









NON-DESTRUCTIVE METHODS FOR DETECTING HEALTH-PROMOTING COMPOUNDS

COST Action 924 Working Group Meeting



Bornimer Agrartechnische Berichte

Heft 54 Potsdam-Bornim 2005





NON-DESTRUCTIVE METHODS FOR DETECTING HEALTH-PROMOTING COMPOUNDS

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Welcome

In the last years a growing interest has appeared in the development and application of non-destructive measuring tools to detect fruit and vegetable quality parameters. These tools have the advantage of being fast and objective and allow repeated measurements on the same fruit. This knowledge can improve the understanding of physiological processes and as well as the modelling of product chains.

The task of this workshop is to inform the participants in detail about innovative optical methods to non-destructively detect health promoting compounds in fruit and vegetables. The main questions which have to be solved are: which compounds are important for human nutrition and how can we detect these substances. The following session is dealing with physical basics of spectrometry and recent hardware developments. The next morning different optical applications are presented followed by a lecture to quality insurance of new analytical methods. In the early afternoon several manufacturers for non destructive measuring devices will present their products.

The workshop offers enough time for discussions which would be extended by a glass of bear or wine in the evening.

Dr. rer. agr. Martin Geyer Head of Department Horticultural Engineering, Leibniz-Institute of Agricultural Engineering Potsdam-Bornim e.V. (ATB)

PROGRAM

07/12/2005				
13:00 – 14:00	REGISTRATION			
14:00	WELCOME by Prof. Dr. Reiner Brunsch Leibniz-Institute of Agricultural Engineering Potsdam-Bornim e.V. (ATB)			
14:15–18:30 Se	ession 1: Background			
Moderator: Martin Geyer				
14:15	OVERVIEW ON HEALTH PROMOTING COMPOUNDS IN FRUIT AND VEGETABLE - IDENTIFICATION OF NUTRIENTS GROUPED ACCORDING TO THEIR CHEMICAL AND PHYSICAL PROPER- TIES Prof. Dr. Regina Brigelius-Flohé German Institute of Human Nutrition Rehbrücke (DifE), Department Biochemistry of Micronutrients			
15:00	NUTRITIONAL IMPORTANCE OF SECONDARY PLANT INGRE- DIENTS Prof. Dr. Florian J. Schweigert University Potsdam, Department of Nutritional Physiology and Pathophysiology			
Coffee break				
16:15	ANALYTICAL METHODS BASED ON FLUORESCENCE SPEC- TROMETRY Franz-Josef Schmitt, Prof. Dr. Ing. Hans Joachim Eichler Technical University Berlin, Institute for Optical Technologies			
17:00	RECENT HARDWARE DEVELOPMENTS REGARDING OPTICAL TECHNOLOGIES Dr. Bernd Sumpf Ferdinand-Braun-Institut für Höchstfrequenztechnik (FBH)			
17:45	HEALTH PROMOTION BY A NEW MICROBIOCIDAL FORMULA- TION COMPRISING ESSSENTIAL OIL FROM CITRUS FRUITS Prof. Dr. Shimshon Ben Yehoshua Volcani Center, ARO Israel			

12/08/2005

8:30 – 12:00 Session 2: Applications

Moderator: Manuela Zude

8:30	OPTICAL PROCESS AND QUALITY SENSING (OPQS): APPLIKA- TIONS IN FOOD SCIENCE Prof. Dr. Hans-Gerd Loehmannsröben, Dr. Michael U. Kumke, N.N. University of Potsdam, Department of Chemistry
9:15	FRUIT QUALITY SENSING BY MEANS OF TIME-RESOLVED RE- FLECTANCE SPECTROSCOPY Prof. Dr. Paola Eccher Zerbini CRA - I.V.T.P.A. (Istituto sperimentale per la Valorizzazione Tecno- logica dei Prodotti Agricoli)
Coffee break	
10:30	NON-INVASIVE ONLINE DETERMINATION OF ANTIOXIDANT SUBSTANCES IN FRUIT AND VEGETABLE BY RAMAN SPEC- TROSCOPIC MEASUREMENTS Prof. Dr. Jürgen Lademann, Dr. M. Darvin, S. Schanzer, H. Richter Charité, Klinik für Dermatologie Berlin
11:15	QUALITY INSURANCE IN DEVELOPMENT AND APPLICATION OF NEW ANALYTICAL METHODS Dr. habil. Thomas Hübert Federal Institute for Materials Research and Testing (BAM)
Lunch	

13:00 – 15:00 Exhibition

COMMERCIAL COMPOUNDS FOR NON-DESTRUCTIVE SENSING

Moderator: Bernd Herold

13:00 Michael Rode, Carl Zeiss Jena GmbH http://www.zeiss.com

> Karsten Stein, IOM Innovative Optische Meßtechnik GmbH http://www.iom-berlin.de

Klaus Haberstroh, ESE GmbH http://www.ese-gmbh.de

Dr. Andreas Bülter, Pico Quant GmbH http://www.picoquant.com

Jessica Gerhart, ETR Elektronik und Technologie Rump GmbH http://www.etr-gmbh.de

Stefan Rieth-Hoerst, RTE Akustik + Prüftechnik GmbH http://www.rte.de

Dr. Monika Zehlicke, Beckman Coulter Sales http://www.beckman.com

Eberhard Friese, Magnettech GmbH http://www.magnettech.de

Philipp Neumann, CP GbR

http://www.cp-info.de

Testing the equipment presented with own samples can be arranged during the session.

R. Brigelius-Flohé

German Institute of Human Nutrition, Potsdam-Rebruecke Department Biochemistry of Micronutrients Arthur-Scheunert-Allee 114 – 116, 14558 Nuthetal, Germany

Introduction

Numerous observational studies provide evidence that a diet high in fruit and vegetables can reduce the risk of some diseases, including cardiovascular diseases, neurodegenerative diseases and cancer. Fruits and vegetables contain so many compounds that are putatively protective that the effects are not likely attributed to a single substance. Rather function the compounds by complementary and overlapping mechanisms. Thus we have to look at all bioactive components simultaneously to understand whether and how a certain diet contributes to disease prevention. The more than 500 dietary compounds so far identified as potential modifiers of disease development comprise, e.g. vitamins, polyphenols, glucosinolates, and organic compounds delivering essential trace elements such as selenium. Examples for these categories will be presented in this overview.

Vitamins

Vitamin E

Vitamin E has for long been considered as an antioxidant only. For this reason it has been tested for its protective effect in the prevention of diseases connected to oxidative stress, mainly atherosclerosis. Numerous experiments with different animal models did not show a consistent inhibitory effect of vitamin E. Results were contradictory, depending on the model used and the feeding modus. In most of the large clinical trials vitamin E consistently failed to prevent atherosclerosis (reviewed in [1]). In addition, there is little evidence that compounds with antioxidant properties in a test tube also inhibit oxidative reactions *in vivo* [2]. Future studies with vitamin E should consider novel functions of vitamin E which include regulation of gene activity. Identifying the vitamin E-regulated genes and the biological processes depending thereon will definitely help to understand the real physiological role of vitamin E and to develop strategies how to use the vitamin to keep an organism healthy.



Vitamin C

Vitamin C is also mainly considered as an antioxidant. It is an electron donor and therefore a reducing agent. Electrons are donated sequentially, i.e. vitamin C is first converted to a radical, the semidehydroascorbic acid or ascorbyl radical. Since the ascorbyl radical is fairly unreactive and relatively stable, ascorbate can react with a more reactive and thus more harmful radical and, therefore, may be considered to be a good free radical scavenger. Loss of a second electron results in dehydroascorbic acid. Vitamin C is, however, required for the function of a number of enzymes. The most important are prolyl and lysyl hydroxylases which hydroxylate collagen, a process required for the adequate formation of the collagen molecular structure. Vitamin C deficiency prevents proper collagen formation, thus leading to scurvy.

In vitro, vitamin C prevents oxidation of LDL, proteins and DNA. In animal experiments it has been shown to have pro- and antioxidant effects, depending on the experimental design [3]. Beneficial effects of supplemental vitamin C have been noted in small studies, while large, placebo-controlled and prospective studies have failed to show benefit in conditions believed to result from oxidative stress. To increase vitamin C plasma levels to putatively protective concentrations corresponding to *in vitro* studies is not possible, since the plasma concentrations are tightly controlled and excess vitamin C.



Oxidation states of ascorbate

Polyphenols

Polyphenols are a wide variety of compounds that occur in fruits and vegetables, wine, tea, extra virgin olive oil, chocolate and other cocoa products. The family is composed of flavonoids, phenolic acids, stilbenes and lignanes.

Flavonoids

Flavonoids are divided into several classes according to the degree of oxidation of the chroman heterocycle and the position of the attached benzene ring: flavones, flavonols, isoflavons, flavanones, flavanols, anthocyanins, and proanthocyanidins [4]. They are present in food as glycosides and largely processed to their aglycones before absorption.

From the most prominent flavonoids quercetin, e.g., is a flavonol, catechin a flavanol, genistein an isoflavone, and cyanidin an anthocyanidin.



Stilbenes

Stilbenes are less ubiquitous. One of them, resveratrol present in red wine has recently received great attention. Its anti-inflammatory and chemopreventive properties have first been attributed to antioxidant activity, Recently, however, an inhibition of NF κ B activation was described [5]. Depending on the tissue investigated this is achieved by an interference with early events in the signaling cascades mediated by Toll-like

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receptors or by a stimulation of Sirt1 histone deacetylase activity that leads to deacetylation of the p65 submit of NF κ B which prevents transcriptional activity.



Although the precise mechanism of resveratrol action is not known yet, the resorcin moiety appears to be essential for the chemopreventive efficacy.



resveratrol

Several polyphenols thus have been demonstrated to exert clear antioxidant *properties in vitro*. However, the concept that these characteristics constitute their diverse pharmacodynamic profiles now appears to be too simplistic. Evidence is indeed accumulating that polyphenols may provide an indirect protection by activating endogenous defense systems, such as the adaptive response(see below).

Glucosinolates

Glucosinolates are a class of about 100 naturally occurring thioglucosides that are characteristic of the Cruciferae and related families in the order Capparales including broccoli, cauliflower, Brussel sprouts, cabbage, mustard, and watercress. The skeleton

of glucosinolates consists of a thioglucosidic link to the carbon of a sulphonated oxime. Glucosinolates appear to have little biological impact themselves, but release biologically active products such as isothiocyanates or indoles depending on the structure of the side chain. Degradation requires myrosinase, an enzyme provided by the plants themselves and released during the disruption of the plant cells during mastication or chopping. Cooking destroys enzyme activity. Alternatively glucosinolates can be cleaved by gastrointestinal bacteria. Common products of glucosinolate hydrolysis are isothiocyanates, nitriles and thiocyanates.

Sulphoraphane

Sulforaphane is released from glucoraphanin and regarding chemopreventive effects one of the best investigated isothiocyanate. It inhibits phase 1 enzymes such as cytochrome P_{450} and induces phase 2 enzymes. The latter function makes it a potent activator of the adaptive response which enables an organism to cope with oxidative/xenobiotic challenge (see below). However, as an electrophile it can also form DNA adducts indicating that the beneficial effect is dose-dependent.



