI-TOF/MS imaging can be applied from the same sample of interest to get knowledge concerning the molecular weight distribution. This method is also suitable for studying the effect of nutrition onto different kinds of diseases, e.g. prostate cancer.

3. Conclusions

The techniques described can be applied according to the scheme depicted in Figure 1. This systematic analytical strategy allows getting multifacial knowledge and insights into food and samples derived therefrom.

Acknowledgements

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