The adaptive response

Induction of phase 2 enzymes is a highly effective strategy for the protection against carcinogenesis and other forms of electrophile and oxidant challenge. Induction is regulated via the Nrf2/Keap1 system. Under unstressed conditions Nrf2 is kept in the cytosol by the association to Keap1 anchored to the action cytoskeleton. Dissociation of the complex is initiated by the modification of thiol groups in the Keap1 molecule. The resulting change in configuration releases Nrf2 and allows its translocation into the nucleus where it binds to its responsive element (ARE = antioxidant responsive element, also called electrophile responsive element, EpRE).

Compounds modifying Keap1, e. g. are isothiocyanates, dithiolethiones, oxidizable hydroquinones, Michael-reaction acceptors, hydroperoxides and heavy metals. Even quercetin, has been shown to activate Nrf2. Target genes are: heme oxygenase-1, GSTs, γ -GCS (γ -glutamyl-cysteine synthetase), thioredoxin, thioredoxin reductase, GI-GPx, NADPH quinone oxidoreductase.



Selenium

In mammals, selenium is incorporated into the peptide chain of selenoproteins as selenocysteine. For this, selenide is required which can be derived from inorganic salts, especially selenites, or from organic selenium compounds. These have to be transformed into selenocysteine from which selenide is released by the β -lyase. The most prominent mammalian selenoprotein families are the glutathione peroxidases, the thioredoxin reductases and the deiodinases. All are involved in redox-regulated cellular processes, however, are not typical antioxidant enzymes.

The chemical nature of the selenium compounds present in food are only scarcely known (for review see [6]). Apart from selenomethionine, mainly methylated seleno amino acids like selenomethylselenocysteine and selenomethylselenomethionine have been identified. Methylated seleno amino acids have been made responsible for the anticarcinogenic effect of selenium. However, it remains to be tested whether they are suitable precursors for selenoprotein biosynthesis.





Se-Methylselenocysteine

Se methionine

Conclusions

Most of the 'antioxidant' components of a healthy fruit and vegetable-based diet proved not to exert their putative health benefits by means of their antioxidant potential. The biological role of 'antioxidant' vitamins has rather to be seen in specific functions as cofactors or regulators of enzymes. Quite a number of polyphenolic compounds and the glucosinolate-derived isothiocyanates such as sulforaphane activate the Nrf2/Keap1 system thus triggering an adaptive response to cope with greater challenge by xenobiotics or oxidants. In part at least, this adaptive response depends on selenoproteins and thus on the intake of adequate bioavailable selenium.

References

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Analytical Methods Based on Fluorescence Spectrometry

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Introduction

The spectrometry of light is a powerful tool for non-invasive analysis of material properties. It is a sensitive method of investigation, because the visible photons which carry an energy of about 2 eV interact with the electrons of molecules without destroying chemical bonds. The chemical structure of different substances influences the specific quality of the absorption and emission spectra of the investigated matter.

This contribution deals with the spectrometry of light emitted from electronically excited states which is called fluorescence spectrometry.

While many chemical substances present in plants or fruits like carbohydrates, proteins and lipids absorb visible light only weakly or convert the absorbed energy to heat, some others, like most pigment molecules, NADPH or aflatoxin show fluorescence. During the past 20 years there has been a remarkable growth in the use of fluorescence in biological sciences. Environmental monitoring, clinical chemistry and DNA sequencing are just a few areas of application.

Because of the sensitivity of fluorescence spectrometry the wavelength and also the time dependency of the emitted light helps to identify and quantify fluorescent substances with high accuracy. So this method is a tool for the investigation of fruit and plant quality parameters represented by the existence of toxic substances or health promoting components. Fluorescence also delivers information about the interaction between different pigments which is an important aspect in photosynthesis research. For that reason the spectrometry of fluorescence is adequate for analysing the living plant including fundamental research of the basic principles of photosynthetic energy conversion.

Basic principles of spectrometry

The interaction of light and matter can be interpreted macroscopic or microscopic. On the macroscopic level surface interactions and interactions inside a medium are distinguished. In this interpretation which leads to geometric optics the optical properties are described by the complex refraction index $\tilde{n} = n + i\alpha$. The real part *n* describes the refraction, the reflection and the transmission from surfaces between different materials. The complex part $\alpha(\lambda)$ which strongly depends on the wavelength λ of the light denotes the absorption inside a medium. Absorption along the axis x is described by Lambert Beer's Law

 $I(x) = I_0 e^{-\frac{\alpha(\lambda)4\pi}{\lambda}x} := I_0 e^{-\kappa x}$, where I(x) describes the intensity of the light inside the medium after propagation of a distance x, I_0 is the initial intensity and κ is the absorption coefficient.

The microscopic interpretation has to assume direct interactions between two quantum objects, the photon and the electron (the optical properties of matter are mostly determined by the electrons). In microscopic interpretation the transmission inside the matter is caused by elastic scattering of light from all different atoms.

The absorption inside the matter is an inelastic scattering of the photons from the electrons. In Bohr's atom model the electron can only stay in certain energy levels called eigenstates of the atom. Only photons with the energy of the difference between two eigenstates can be absorbed with high efficiency. Absorption spectrometry is measuring the typical absorption energies which appear as dark "lines" in the spectrum representing a "fingerprint" of the observed matter.

Excited states of molecules and average lifetimes of excited states

After light absorption an atom or molecule is "excited". The lifetime of the excited states strongly depends on the electronic structure of the molecule. For pigments showing high fluorescence a typical lifetime of the first excited state is some ns (i.e. 10^{-9} s).



Figure 1: absorption and fluorescence of Chl a in solution.

In molecules the fluorescence light has not the same wavelength as the absorbed light. It is normally red shifted to the absorbed light and the spectral properties of the fluorescence also depend on the electronic states in the molecule and the possible transitions between these states.

When we look at chlorophyll then there is a big delocalised Pi-electron system. The first excited state of this system has a lifetime of about 5 ns for isolated chlorophyll molecules. The energy is partially converted to heat and mainly emitted as fluorescence and phosphorescence light with a maximum at 680 nm (**Figure 1**). For that reason green leaves emit red fluorescence.

Fluorescence spectrometry: a tool for molecule identification

Fluorescence spectrometry helps to understand the electronic properties and the transition processes in molecules but it is also a useful tool for the identification of molecules by their typical fluorescence spectrum.

When matter which is composed by several substances is exited by a light source the emission of all fluorescent substances will mix with the light of the source. To avoid an unspecific excitation of several molecules, fluorescence spectrometry is often done with a laser system or another monochromatic light source, which helps to excite molecules specifically.

At room temperature the electrons are not localised in a certain state but they are temperature dependent distributed to several states. For that reason the fluorescing molecules do not emit a sharp frequency but a fluorescence emission band with a certain bandwidth.

Low temperature measurements can help to avoid this temperature induced fluorescence line broadening. But in this case a biological sample is destroyed by the freezing process. Another tool for specific pigment identification is the variation of excitation wavelength. If there are no energy transfer processes between different fluorescing pigments only the fluorescence of the pigments that can absorb the excitation light will be observed. If the absorption spectra of the possible molecules in the sample are known, a variation of excitation wavelength can also help to identify a specific fluorescing pigment without line narrowing just by observation of the intensity of fluorescence signals in a certain spectral bandwidth after excitation.



Figure 2: pathway of light in pigment protein complexes of an antenna system in a photosynthetic organism.

In some cases, when the distance between different fluorophore molecules is in the nm area, there occur energy transfer processes. The excited state of one fluorophore can be transferred to another one and induce a fluorescence signal in a spectral range of the latter fluorophore which was not directly excited. These energy transfer processes play an important role nature where these processes are important in biological terms. Especially in photosynthesis the transfer of excitation energy from antenna pigments to a reaction center where charge separation is possible is important for the plant physiology (**Figure 2**). So the kinetic of the fluorescence decay of a donor pigment or temporal fluorescence rise of a molecule that gets energy from the donor (acceptor pigment) is an evidence for energy transfer. These processes can be analysed by time resolved fluorescence spectrometry.

Mathematical aspects of fluorescence dynamics

The fluorescence of a single molecule without energy transfer processes is decaying in time with a certain rate constant k_{Fluor} if no other relaxation processes are possible. k_{Fluor} is the hypothetical fraction of excited electrons falling to the ground state per time interval and emitting fluorescence light that would be observed if relaxation could only occur as fluorescence. If other energy dissipation processes (for an example also photochemical quenching in photosynthetic organisms) are possible with a rate constant k_{Diss} , the time constant of the fluorescence decay depends on all possible energy dissipation.

pation processes. Then the change of excited states on time $\frac{d\langle N_1 \rangle}{dt}$ is given by the product of the sum of all rate constants of the possible relaxation processes and the concentration of excited states $\langle N_1 \rangle$:

$$\frac{d\langle N_1 \rangle}{dt} = -\left(k_{Fluor} + k_{Diss}\right) \langle N_1 \rangle \tag{1}$$

The integration of eq. (1) delivers the time dependency of the excited states $\langle N_1 \rangle (t)$:

$$\langle N_1 \rangle (t) = \langle N_1 \rangle (0) \cdot e^{-(k_{Fluor} + k_{Diss})t} = \langle N_1 \rangle (0) \cdot e^{-\frac{t}{\tau}}$$
(2)

 $\langle N_1 \rangle$ (0) denotes the initial concentration at t=0.

.

The inverse sum of all rate constants is the fluorescence decay time $\tau = \frac{1}{k_{Fluor} + k_{Diss}}$.

The fluorescence intensity decays exponentially in time, because it follows proportional to the concentration of excited states by

$$F(t) = k_{Fluor} \cdot \langle N_1 \rangle(t) \tag{3}$$

while the non fluorescent energy relaxation is competing with the rate constant k_{Diss} .

For that reason the fraction of the energy relaxation by fluorescence and the total energy relaxation, which is called "fluorescence quantum yield" denotes as

$$\Phi_F = \frac{k_{Fluor}}{k_{Fluor} + k_{Diss}} \tag{4}$$

At room temperature energy transfer between two coupled pigments occurs from the donor molecule to the acceptor with a rate constant k_{12} but also backwards from the acceptor to the donor with a rate constant k_{21} . In that case the fluorescence decay has to be calculated from the solution of a system of coupled linear differential equations 1st order (one equation for each pigment in an energy transfer chain of an arbitrary number of coupled molecules):

$$\frac{d\langle N_1 \rangle}{dt} = -\left(k_{Fluor(1)} + k_{12}\right)\langle N_1 \rangle + k_{21}\langle N_2 \rangle$$

$$\frac{d\langle N_2 \rangle}{dt} = -\left(k_{Fluor(2)} + k_{21}\right)\langle N_2 \rangle + k_{12}\langle N_1 \rangle$$
(5)

This system is solved by a sum of two exponential functions with different time constants. The decay of the fluorescence is no longer monoexponential or monophasic but it is biphasic:

$$\langle N_1(t) \rangle = A_1 e^{-\frac{t}{\tau_1}} + A_2 e^{-\frac{t}{\tau_2}} \qquad \langle N_2(t) \rangle = B_1 e^{-\frac{t}{\tau_1}} + B_2 e^{-\frac{t}{\tau_2}}$$
(6)

So the time dependency of the fluorescence from both pigments is given by putting the solution (6) in eq. (3). The measured fluorescence signal can be analysed by matching two exponential functions to the measured data and the best fit delivers the time constants τ_1 and τ_2 . If more than two pigments are coupled the system becomes more complex (multiphasic decays) [1].



Time resolved fluorescence spectrometry

Figure 3: Colour intensity plot of a time- and wavelength resolved fluorescence measurement and the decay of fluorescence at 661 nm and 725 nm.

Time resolved fluorescence spectrometry can be carried out by direct monitoring of the fluorescence signal with a fast photodiode and an oscilloscope. In that case a strong signal is necessary to reach a high signal to noise ratio.

Modern techniques use single photon counting for time resolved spectrometry. In that case averaged only one photon during 100 excitation processes is detected. Comparing the time difference between the excitation pulse and this first incoming photon the fluorescence decay can be resolved with high resolution if enough (at least several hundred thousands) photons are collected. This technique needs pulsed laser systems with high excitation repetition rates in the MHz area to reach the final number of detected photons in an accurate time (of several minutes or hours). Using time- and wavelength-resolved

fluorescence spectrometry one gets the time dependency of the whole fluorescence spectrum. **Figure 3** shows the original data which is collected in a 2-dim plot of the intensity (pictured by the colour) in dependency of wavelength and time. The analysis of this data shows the time constants and the wavelength dependent initial amplitudes of the resolved four exponential components which are shown in the form of "Decay associated spectra" by **Figure 4**.





Non-invasive investigation of photosynthesis: Acaryochloris marina (A.marina)

The time resolved fluorescence spectrometry is widely used for monitoring the energy transfer processes in photosynthesis. In Optical Institute of TU Berlin actually an interesting cyanobacterium called *A.marina* is investigated that rose strong attention of the scientific community after the discovery in 1996 [2]. *A.marina* is the only known organism containing chlorophyll *d* as the mayor pigment in the membrane intrinsic antenna system. The energetic state of excited Chl *d* is lower than Chl *a* or Chl *b* which are mostly found in green plants. So *A.marina* absorbs light in the near infrared region which is not useful for other organisms. The mechanism of water oxidation process with the lower energy of Chl *d* remains still unclear. The data of **Figure 3** and **Figure 4** are measurements performed on *A.marina*.

The described method of time resolved fluorescence spectrometry showed, that the energy which is absorbed by additional pigments called phycobiliproteins (PBP) is transferred to Chl *d* with a time constant of about 60 ps (denoted as 64 ps component in **Figure 4**), much faster than the energy transfer from PBP to Chl *a* in other Cyanobacteria like Synechococcus [3].

In **Figure 4** the energy transfer appears as a fast fluorescence decay component in the emission band of PBP (640 - 680 nm) and a corresponding rise-term (i.e. a fluorescence component with a negative amplitude) at the wavelength of ChI *d* emission (725 nm) [4].

Non-invasive detection of Aflatoxin fluorescence

The detection of fluorescence signals without the need of a high time resolution is more easy in general. For an example aflatoxin pigments show high autofluorescence suggesting the use of such fluorescence signals for non-invasive aflatoxin detection.

If several laser systems can be used for excitation the wavelength dependency of the broad fluorescence signal itself, the dependency of the excitation wavelength and also the time constant of the fluorescence decay can be used for specific detection, for an example of aflatoxins, in contamination control processes.

Figure 5 shows the colour intensity plot of a measurement on mould cells. Mould shows strong fluorescence signals around 670 nm and 720 nm (**Figure 6**) which are easy to detect also in low concentrations. These two emission maxima indicate the presence of two different fluorophores.



Figure 5: Colour intensity plot of a measurement on mould.



Figure 6: time integrated fluorescence spectrum of Figure 5.

Effect of toxic substances on photosynthesis

While the detection of aflatoxin fluorescence is performed by measuring the spectral dependency of fluorescence intensity, the time resolved fluorescence spectrometry can be used to study the effect of toxic substances on photosynthesis like the effect of the inhibitor 3-(3',4'-Dichlorphenyl)-1,1 -Dimethylharnstoff (DCMU). DCMU blocks the reoxi dation of reduced plastoquinone (Q_A^-), which is the secondary electron acceptor in the reaction center of photosynthetic organisms.



Figure 7: Effect of DCMU on the fluorescence dynamics of A.marina at 725 nm.

For that reason the energy transfer chain is blocked and the rate constant for photochemical energy conversion vanishes (the reaction centers are "closed") According to eq. (4) this will result in a rise of the fluorescence quantum yield when charge accumulates on Q_A during illumination of the inhibited organism.

The time resolved spectrometry (**Figure 7**) shows a much longer decay time of the chlorophyll fluorescence if the reaction centers are closed.

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Recent Hardware Developments Regarding Optical Technologies

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Introduction

Diode lasers are widely used compact and robust light sources for different purposes. Beside high-power applications in material processing (welding, soldering, etc.) and as pump sources for other lasers, they are also used in medical and sensor applications. The latter includes the direct application of these devices as well as the use of diode lasers as pump sources for other more complex laser systems.

Different semiconductor materials allow to manufacture laser devices from the ultraviolet spectral range [1] up to the mid-infrared [2]. The devices are based on compound semiconductors of the type $A_{III}B_V$ (GaN, AIN, GaAs, GaP, InP, etc.) for the short wavelength range or $A_{VI}B_{VI}$ (PbS, PbSe, PbTe, SnTe, etc.) for the mid-infrared. The use of ternary mixtures, e.g. $In_{1-x}Ga_xP$, GaAs_{1-x}P_x, PbS_{1-x}Se_x, allows to adjust the wavelength of the devices to requested values.

Gaps in spectral coverage can be filled by applying non-linear processes, like second harmonic generation (SHG), sum frequency generation (SFG) or difference frequency generation (DFG).

Diode lasers can be ideally used as narrow-linewidth fixed frequency light sources for the excitation of fluorescence or Raman scattering. Due to their tuneability over a range of more than 10 nm simply by changing temperature and current, they can ideally be used in absorption or photo-acoustic spectroscopy.

In this paper, a short overview of diode lasers for spectroscopic applications in the VISand NIR-range will be given. This includes the presentation of the properties of narrow linewidth DFB-light sources at an output power up to 500 mW, hybrid narrow linewidth high-power devices with a maximum output power of 7 W, and light sources using nonlinear frequency conversion with diode lasers as pump sources.

Narrow-linewidth light sources – RW-DFB lasers

In our institute, monolithic narrow-linewidth light sources are manufactured in the spectral range between 760 nm and 1100 nm. Wavelengths of special interest are:

- 760 nm 765 nm: absorption spectroscopy of oxygen
- 780 nm: atomic clocks ⁸⁷Rb
- 785 nm: excitation sources for Raman spectroscopy
- 852 nm: atomic clocks ¹³³Cs
- 860 nm: SHG for digital film exposure at 430 nm
- 920 nm: SHG for laser display technology at 460 nm (blue)
- 935 945 nm: absorption spectroscopy, remote sensing of H₂O vapour
- 976 nm: SHG for replacement of Ar⁺-Laser at 488 nm
- 1060 nm: SHG for laser display technology at 530 nm (green)



Figure 1: Schematic view of a high power DFB laser.



Figure 2: Power-current characteristic of a 940 nm DFB-laser at $T = 20^{\circ}$ C. The dips in the curve are originated by absorption lines of water vapour.



Figure 3: Water vapour spectra measured with the 940 nm DFB-laser in comparison to the well-known spectroscopic data from the HITRAN-database.

The frequency selective element in these structures is an internal grating. To obtain high power fundamental lateral mode operation the grating has to be integrated into the epitaxial layer structure, which requires a two-step epitaxy.

A schematic view of a DFB diode laser is presented in **Figure 1**. The layer structure was fabricated with metal organic vapour phase epitaxy (MOVPE). The internal grating was manufactured by holographic lithography and wet-chemical etching [3]. The laser length *L* is typically 1.5 mm. The reflectivity of the front facet is $R_{\rm F} \approx 10^{-3}$, the rear facet reflectivity $R_{\rm R} \approx 0.95$.

In **Figure 2** a power current characteristics of a 940 nm DFB-laser at $T = 20^{\circ}$ C is shown. The threshold current I_{th} is about 35 mA, the slope efficiency S = 0.9 W/A. The maximum output power is 500 mW. In the curve pronounced dips are observable. These dips are due to water vapour absorption in the measurement path.

The same measurements were performed at 20°C and 50°C. From the data with and without absorption the water vapour spectrum can be calculated using Lambert-Beerslaw. In **Figure 3**, the obtained spectra were compared with the data from the HITRAN database [4]. The agreement between the database and the measured spectra is excellent. It illustrates that at one selected temperature a continuous current tuning over 5 nm is possible.

DFB-lasers suitable for absorption spectroscopy were also manufactured for 780 nm ($P_{max} = 300 \text{ mW}$) [5], 850 nm [6], 860 nm ($P_{max} = 400 \text{ mW}$) [7], and 980 nm ($P_{max} = 900 \text{ mW}$) [8].

High-power narrow-linewidth diode lasers with an output power larger than 150 mW at λ = 785 nm [9] meet the requirements for Raman spectroscopy. A typical power current characteristic for such a diode is shown in Figure 4. This laser was used for in situ surface enhanced Raman spectroscopy (SERS) of polycyclic aromatic hydrocarbons [10]. Typical SER- spectra for pyrene are shown in Figure 5. With a field operable NIR-Raman setup, it was possible to detect 1 nmol/l of this substance in artificial seawater.



Figure 4: Power-current characteristic of a 785 nm DFB-laser at T = 35°C. The wavelength at 150 mW is 785 nm as necessary for common Raman-set-ups.

Figure 5: SER spectra of pyrene in seawater measured with the DFB laser emitting at 785 nm with 25 mW at sample and an integration time of 10 s.

1626

1592

1600

The maximal output powers of the investigated RW-DFB-laser in the spectral range from 750 nm up to 1080 nm fulfil the demands for the different spectroscopic applications.

High-power light sources

Some applications require not only narrow spectral linewidth but also higher output powers in the Watt-range. One interesting medical application is for example the lung diagnostics using polarized helium. This requires laser sources emitting at about 1083 nm with a spectral linewidth matched to the Doppler-width of the low pressure Hegas, i.e. 0.01 nm, a good beam guality, and an output power of 4 W. DFB-laser are limited to about 500 mW output power. Therefore a hybrid master-oscillator poweramplifier (MOPA) system was developed. The master oscillator, e.g. a DBR-laser was tuned to the desired seed wavelength by varying temperature or current. As master oscillator a highly anti-reflection coated tapered devices was used. A scheme of the set-up is shown in **Figure 6**.



Figure 6: Scheme of the experimental set-up of the MOPA system; L_1 , L_2 – lenses; FI – Faraday isolator.

The master-oscillator was a DBR laser with a total length of 2 mm. The lengths of the individual sections are 1000 µm, 500 µm, and 500 µm (gain, phase, DBR). The frontand back facets are coated to 10% and 0% reflectivity, respectively. The wavelength of $\lambda = 1083$ nm was obtained at T = 40°C. The threshold current is $I_{th} = 42$ mA and the slope efficiency is S = 0.5 W/A. The beam of the DBR-laser was focussed via two lenses into the power amplifier (PA). To prevent back-reflections, a 60 db Faraday isolator was inserted between the two lenses. A seed power of 36 mW was used in the MOPA experiment.

The power amplifier (PA) was a 4 mm long anti-reflection coated tapered laser. The power-amplifier-current characteristic was measured at $T = 20^{\circ}$ C. The result is shown in **Figure 7** a maximum output power of 7.3 W was measured.



Figure 7: Power-current characteristic of the 1083 nm MOPA system. $P_{seed} = 36 \text{ mW}, T = 20^{\circ}\text{C}.$

Figure 8: Spectrum at $T = 20^{\circ}C$ and P = 7 W.

At P = 7 W the side-mode suppression rate is about 42 dB and herewith comparable to the features of the DBR-laser. The beam quality was measured up to an output power

of about 4 W [11]. The output beam of the MOPA was nearly diffraction-limited with a beam propagation ratio $M^2 = 2$. Together with the reached output power the spectral features and the beam quality fulfil the demands of the requested application.

Beside this rather large system developments towards a compact micro-bench design are carried out. In a first experiment the two lenses and the Faraday isolator were replaced by a single GRIN-lens. In this compact set-up an output power of 3.1 W was reached [12]. Such a MOPA will be placed on a compact holder with the dimensions 25 x 25 mm².

Light sources based on non-linear optics



Figure 9: Setup of the SHG-experiment:

1) 976 nm DFB-RW laser, $P_{seed} = 40 \text{ mW}$; 2) Lens L1;

3) Optical isolator; 4) Lens L2; 5) Tapered amplifier – 4 mm long; 6) Lens L3; 7) Lens L4;

8) Lens L5; 9) PPMgLN bulk crystal;10) Short pass filter.





Beside the direct application, diode lasers are also interesting as pump sources for nonlinear processes. Using RW-DFB lasers as light sources the SHG in bulk material [13] or wave-guide crystals [14] is known. The output power in these applications is limited to about 100 mW. Higher output power can be reached by using high power MOPAsystems for the excitation and of periodically poled material (e.g. lithium niobate) for the frequency conversion.

The single-path-set-up used in our experiment is shown in **Figure 9**. At a temperature of 10°C the 976 nm MOPA reached output power of about 3.6 W. This light was focussed into the periodically poled MgO:LiNbO₃ (PPMgLN) crystal. The dependence of the 488 nm output power from the 976 nm pump power is shown in **Figure 10**. At a pump power of 4 W a maximum output power of 600 mW at 488 nm was achieved with a conversion efficiency of 15 % and an overall wall-plug efficiency of 4 %. This is to the best of our knowledge the highest output power obtained with a single path SHG in a bulk material using a diode laser as excitation source [15]. The reached output power would allow to replace an Argon-ion laser by this laser.

Beside the spectroscopic application in the VIS- and NIR-range, also measurements in the mid-infrared region, where the majority of substances have characteristic spectroscopic features are interesting. Unfortunately, semiconductor lasers (lead salt lasers, quantum cascade lasers) often require cryogenic cooling. Therefore the use of difference frequency generation using VIS- or NIR-diode lasers as pump sources is also a promising approach.

The setup of an all diode laser pumped DFG-system for the monitoring of carbon monoxide at 4.7 μ m is shown in **Figure 11**. An 810 nm diode laser with 150 mW output power and a 690 nm diode laser with 30 mW was combined by a dichroitic beam combiner and focussed into a 4 cm long AgGaS₂ crystal [16]. This system was used for the detection of carbon monoxide at ppb-level [17]. A result for such a measurement is given in **Figure 12**.



Figure 11: Setup of the difference frequency spectrometer DM: dielectric mirror OAP: off-axis parabolic mirror Ge: Germanium filter

BS: beam splitter.





volume of traffic.

Conclusions

Diode lasers are compact, stable, and powerful tools for several spectroscopic applications. They can act as fixed-frequency excitation sources for fluorescence and Raman set-ups and as tuneable light sources in absorption spectroscopy. With output powers larger than 100 mW, they also can be used as pump sources for non-linear processes like SHG or DFG.

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Fruit Quality Sensing by Means of Time-Resolved Reflectance Spectroscopy

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Introduction

Time-resolved reflectance spectroscopy (TRS) is a non-destructive method for optical characterisation of highly diffusive media. It has recently gained increasing use in bio-medicine for the non-invasive investigation of biological tissues [16]. Similarly, it has been used for optical characterisation of fruit [3,4]. Main features of TRS as regards application in fruit have been described by Cubeddu and co-workers [2].

Why time-resolved methods

In the visible region of the spectrum, colorimetry has been used to determine the colour of the skin of peaches and, in the near infrared region, the spectrum of re-emitted light has been studied mainly to estimate the total sugar content. Referring to the optical technique, a key limitation is that the total reflected intensity yields the attenuation coefficient $\mu_t = \mu_a + \mu_s$ representing the photon loss due to absorption and to photons scattered into different directions than the one of observation, without separating the effects of these properties. Absorption and scattering contain distinct information on the medium. Absorption is determined by the pigments and constituents of the pulp, while scattering is due to cellular structure (cell walls, membranes, air, vacuoles, etc.). The effect of scattering can be properly taken into account by direct measurements of photon pathlength, which is directly related to time of flight in the medium: so the natural choice is to perform time-resolved measurements.

Principles of time-resolved reflectance

Consider the injection of a short pulse of monochromatic light within a diffusive medium. By using a simplified description we can regard the medium as consisting of scattering centres and absorbing centres, and the light pulse as a stream of particles, called photons. Whenever a photon strikes a scattering centre it changes its trajectory and keeps on propagating in the medium, until it is eventually re-emitted across the boundary, or it is definitely captured by an absorbing centre (see **Figure 1a**). The characteristic parameters of light propagation within the diffusive medium are the scattering coefficient $\mu_s = 1/L_s \text{ (cm}^{-1})$ and the absorption coefficient $\mu_a = 1/L_a \text{ (cm}^{-1})$ which indicate the scattering probability per unit length and the absorption probability per unit length, respectively. To account for non isotropic propagation of photons the reduced scattering coefficient $\mu_s' = (1-g) \mu_s$ is commonly used, where g is the anisotropy factor, i.e. the mean cosine of the scattering angle. Following the injection of the light pulse into a turbid medium, the temporal distribution of the re-emitted photons at a distance ρ from the injection point will be delayed, broadened, and attenuated. The volume probed by a TRS measurement is a "banana shaped" region connecting the injection and collection points (see **Figure 1b**) [10]. The photon paths are more densely packed in the banana region but can be distributed in the whole medium.



Figure 1: (a) Photon migration in turbid media: photon paths, scattering (o) and absorbing (•) centres. (b) The banana shaped region.

Materials and methods

Instrumentation for TRS

Photon migration measurements in the time domain rely on the ability to extract the information encoded in the temporal distribution of the remitted light, following the injection of a short monochromatic pulse in a diffusive medium. The two key points in the designing of a system for time-resolved measurements are temporal resolution (events in the range 1-10 ns) and high sensitivity (ratio of detected to injected power is about – 80 dB).

Two different systems for time-resolved reflectance measurements based on the timecorrelated single photon counting technique (TCSPC) [1] have been developed at the Politecnico di Milano - Department of Physics. The first one is a fully automated laboratory set-up for broadband time-resolved reflectance measurements in the spectral range 600-1000 nm. The second system is a portable prototype for time-resolved reflectance measurements at discrete wavelengths. A detailed description of the two systems can be found in [3, 4]. Results from the two instruments will be presented in the following paragraphs. The time required for one TRS measurement is now 1 s with the portable prototype, but the technique could be adapted for on-line measurement, reducing acquisition time to 10 ms without loss of accuracy.

While laser sources, photo-detectors and electronics for TCSPC can be separately bought from different dealers, to our knowledge no commercial systems for TRS measurements are nowadays available on the market.

Data analysis for TRS

The temporal profile of the time-resolved reflectance curve is analysed using a solution of the Radiative Transport Equation under the Diffusion approximation for a semi-infinite homogeneous medium [11, 14]. This allows for the simultaneous estimate of μ_a and μ_s '. A detailed description of the fitting procedure can be found in [3].

Results of TRS measurements of fruit

Effect of skin on time-resolved reflectance measurements

Measurements were performed on apples (Golden Delicious, Granny Smith, and Starking Delicious), peaches, nectarines, kiwifruit, and melons. The peeling of the skin did not alter markedly the results, at least for fruits with thin skin, confirming that TRS is sensitive mostly to the internal features [2].

Penetration depth of time-resolved reflectance measurements

A series of measurements were performed on a Starking Delicious apple where slices of pulp were cut from the side opposite to the measurement site. Spectra were taken on the whole apple, and then slices were removed to yield a total thickness of 4.1, 2.7, 2.1, and 1.5 cm. Both the absorption and the scattering coefficients were unchanged down to a thickness of 2.7 cm. Overall, the data show that the TRS measurement is probing a depth of at least 2 cm in the pulp. Of course this is a rough estimate, yet it confirms that the TRS measurement is not confined to the surface of the fruit. Moreover, the penetration depth can be somehow dependent on the optical properties, and we expect deeper penetration in less absorbing and/or scattering fruit [2].

Optical properties of fruits and vegetables

Measurements were performed on different fruit (apple Starking Delicious, yellow peach, tomato and kiwifruit).

Absorption and tissue components

Typical absorption spectra are reported in **Figure 2** (**a**). The absorption spectrum of the apple is dominated by the water peak, centred around 970 nm, with an absolute value of about 0.4 cm⁻¹. Minor absorption features of water are usually detected around 740 and 835 nm, where the absorption coefficient is low (0.05 cm^{-1}) . A significant absorption peak $(0.12-0.18 \text{ cm}^{-1})$ at 675 nm, corresponding to chlorophyll-a, is found. Absorption spectra of peach and tomato are quite similar to those of apples. However, for kiwifruit, chlorophyll-a absorption is considerable, with a maximum value up to 2 or 3 times the water maximum in the infrared. Information on the water content can be obtained by considering the absolute values of the absorption at 970 nm. In agreement with the different water/solids ratio in distinct species, a higher absorption was detected in tomatoes (~0.5 cm⁻¹), than in peaches and kiwifruits (~0.45 cm⁻¹), and in apples (~0.4 cm⁻¹). By fitting the absorption spectrum of an apple with the line shape of water and chlorophyll-a, the percentage volume of water and the chlorophyll-a content in the fruit flesh could be estimated [2].



Figure 2: Absorption (a) and scattering (b) spectra of apple, peach, tomato and kiwifruit.

Scattering and tissue structure

The value of the reduced scattering coefficient decreased progressively with increasing wavelength (**Figure 2.b**) The transport scattering spectrum of the kiwifruit was noisier than the spectrum of other fruits, particularly in the 675 nm region where the high absorption of chlorophyll reduced the accuracy of the evaluation of transport scattering. Even though marked variations in the absolute values were noticed depending on variety and ripeness, kiwifruits and tomatoes are usually characterised by a lower scattering than other species. Further information could be obtained by interpreting the transport scattering spectra with Mie theory [3]. For a homogeneous sphere of radius r, Mie the-

ory predicts the wavelength dependence of the scattering and the relation between scattering and sphere size. Under the hypothesis that the scattering centers are homogeneous spheres behaving individually, the relationship between μ'_s and wavelength (λ) can be empirically described as $\mu'_s = a \lambda^b$ where *a* and *b* are free parameters. In particular, *a* is proportional to the density of the scattering centers, and *b* depends on their size [13].

Scattering and pectin content in apples

Pectin content, firmness, internal space volume and scattering at 630 and 750 nm were analyzed during storage and shelf life in Jonagored apples. Scattering at 750 nm (μ'_s 750) was correlated to internal air space volume and to firmness and pectin composition: at harvest, μ'_s 750 was directly related to water soluble pectin (W) and inversely to oxalate fraction (O) and residual insoluble pectin (R); after storage it was inversely related to firmness, R and protopectin index (R/W+O) [15]. Further studies will explore the possibility of using scattering coefficient for non destructive estimation of firmness.

Brown Heart in Conference pears

Brown Heart (BH) is an internal disorder sometimes shown by pears during controlled atmosphere (CA) storage. The symptoms are in no way recognizable from the outside of the fruit and are visible only after cutting the fruit. 'Conference' pear fruits were measured after storage with TRS at 690 nm and 720 nm on 8 points around the equator. The absorption coefficient μ_a both at 690 and at 720 nm discriminated between sound and brown heart affected tissue in the intact fruit, while the development of over ripening and of bruises (translucent tissue) was detected by the scattering coefficient μ_s ' [5]. An example is reported in **Figure 3**, where the TRS measurements in the intact fruit are compared with the photograph of the equatorial section of a pear.



Figure 3: Absorption coefficient (middle column) and reduced scattering coefficient (right) at 690 nm as a function of the position around the equator of an intact pear. Reported measurements were performed at the end of storage (black diamond) and at the end of shelf life (grey diamond). After cutting the fruit, the photograph of the equatorial section of the fruit (left column) shows that the pear was partially affected by brown heart (upper left) and by a bruise (on the right), without any visible symptoms outside. Units for absorption and scattering are cm⁻¹.

Chlorophyll absorption as a maturity index

Chlorophyll absorbs light with a peak at 672 nm. The use of absorption at a wavelength near the chlorophyll peak (between 630 and 690 nm) as a maturity index has been checked in different fruit species, by measuring μ_a at harvest and then checking fruit quality after storage and shelf life.

Apples

Jonagored apples were harvested at two dates and classified at harvest by μ_a at 630 nm (μ_a 630). The absorption coefficient μ_a 630 was significantly higher in first harvest apples, indicating a less mature fruit. Apples with higher μ_a 630 had lower fruit mass and lower per cent blush. Fruits classified as more mature by TRS had less titratable acidity at harvest and more soluble solids after storage; at sensory analyses these fruits were significantly sweeter, more aromatic and pleasant. However flesh firmness was not affected by maturity classification [15].

Pears

Abbé Fétel pears were classified at harvest by $\mu_a 690$. At harvest, fruit with high $\mu_a 690$ (i.e. less mature) had lower mass, soluble solids and titratable acidity, whereas skin colour (hue), starch and firmness were not different between the maturity levels. Significant correlations were found between $\mu_a 690$ and soluble solids (r=0.72), acidity (r=0.49) and mass (r=0.54) (n= 30 fruits) [7].

Nectarines

Nectarine fruit of two sizes (A and B) were classified at harvest by μ_a670 , then firmness and other quality parameters were measured during shelf life. On the average fruit mass was significantly lower in fruit with higher μ_a670 . Smaller size (B) fruit had a significantly higher μ_a670 than larger size (A) fruit. In fruit with lower μ_a670 at harvest (i.e. more mature), the soluble solids content and per cent blush was higher, firmness and acidity lower than in fruit with high μ_a670 . As regards sensory attributes, more mature fruit (i.e. with lower μ_a670 at harvest) were perceived significantly less firm and more juicy, sweet, pulpy and aromatic and were more appreciated by the assessors [6]. Sugar and acids composition was also affected by μ_a670 : total sugars, per cent sucrose, per cent malic and quinic acid increased, while per cent fructose and sorbitol, total acids and especially citric acid decreased with decreasing μ_a670 , i.e. with increasing maturity at harvest [12].

Perhaps the most interesting result obtained so far from the use of $\mu_a 670$ for the evaluation of maturity, is its predictive power as regards softening of nectarine fruit. Despite firmness is a destructive measurement, by selecting at harvest fruit of the same maturity level, it was possible to follow softening in different fruit of the same maturity [8,9]. Softening occurred earlier in more mature fruit (lower $\mu_a 670$ at harvest) and later in less mature fruit (higher $\mu_a 670$ at harvest), with the same sigmoid pattern in time. Softening of nectarine fruit during the shelf life at 20°C was fitted by a logistic model with $\mu_a 670$ and time as independent variables, which explained 85 % (cv 'Spring Bright') and 75 % (cv 'Ambra') of the variation in fruit firmness. It is remarkable that the parameters of the model were practically the same for the same cultivar in two seasons despite different climatic conditions; also the differences between cultivars were limited. Through this model, it is possible to predict the onset of softening and the softening rate at 20°C of nectarine fruit, if their $\mu_a 670$ is measured at harvest.

Conclusion

Compared with other optical techniques, TRS has the merit of measuring well-defined, physically based properties. Moreover, TRS explores a volume within the fruit, without being affected by surface properties. By using the coefficient of absorption μ_a at a wavelength near 670 nm, the maturity of individual fruit can be measured, which is one of the main determinants of the postharvest quality. The maturity level can be discriminated by TRS even if fruit are extensively covered by intense blush. Scattering is related to internal structure, defects, firmness and pectin composition. Absorption at 720 nm is related to the presence of Brown Heart in pears. TRS technique, by means of the absorption and scattering coefficients μ_s ', allows a description of the virtual appearance of the internal tissue in the intact fruit to a depth of about 2 cm, of the presence of defects and of their position inside the fruit, as it can be visually assessed only after cutting the fruit.

Possible applications of this non-destructive technique for commercial purposes that until now can be envisaged are: a) to detect internal defects in fruit, even following up their formation; b) to grade fruit according to maturity, predicting their potential shelf life as in the case of nectarines. For on-line grading of fruits, some aspects still need to be studied (non-contact measurement, number of points, etc.), but a great deal of potential applications can be envisaged for this technique when a simpler instrumentation will be available.

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A Non-Invasive Optical Method for the Determination of the Carotenoids Beta-Carotene and Lycopene in Foodstuffs

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Abstract

Resonance Raman spectroscopy was used for the fast in vivo determination of carotenoid antioxidant substances in foodstuffs and their dynamics subsequent to external influences. Carotenoids of the yolk of eggs, peel and pulp of potatoes, bananas, apricots, plums, yellow and red peaches and nectarines were measured.

It was shown that the peel and pulp of fruits and vegetables contain different amounts of carotenoids, are dependent on the type of measurement.

Potato peel provides a defense effect on the carotenoids of the pulp during boiling. The same tendency can be expected with the other fruits and vegetables.

The yolks of "simple" and "bio" eggs were investigated. It was found that "bio" yolk has approx. twice the level of carotenoids compared with the "simple" yolk.

Introduction

Carotenoids are natural antioxidant substances, which effectively neutralize free radicals and other reactive species [1]. The production of free radicals and other reactive species in the living organism is a natural and essential component of cellular metabolism. These molecules have unpaired electrons that give rise to their high oxidative activity. Approximately 3 % of oxygen used in metabolic processes in the mitochondria is converted to oxygen free radicals. Part of them act in the same way as biological weapons against viruses, bacteria and cancer cells, while the remaining part can damage the DNA, proteins and lipids of the living cells and disintegrate them [2-8].

If the number of radicals formed in the tissue is significantly increased, the defense mechanism of the organism will be overloaded and is not able to neutralize all these reactive molecules. In this case, chain reactions start, which again form high amounts of free radicals [3, 7]. This is the case if the living organism is irradiated with a high dose of UV light or contacted with environmental hazards. The action of the free radicals causes

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oxidative cell damage [4, 5], which can be the main reason for aging [2, 3] and even for the formation of different types of cancer [6-8].

Biological organisms and plants have developed natural defense mechanisms against the destructive action of free radicals and other reactive species, which act effectively and are based on the action of antioxidant substances such as enzymes (superoxide dismutase, catalase, glutathione peroxidase, etc.), vitamins (A, C, E, etc.), carotenoids (beta-carotene, lycopene, lutein/zeaxanthin, beta-cryptoxanthin, etc.) and others (flavonoids, lipoic acid, uric acid, Q10, etc.), which form an antioxidant network of living organisms. Most of the antioxidants in the human organism have to be uptaken from food.

Nature uses carotenoids as effective antioxidants for the protection of living organisms and plants against aggressive influences of the environment. Carotenoids can be found not only in fruits and vegetables, but also in eggs and some types of meat.

The standard technique for measuring carotenoid antioxidant substances is highpressure liquid chromatography (HPLC).

For the fast in vivo measurements of the carotenoid relative concentrations in foodstuffs (fruits and vegetables), we have used a portable laser setup, which operates using the resonance Raman spectroscopy method.

Materials and methods

We have used resonance Raman spectroscopy, as a fast and non-invasive optical method to measure carotenoid antioxidant substances in foodstuffs (fruits and vegetables were measured) [9].

Our device was calibrated for the determination of beta-carotene and lycopene antioxidant substances in the skin [10]. Fruits and vegetables possess different optical parameters, compared with each other and with the skin. In this case, it is impossible to make a quantitative comparison between them. Moreover, we cannot separate carotenoids from each other and can only measure the total carotenoid content in the foodstuffs. The pulp and peel of fruits and vegetables were measured.

Carotenoid antioxidant substances have approximately the same absorption spectra in the blue visible light region (**Figure 1**). As a result, they have the same resonance Raman activity in this spectral region.

A typical spectrum obtained from fruits/vegetables with an excitation wavelength at 488 nm is presented in **Figure 2**. The three Raman bands of the carotenoids can be well recognized and evaluated on a fluorescence background.



Figure 1: Absorption spectra of solutions of beta-carotene (solid line) and lycopene (dotted line) carotenoid antioxidant substances in ethanol.



Figure 2: Typical Raman spectrum obtained from fruits or vegetables with an excitation wavelength at 488 nm.

The Raman bands at 1005 cm⁻¹, 1156 cm⁻¹ and 1523 cm⁻¹ originate, respectively, from the rocking motions of the methyl groups, from carbon-carbon single bond and carbon-carbon double-bond stretch vibrations of the conjugated backbone of carotenoid molecules.

The strong 1523 cm⁻¹ carbon-carbon double-bond stretch vibration Raman line, corresponding to the 488 nm excitation, were used for the detection of carotenoids relative concentration in the fruits/vegetables.

Carotenoids of eggs, peel and pulp of potatoes, bananas, apricots, plums, yellow and red peaches and nectarines were measured.

Experimental arrangement

The radiation of an Ar^+ multiline laser (1), which operated at 488 nm is focused into an optical fiber (2). This fiber is connected to an optical imaging system (3) where the light is filtered and focused onto the measured subject. The Raman signal from the measured subject is collected by a lens system and transferred into a fiber bundle (4), which is connected to a spectrograph (5). The spectrum is recorded by a CCD camera (6) and transferred to a PC (7). The experimental arrangement for the measurements of carotenoid antioxidant substances is presented in **Figure 3**.



Figure 3: Diagram of the experimental setup: (1) Ar⁺ multiline laser, (2), (4) optical fibers, (3) optical imaging system, (5) spectrograph, (6) CCD camera, (7) personal computer.





Figure 4: Relative content of carotenoid antioxidant substances in various fruits.



Figure 5: Dynamic of degradation of the total content of carotenoids in the peel and pulp of potatoes during boiling.

Figure 4 shows the relative concentration of carotenoid antioxidant substances in the peel and pulp of various fruits. As can be seen, all of them contain carotenoid antioxidant substances at different concentrations.

Figure 5 indicates the dynamic of degradation of the total content of carotenoids in the peel and pulp of potatoes during boiling. It is well known that high temperatures decrease the carotenoid content. Surprisingly, that potato peel provides a defense effect on the pulp carotenoids. The same tendency can be expected with the other fruits and vegetables.



Figure 6: Differences between carotenoid contents in "bio" and "simple" egg yolks.

The yolk of egg contains many carotenoid antioxidant substances. Most of all, lutein/zeaxanthin. Carotenoids of the "simple" and "bio" egg yolks were investigated. It was found that "bio" yolk has approx. twice the level of carotenoids compared with the "simple" yolk (**Figure 6**). This behavior is understandable, because "bio" hens have become naturally rich in carotenoids on account of their feed. In this case, the carotenoid accumulation in the organism of the hen occurs, which gives rise to an increase in the carotenoid levels of the eggs.

Conclusions

The standard technique for measuring carotenoid antioxidant substances is highpressure liquid chromatography (HPLC) [11]. The majority of the authors have used the HPLC method for determination of carotenoids in foodstuffs, in biological liquids and tissues.

For the fast in vivo measurements of the carotenoid relative concentrations in foodstuffs (eggs, fruits and vegetables), we have used a portable laser setup, which operates using the resonance Raman spectroscopy method [9, 10].

One of the main purposes of the food industry is the preservation of the natural level of nutrients, such as carotenoids, after the heating and chemical treatments. In this case, the fast and non-invasive methods of measuring carotenoids are effective and up to date.

The Raman spectroscopic method, as a fast non-invasive method, is well suited for the quantitative determination of carotenoid antioxidant substances in foodstuffs.

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Introduction

Analytical procedures can be classified in four types. These are identification tests, guantitative tests for impurities' content, limit tests for the control of impurities and guantitative tests to measure one or several analytes [1]. In a wider sense, the target of an analytical procedure can be a substance-based parameter, like aroma, odour, freshness or ripeness of organic materials. Nevertheless, the results of analytical measurements have to be true, precise, repeatable, reproducible, traceable and documented. Therefore it is necessary in the framework of a quality management system to perform calibration and validation, to use standards, reference methods and materials, to determine uncertainties and make documentations. This is well accepted for routine procedure, but has an increasing importance for research and development of new methods, too. New analytical methods were required to detect new substances, compounds and quality parameters or to improve existing analytical methods and devices. The new method should be more accurate, precise, sensitive or specific. Also further aspects, like easier use, more economic, non-destructive or environmental friendly, are of importance [2]. The aim of the paper is to introduce to quality assurance, validation and certification procedures as supporting tools for the development of valid and accepted new analytical methods.

Quality assurance system

Quality assurance contains all the planned and systematic actions implemented within the quality system to provide adequate confidence that an entity will fulfil requirements for quality [3]. An extensive number of guidelines and standards were published in order to define, explain and describe all actions. The efforts aim finally to establish confidence of the customers [4-6]. Quality activities can be discussed in a shell model (see **Figure 1**).



Figure 1: Nested structure of quality activities [7].

The organisational quality elements refer to the quality policy of the organisation, the quality management and the administrative and technical planning of work, staff qualifications, training and supervising, monitoring of the quality system. It concerns the procedures of record keeping and document control. The technical quality elements relate to the technical capabilities of a laboratory, policy and procedures for instrument calibration and performance checks, use of calibrants and statistical procedures. It involves the methods of method validation and determination of measurement uncertainties. The analytical task quality elements represents activities in particular projects and includes planning, control and reporting during and at completion of R&D work. The research plan defines goals, tactics, resources and time schedule. Further items are resource management, progress review, data verification, technology transfer, publication and archiving.

Calibration of analytical methods

The calibration is a set of operations which establish, under specific conditions, the relationship between values indicated by a measuring instrument, measuring system or material measure, and the corresponding known values of a measurand [3]. It is the task in quantitative chemical analysis to provide a mathematical calibration function between a signal *y* and the chemical concentration of the analyte *x*, y = F(x) (direct calibration), or an analytic function x = G(y) (inverse calibration), what has better applicability. The calibration experiment demands defined program and conditions. This concerns the concentration range of the analyte, constant ambient conditions, the mathematical type of the calibration or analytic function and the number and distribution of calibration points. A linear function is preferred because only 3 (recommended 5) calibration points were needed. The applicability of a linear function can be proofed by statistic tests [8]. A linearization can be achieved by a transformation procedure of the analyte or signal data. If there are several dependent and independent variables, a multivariate calibration has to be performed. Whereas for a linear calibration, samples of known concentration of the analyte (prepared by dilution or addition) were analysed, in the case of several variables one can not waive the use of statistical planed experiments.

Validation of analytical procedures

A successful validation process gives a confirmation by examination and provision of objective evidence that the particular requirement for a specified end use are fulfilled [3].

This includes the three steps of

- i. determination of relevant parameters and characteristics of the method,
- ii. comparison of the results with the requirements of the customer and
- iii. conformation of the suitability for the specific application.

The validation process has to consider the following characteristics (see **Table 1**):

Validation of empiric calibrations

The calibration function can be proofed by statistic methods. These include the test of Gaussian distribution of the data, test of linearity (if such a function was assumed), or the discussion of residues, test of homogeneity of the variances of x and y, test of outliers.

Determination of uncertainties

The uncertainty u(y) of a measurement results y is the estimated standard deviation of y. Uncertainties can be estimated according to GUM [9] in two variants from the propagation of uncertainty contributions of relevant parameters or by estimation of $u(x) \approx s_R$ based on a validation of the method. In many cases adapted mixed variants were applied.

Selectivity (Specificity¹)

The selectivity is the ability to assess unequivocally the analyte without or low disturbance by other present components. In most cases several components can influence the results or the signal of a measurement. Therefore the specificity of an analyte A in

¹ Specifity means 100% selectivity. IUPAC recommended using not the term "specifity", because no method is absolute specific.

relation to further components (B, C,..., N) in concentration x_J can be estimated by comparing the values of partial sensitivities $y_{IJ} = \frac{\partial y_I}{\partial x_J}$ of the signal *y* [10].

Range

The range of an analytical procedure is the interval of upper and lower concentration of the analyte for which has been demonstrated that the method has a suitable level of precision and accuracy [1].

Accuracy

The accuracy of an analysis is given by the trueness and the precision. The trueness is given by the systematic deviation from the true or accepted value and the precision as a measure of the scatter of the determined results by the standard deviations [11].

Precision

The precision may be considered at three levels: repeatability, intermediate precision and reproducibility can be given by variance or standard deviation [1].

The *repeatability* expresses the precision under the same operating conditions. A minimum of 9 determinations covering the concentration range of the analytical procedure were suggested.

The *intermediate precision* expresses variations of results in a laboratory due to different analysts, equipment of days.

The *reproducibility* expresses the precision between laboratories and can be accessioned by means of inter-laboratory comparisons.

Quantitation limits

The smallest detectable concentration difference can be characterised by the signal-to-

noise ratio $N = \frac{y}{\Delta y}$. A typical ratio is 10:1. The quantitation limit can be expressed as a

ration of standard deviation of the signal to the slop of the calibration curve, too. An investigation of blind samples without analyte is necessary. There were three different parameters specified, which consider statistic considerations (see **Table 1**).

parameter	explanation	mathematical approach	Ref.
uncertainty	parameter of a meas- urand which character- ises the scatter of the values	$x = F(a, b, c,) \Rightarrow$ $u(x) = \sqrt{\left[(\alpha \cdot u(a)\right]^2 + \left[\beta \cdot u(b)\right]^2 + \left[(\gamma \cdot u(c)\right]^2 +}$	[9]
precision	standard deviation from an estimated value	$s_{y,x}^{2} = \sum_{i=1}^{n} \frac{(y_{i} - y_{i})^{2}}{n-2} = \sum_{i=1}^{n} \frac{(y_{i} - a_{x} - b_{x}x)^{2}}{n-2}$	[8]
Selectivity (specifity)	measure how the sig- nal is influenced by the analyte in comparison to further components	$sel(A/B,,N) = \frac{S_{AA} \cdot x_A}{\sum_{J=A}^{N} S_{JA} \cdot x_j}$	[10]
sensitivity	slop of the calibration function	$b = \frac{\partial y}{\partial x}$	[8]
linearity	capability of a proce- dure to deliver results which are direct pro- portional to the con- centration of the ana- lyte.	$\hat{y} = a + b \cdot x$	[8]
quantitation limit	ratio of standard devia- tion of response to slop	$ql = \frac{10\sigma}{b}$	[1]
limit of detection limit (Nachweis- grenze)	smallest value of the analyte x_{NG} which could be discriminated from a blind value	$x_{NG} = s_{x,y} \cdot t_{f,\alpha} \sqrt{\frac{1}{m} + \frac{1}{n} + \frac{(\bar{x})^2}{(x_i - \bar{x})^2}}$	[12]
method detection level (Erfassungs- grenze)	$2 \cdot x_{_{NG}}$	$x_{EG} = x_{NG} + s_{x,y} \cdot t_{f,\alpha} \sqrt{\frac{1}{m} + \frac{1}{n} + \frac{(\bar{x})^2}{\sum (x_i - \bar{x})^2}}$	[12]
limit of determi- nation, quantita- tion (Bestim- mungsgrenze)	lowest amount of analyte which can quantitatively determined with a suitable precission and accuracy	$x_{BG} = k \cdot s_{x,y} \cdot t_{f,\alpha} \sqrt{\frac{1}{m} + \frac{1}{n} + \frac{(x_i - x)^2}{\sum (x_i - x)^2}}$	[12]
confidence (pre- diction) interval (Vertrauens- bzw. Vorhersa- gebereich)	interval containing the determined analyte concentrations with a certain probability (95 %)	$\pm s_{x,y} \cdot t_{f,\alpha} \sqrt{\frac{1}{m} + \frac{1}{n} + \frac{(x_i - \bar{x})^2}{\sum (x_i - \bar{x})^2}},$	[13]

Table 1: Characteristics for validation of an analytical method.

Traceability

The traceability insures the trueness and comparability of results and is based on an uninterrupted chain of comparisons (calibrations) on standards back to the National standard or to SI units. To realise traceability it needs an accepted and documented procedure performed by competent calibration laboratories [14]. An essential contribution to the validation of methods gives inter-laboratory comparisons.

Robustness

Robustness is the insensitivity of a method against deviations of working conditions and is an important parameter for capability of technical application. It can be estimated in inter-laboratory comparisons or by experiments planed by statistic methods. The estimation of precision and trueness of the method is a precondition for the determination of the robustness.

Analytical methods in application

Analytical methods were applied for the monitoring of industrial processes in order to detect deviations of the measurand. In many cases limits of variation of the process value (T - tolerance) are defined (specification and control limits) [15, 16]. It has to be checked that the applied method has a precision which permits the detection of significant deviation of the measurand (see Figure 2). For that purpose quality parameter were defined [13].





The validation will be completed by a proof of fulfillment of the agreed requirements. The evaluation and assessment of experimental data are given in a written report, that obtains parameters express the requirements or costumer's demands.

Certification

The certification is a procedure by which a third party gives written assurance that a product, process or service conforms to specified requirements [18]. The specific requirements are defined in standards, guidelines (for example CE for electromagnetic compatibility, medical devices or measurement instruments) or in agreed demands. The procedure for declaration of conformity refers to different phases of the product cycle. It is modularly structured and can be focused on design and development, test of products (prototype), quality system of the production, or the testing of product properties.

The certification procedure will be performed by an independent third body which has to be competent and qualified and accredited according to DIN 45011 and consists of the

- i. test of technical and quality documents, audit at the manufacturer, experimental test of the quality of the method in an independent laboratory
- ii. assessment of the results and
- iii. formal document of certification of approval [19].

The testing laboratory has a competent and qualified staff. It runs a quality system and should be accredited according DIN ISO EN 17025 [20]. The evaluator is an expert who coordinates the tests and performs an assessment of the results. The course of certification includes

- information about the procedure (inquiry)
- test of certification conditions (standards, requirements)
- offer (draft of agreement, estimation of costs)
- application, conclusion of agreement
- testing and assessment
- certification, invoice, publication continued monitoring

Certification contributes in general to technical harmonisation (of product quality especially with regard to security and human and environmental safety), and to overcome of technical trade inhibitions in a global market. In particular it supports the acceptance on new analytical methods. The efforts of quality assurance in analytics can be summarised as follows:

Principles for making valid analytical R&D measurements [1]

- Analytical measurements should be made to satisfy an agreed requirement.
- Analytical measurements should be made by using methods and equipment which have been tested to ensure that they are fit for purpose.
- Staff making analytical measurements should be both qualified and competent to undertake the task.
- There should be regular independent assessment of the technical performance of a laboratory.
- Analytical measurements made in one location should be consistent with those made elsewhere.
- Organisations making analytical measurements should have well defined quality control and quality assurance procedures.

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Chlorophyll Fluorescence Analysis in Horticultural Research

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The chlorophyll fluorescence analysis is a valuable, non-invasive tool to investigate the photosynthetic reaction of plants to various biotic and abiotic stressors. In a straightforward manner, it helps to study spatial and temporal dynamics of the metabolic activity of greens and some fruits.

Chlorophyll fluorescence analysis provides different information on different physiological aspects that are differently affected by certain constraints. For a proper application a comprehensive knowledge of this method may be helpful. Different stressors largely enhance the inherent physiological heterogeneity of a produce. Here, whole leaf chlorophyll fluorescence imaging analysis is advantageous over spot measurements with a normal fluorometer. A number of fluorescence parameters may be used to characterize produce quality or help to identify negative pre harvest and postharvest conditions. In horticultural research it has successfully been applied to study the effects of chilling, drought, heat, high light, anoxia, phyto-pathogenic infection, herbicides or senescence.

Because consumers' demand for quality monitoring of horticultural products has largely increased during recent years, this method may be applied to objectively, rapidly, automatically and non-destructively assess postharvest quality and its changes of green produce at many steps of the entire postharvest chain. The product-oriented quality may be related to physiological or biochemical capabilities of a product assuming that a high (actual or potential) activity of metabolism indicates a high internal quality. However, for an effective use of chlorophyll fluorescence imaging analysis in online application, instrumentation, and data processing and data evaluation needs to be improved. Combining chlorophyll fluorescence imaging analysis with other optical imaging methods may largely increase the reliability of quality analysis.





Developing a Novel Environmentally Friendly Microbiocidal Formulation from Peel of Citrus Fruit

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A new formulation of a microbiocide was developed from natural compounds extracted from citrus flavedo. The formulation prevented the development of decay in P. digitatum - inoculated fruits, and markedly reduced decay in non-inoculated citrus fruits. Similar good control was shown for other fruits and against several other fungi and bacteria. These natural compounds were found to be active against human pathogens such as Staphylococcus aureus, Candida albicans and others. A major advantage of this new formulation is its own non -toxic food grade characteristics and high chances of easy approval for use on fresh agricultural produce by the health authorities. The new formulation has both a direct fungicidal activity and elicits the fruit to build its endogenous defense mechanisms against decay. These combined modes of activity of both direct microbial inhibition, as well as elicitation of the endogenous resistance mechanisms against the pathogen, greatly improve the potential to control decay. Several other uses in household hygiene and in reducing contamination with this fragrant environmentally friendly food grade new product are possible. The active ingredients of this formulation were identified and comprise essential components and hydroperoxide. A procedure for the synthesis of the most active biocide has already been developed. This novel finding was acquired by Janssen Pharmaceutica and patents are pending in over 20 countries.

Chlorophyll Fluorescence as a Tool for Estimation of the Flavonoid Content in Apples

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Background: Flavonoids are assumed to have beneficial effects on human health and are widely distributed in plant foods. Due to an increased interest in these compounds, a need for new analytical methods has emerged. In all plant organs that contain chlorophyll, a small part of absorbed radiation will be emitted as red fluorescence. In context to plant protection against solar radiation, this chlorophyll fluorescence (ChIF) has been introduced as a method for assessment of UV-absorbing compounds in the epidermis of plant leaves [1,2].

Objectives: The aim of this study was to test ChIF measurements for rapid, nondestructive estimation of the flavonoid content in apple skin.

Methodology: Apples (cv. Aroma) with various concentrations of flavonoids were used as plant material. ChIF measurements were performed directly on the apple skin using portable fluorometers. The relative flavonoid content in the apple skin was calculated from the ratios between ChIF signals obtained with excitation beams absorbed (375 nm and 470 nm) and not absorbed (655 nm) by the various flavonoids. For reference, the same area of the apples as subjected to the ChIF measurements was analyzed with reflectance spectroscopy and HPLC-DAD.

Results: The results show that the relative absorbance by flavonoids and anthocyanins calculated from ChIF measurements was well correlated with the content of these compounds detected with HPLC-DAD and reflectance measurements.

Conclusion: ChIF can be used as a tool for rapid, nondestructive estimation of both anthocyanins and UV-absorbing flavonoids in apple skin.

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COST Action 924 Workgroup: Non Destructive Methods for Quality Assessment, Dec 7.-8. 2005, Potsdam, Germany

estimation of the flavonoid content in apples Chlorophyll fluorescence as a tool for

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INTRODUCTION

and anthocvanins. Flavonols, flavanols and dihydrochalcones absorb UV radiation whereas the anthocvanins absorb blue light. In this study mostly concentrated in the skin of the apples. The major classes of flavonoids present in apples are flavonols, flavanols, dihydrochalcones Flavonoids constitute an important group of polyphenols and are assumed to have beneficial effects on human health. The flavonoids are chlorophyll fluorescence (ChIF) was tested as a tool for non-destructive estimation of the flavonoid content in the skin of apples.



METHOD PRINCIPLES



chlorophyll

MATERIALS AND METHODS

Plant material: Ripe apples (Malus domestica Borkh., cv. Aroma) with various amounts of flavonoids were harvested from the orchard of the Norwegian University of Life Sciences. Fluorescence imaging: Cross-sections (50 µm) from the outer 5 mm of the apple were cut with a cryotome and studied under epifluorescence microscopy. The sections were then stained with Naturstoffreagenz A (diphenylboric acid 2-aminoethylester) and washed before re-examination.

Fluorescence spectroscopy⁰: All ChIF measurements were performed directly on apple skin using the portable fluorometers UV-A-PAM (Gademann Instruments, Germany) and PAM-2000 (Walz, Germany). ChIF was excited with UV-A (375 nm), blue-green light (470 nm) and red light (655 nm); F(UV), F(B) and F(R) respectively. Relative absorbance was calculated from the equations -logF(UV)/F(R) and -logF(B)/F(R).



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Relationship between Non Destructive Firmness Measurements and Texture Analyzer Measurements in Golden Smoothee Apples

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Apples (*Malus domestica* cv. Golden Smoothee) grown in Lleida, Spain were harvested at two different maturity stages on 27st September (less maturity stage) and 8th October (higher maturity stage), 120 fruits in total. For each date, six samples of 10 fruits were selected and stored at 1°C of temperature and 92 % R.H. At predetermined storage durations (0, 30, 60 days), twin samples from both ripeness stages were removed from cold storage using half of the samples for dehydration with hot forced air during 48 hours. For all samples, measurements on non-destructive firmness by AFS– AWETA, non destructive impact (LPF-TAG) and Sinclair firmness tester, as well as and reference tests for firmness (ball compression and Magness Taylor), and turgency (deformation at skin puncture) were carried out. Statistica® software was used for analyzing differences in ripeness stage, storage duration and dehydration treatments. Also a study on the relationship between measured variables was performed with special emphasis on non-destructive techniques and its affection with controlled sources of variation.

In general, reference and non-destructive firmness variables discriminate better between storage periods, and stages of hydration than between different maturity stages at harvest, except for Magness-Taylor. Contact measurements for firmness such as impact duration, Sinclair firmness and deformability modulus with compression ball test show to be strongly related one-another. Acoustic firmness (AWETA) losses its relationship with those contact measurements due to an erratic behaviour for extreme conditions (dehydrated at harvest and hydrated after 6 months storage) as stated with Principal Component Analysis. It has to be taken into account that apples in Spain are harvested with lower values and consumer preference for firmness is stressed towards lower firmness values than in northern countries. The unexpected behaviour of AFS-AWETA may be associated to a miss identification of the main frequency peak and so further research is ongoing in this direction.

Apple Firmness Assessed by Non-Destructive Technology Compared to MT Firmness and its Consumer Rating

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Three apple cultivars `Diwa`, `Elstar` (1-MCP treated and controls) and `Idared` were stored under ULO conditions for 9 months. After removal from storage, apples were held at 20°C for 7 days. Firmness of the apples was assessed by non destructive measurements using a Greefa (IFD unit) as well as an Aweta (acoustic unit) device. Apples were then subjected to consumer testing (one apple per person and each tasting one slice of an apple). The remaining apple was evaluated using an MT firmness measurement. In parallel, soluble solids content and acidity were determined. Correlation was weak between non-destructive and MT firmness, whereas measurements based on Greefa and Aweta units concurred. MT firmness was a more reliable predictor of consumer acceptability than the non-destructive techniques. 1-MCP Elstar were firmer than the untreated ones and were preferred by the consumers.





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Non-destructive spectraloptical measurements are suited to monitor continuously the development of fruit on the tree. Picking of every time new fruit samples is not necessary, because measurements can be repeated on the same fruit.



that is fixed at the forearm. One hand is used to carry the sensing probe to the fruit surface, the other one is free to hold the fruit in fixed Currently, a backpack device developed for research work is used for the measurements. This device is operated by means of keys on a box position. The sliced fruit shows how the light is penetrating the fruit tissue. A glass-fibre picks up the back scattered light that is finally measured.

The spectral optical measurement in visible and near-infrared range provides in non-destructive way simultaneously informationen on several fruit parameters:

- Index of chlorophyll decrease (correlated with ground colour) Moreover, additional quality related components can be determined in near-infrared wavelength range Index of anthocyanin development (correlated with blush colour)



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Assessment of Health-Related Quality in Broccoli by a Non-Invasive Chlorophyll Fluorescence Method

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Fresh vegetables and fruits have a limited postharvest shelf life. Already before there are signs of senescence health beneficial properties could be reduced. In addition, there has been documented in some vegetables (e.g. *Brassica oleracea*) a large variation in the contents of nutrients and secondary metabolites between individual plants of the same variety and between varieties and subspecies. Rapid and non-destructive methods to assess the actual health-related quality are therefore needed. Chlorophyll fluorescence excited by two different wavelengths has proven to be usable for estimation of the content of UV-absorbing epidermal flavonoids in green leaves [1,2], Our intention was to test whether the method could be used to assess the content of flavonoids in a bulky vegetable and whether there could be a positive correlation between any fluorescence derived parameter and the total antioxidant capacity (ORAC method).

Broccoli heads were treated in the cold 6h per day for 12 days with three wavelength ranges of UV-VIS radiation (24 h darkness control) in order to induce differences in the flavonoid level. However, since the radiation-induced changes were modest, instead the great natural variation in flavonoid level between individual broccoli heads was used. With a reference excitation beam at 685 nm there was a positive correlation between the relative epidermal absorbance of blue light (450 nm) and the content of total flavonoids (r=0.69, p<0.001), as measured by HPLC after extraction and hydrolysis. For quercetin the correlation was higher (r=0,77, p<0.001). A relation between fluorescence derived parameters and the total antioxidant capacity was lacking, whereas there was a very weak correlation between flavonoid content and the ORAC value (r=0.37, p<0.05). Thus, flavonoids, but not total antioxidant capacity, could be assessed in broccoli by the fluorescence method tested.

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Fluorescence Spectroscopy for Monitoring EVOO Quality during Heating

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Extra virgin olive oils (EVOO) were analysed qualitatively and according to their contents of α -tocopherol and polyphenols by means of chromatographic and spectrometric techniques. EVOO was chosen for the experimental series, since it provides valuable sources of native α -tocopherol and polyphenols with high antioxidative capacity.

Variation in the spectral fingerprint of olive oil was mainly found in the UV wavelength range (260-380nm), where some intensity decreased, while other new peaks appeared. PLS calibration models addressing relative changes of EVOO due to the heat impact were developed using different methods of data pretreatment as standard normal variate (SNV), orthogonal signal correction (OSC), Full-Contrast and Reduced Contrast data pretreatments. The OSC pretreatement when removing one orthogonal component led to lowest errors and highest coefficient of determination of PLS calibration models for evaluating the impact of heating on the oil quality.

Quantitative analysis of olive oil was carried out based on oil (diluted in hexane) fluorescence spectra (exc285nm/emi330nm) and α -tocopherol content measured by means of HPLC. Two commercial EVOO were analysed by HPLC for determining changes in EVOO α -tocopherol and polyphenols due to heating at 170°C for 3 hours. This thermal oxidation of EVOO caused an exponential decrease in hydroxytyrosol and α -tocopherol (r²=0.90 and 0.93, respectively), while tyrosol content was relatively stable. With PLS regression analysis (#LV=2) r²=0.69 and SECV=1.14 [mg/100g] (6.9 %) were calculated for non-invasive α -tocopherol analysis.

Agrartedhilk Bornin	explained by the presence of ation in production processes. he evolution of polyphenols minutes for further analysis. ar, Jobin-Yvon, France). Two hydroxytyrosol (OH-Tyr) and of heating, and second to of heating, and second to of the standard and dividing by the standard and dividing by the standard and dividing by the standard to m (m-1) / 2, m being the g the highest correlation with	in methods for nt to improve the PLS
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The Norwegian Crop Research Institute



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 effect in stone fruit
 –
 Preliminary results

 Vangdal, Eivind ^{1*}, Slimestad, Rune² and Sekse, Lars¹
 1

Phenolics and other compounds with antioxidative

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Fig. 1. Reeves a cultivar with medium content of health related compounds

The consumers are growing more aware of health related compounds in the diet. Fruits, berries, nuts and vegetables contain large amounts of several compounds known for their high antioxidative effect. The antioxidative effect of these products show a wide range from 0.06 mmol/100g FW in carrots to 39.46 µmol/100g FW in hips (Halvorsen et al. 2002). They found the antioxidative effect of plums to be 1.02 and in sweet cherries 1.42 mmol/100g. A study of sweet cherries has shown a wide range in content of phenolics and anthocyanins in Canada (Gao and Mazza, 1995). The results from a Norwegian study of these compounds and antioxidative effect in sweet cherries and plums are presented below.

Materials and methods:

The fruit samples were picked in the experimental orchard at Planteforsk Ullensvang Research Centre. The samples were frozen before transportation to the laboratory. The analyses were performed according to standard methods for total phenolic content, content of anthocyanins and antioxidative effect (FRAP-method). Sweet cherries have been analyzed both in the 2002 and 2003 season. The results with plums are from the 2003 season. All analyses are performed twice on three different fruits.

Table 1. Content of anthocyanins, phenolics and antioxidative effect in 9 sweet cherry cultivars. (Two replicate analyses of three replicate fruits)

	Anthocyanins	Phenolics	Antioxidative effect (FRAP)
Cultivar	(mg/100 g)	(mg/100g)	(mmol/100g)
Sue	0.56	23	0.44
Merton Glory	1.4	37	0.71
Sunburst	16.1	38	0.78
Chelan	22.9	56	1.08
Lapins	16.8	<i>L</i> 4	1.16
Van	31.3	75	1.48
Ulster	44.0	87	1.58
Kordia	60.9	109	2.20
Agila	120.6	168	2.67

Results and discussion:

Table 2. Content of anthocyanins, phenolics and antioxidative effect in 9

plum cultivars (Two replicate analyses of three replicate fruits)

The contents of phenolics were higher in cultivars with dark red juice than cultivars high in phenolics than in the cultivars with low phenol content. ranged from 0.44 mmol/100g in 'Sue' to 2.67 mmol/100g in 'Agila'. A The total content of phenolics was more than seven times higher in the in cultivars with yellow juice. The total antioxidative effect (FRAP) total of 41 cultivars were analysed.

Van the total phenolic content increased from 31 mg/100 g in slightly ripe During maturation the content of anthocyanins and phenolics increase. In fruits to 75 mg/100 g in fully ripe fruits. In plums fruits of 9 cultivars were analysed. The total content of phenolics 'Victoria'. A close correlation between FRAP values and total contents of ranged from 27 mg/100g in 'Reine Claude Souffriau' to 54 mg/100g in 'Victoria'. The antioxidative effect (measured by the FRAP-method) ranged from 0.61 mmol/100g in 'Souffriau' to 1.28 mmol/100g in phenolics was found.

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Lofthus, Norway

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-	Anthocyanins	Phenolics	Antioxidative effect (FRAP)
Cultivar	(mg/100 g)	(mg/100g)	(JUU0/ 100g)
Souffriau	5.0	27	0.61
Opal	1.6	29	0.66
Excalibur	0.9	30	0.75
Reeves	0.9	39	0.75
Mallard	4.0	39	0.84
Avalon	2.8	35	0.86
Jubileum	4.8	39	0.90
Edda	10.8	52	1.03
Victoria	0.7	54	1.28
Conclusions: •A wide range in antioxidative effe •In plums 'Victon highest antioxida	contents of anthocy set were found in sv cia? had the lowest tive effect of the tes	ranins and phenolic reet cherries and ph content of anthocyau ted cultivars	s and ims in Norway nins, and yet the





An Approach towards Rapid Optical Measurements of Antioxidant Activity in Cultivated Blueberry Cultivars

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Blueberries are well known for their high antioxidant levels. Compared to bilberries with higher antioxidant activity and more intensive blue colour throughout the whole berry, highbush blueberries have almost colourless fruit flesh and the blue pigments concentrated in the skin. Highbush blueberry skins have been found to contain a very high content of phenolic compounds. To measure the total antioxidant activity in blueberries, several methods, mostly destructive including the FRAP assay, have been used. This work is an initial approach towards a simple and rapid method by combining optical and antioxidant activity measurements.

Highbush blueberry (*Vaccinium corymbosum*) cultivars 'Bluecrop', 'Hardyblue', 'Patriot', and lowbush cultivars 'Putte' (*V. angustifolium*) and 'Aron' (*V. corymbosum* x *V. uligino-sum*) were grown at the Norwegian University of Life Sciences (59° 40'N). Berries were harvested at commercial blue-ripe stage of maturity. Fresh berries were cut horizontally and placed on a scanner in order to examine berry size and skin thickness. Berries were weighed, and analysed for antioxidant activity using the FRAP (Ferric Reducing Ability of Plasma) assay. The FRAP assay is a non-specific method based on absorption changes following a reduction of a ferric- to a ferrous-complex in the presence of antioxidants.

Own previous results have shown that antioxidant activity and berry weight varied between cultivars. Small berries had higher antioxidant activity compared to larger berries. In this follow-up project, skin thickness and berry diameter were measured by using an image processing program. The total cross section and skin area were correlated with the antioxidant activity.

HESPERIDES: Highly Efficient Sensors for Perishable Fruit Products to Evaluate the Role and Impact of Technologies on the Nutritional Damage and Elaborate Optimizing Strategies

M. Zude¹, M. Geyer¹ and D. Treutter²

Evaluating the impact of processing technologies for fresh and ready-to-eat fruit products on their antioxidant nutrient content is an important task to determine strategies for quality optimization and monitoring. For this purpose, the fruit product quality changes need to be examined by rapid non-invasive methods. Fluorescence and absorbance spectrometry may provide efficient measuring techniques for monitoring polyphenol, vitamin and provitamin. However, these techniques must be adapted to the specific physicochemical characteristics of the products, and further, evaluated taking into account their accuracy and feasibility.

In the frame of a new DFG-project "Hesperides", non-invasive spectral-optical methods for analyzing fluorescent nutrients will be developed. Therefore calibrations including correction algorithms regarding the physicochemical product properties will be built. Data acquired by means of conventional chromatographic techniques and non-invasive spectral-optical methods will be used to measure the nutritional damage of fruit due to environmental conditions, advanced maturity, and minimally processing. The study will be carried out on two fruit models: fresh and minimally processed tomatoes (Lycopersicon esculentum) and strawberries (Fragaria ananassa). Such fruit have high market shares and are well described regarding their antioxidative compounds. Tomato is rich in carotenoids and contains vitamin E. Strawberry has high contents in polyphenols belonging to the groups of hydroxycinnamic acids and flavonoids, including flavonols, catechins, proanthocyanidins and anthocyandins. Such different compositions will require specific correction regarding reabsorption and quenching effects, but will allow to widely exploring the potential of spectral-optical methods evaluated in the present project. Furthermore by using these two models, the opportunity appears to study the influence of climacteric and non-climacteric behaviour during processing.

Results of conventional and alternative methods will be compared. Feasible monitoring methods developed in the project will be outlined by means of technical requirement profiles for specific, inexpensive sensor solutions.

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Drought stress suppresses plant growth, because plant cell and tissue enlargement is mainly due to irreversible water storage. Thus water uptake and water transport in plants is a good indicator of the efficiency of plant growth and metabolism. Conventional methods to obtain information on water transport in plant, such as heat balance techniques, pressure bomb or pressure probe have only a macroscopic point of view. Otherwise, the field of view of advanced approaches like magnetic resonance imaging (MRI) is too small. In contrast, 3D imaging of neutron and X-ray have a high resolution and a relatively large field of view. These methods should be excellent tools to observe the full scale of water transport in plants. It would solve an interesting question of how drought stress at a part of a plant affects the whole plant. Hence, the purpose was to evaluate the potential of neutron radiography and X-ray imaging to observe water transport in plants. We introduced a trial application of a D_2O probe for neutron radiography, to observe water uptake and water transport in tomato and corn seedlings. Using the PIV (Particle Image Velocimetry) method it was possible to convert the contrast created by D₂O probes to the velocity of the water stream in a seedling stem. With this system, the process of D₂O absorption in the root was clearly observed. Furthermore, the PIV method obtained vectors of the D₂O absorption. Furthermore, water and D₂O transport in corn seedling was clearly observed using CONRAD which is a new neutron radiography and tomography facility at the HMI. The new facility provides important answers about water transport in plant roots and stems, which are unclear yet. In addition to of neutron tomography, the potential X-ray imaging as an observation method of water transport in plants was studied. Development of contrast medium of x-ray for water transport in plant should provide a great future in the research field of plant-water relations. Both the D₂O probe approach of neutron radiography and optimized contrast media for x-ray imaging may provide new insight into processes of water uptake and water transport in plants at the tissue and plant organ level.

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