



Theses of Ph.D dissertation

**Laser diffuse reflection method to characterize the state of crops**

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## ***1. Introduction***

Due to the recent rapid development of technology, new methods have been introduced for testing food ingredients. Advanced equipments are able to provide fast and reliable quality prediction of crop quality. Over the past decades the application of vision system was increasingly common to replace manual technics and for the automation of processes that includes classic, hyper- and multispectral vision system as well.

In my present research the aim was to predict inner properties and nutritional characteristics. One of the most widely used method for testing is near-infrared (NIR) technique. Using this method, the product is exposed to near infrared radiation and reflected or transmitted radiation is measured. While the radiation penetrates into the product, its spectral characteristics are changing based on wavelength-dependent scattering or absorption processes. The changes are related to the chemical composition, as well as the light-scattering properties which relate to microassemblies. Another novel method is the diffuse reflectance measurement, wherein we can obtain information related to the physical structure and chemical composition of the crop. Based on the scientific literature I designed and constructed the measurement system to monitor the changes of crops under storage. Measurements were performed on several different types of food and crops, including experiments of apple drying, potato and pear storage. For measurement system, testing the applicability and optimal settings are of great importance.

## ***2. Literature***

Researchers has long been dealt with NIR technique and nowadays it is used as a routine method in the food industry. Several articles have been published in this field to monitor changes of different physical and inner properties. The role of hyperspectral imaging is increasing in food and agricultural product's quality and safety assessment. This technique unifies spectroscopy and imaging thus spectral and spatial information can be obtained of the studied sample. Based on the literature only a few researcher deal with the adaptation of diffuse reflection method to predict quality properties of crops. In most of the cases hyperspectral camera is applied which allows to measure on the spectral range of the camera therefore with the help of a focused light source measurements with high spectral sensitivity can be obtained. My opinion is that systems with hyperspectral cameras cannot be spread because of their price. Measurements performed

by this type of devices establish typical wavelengths' selection method that help the construction of cheaper measuring systems, therefore the opportunity of a handheld device. Evaluation of profiles is distinct, not well established, researchers are using different methods to describe profiles obtained by measurement. Among others by fitting different models that is really time consuming and requires a lot of calculations. Based on the evaluated literature (Scopus, Science direct and Google scholar) there were no information about prediction of known optical parameters obtained by simulation. I found it interesting what kind of relationship describes the changes of parameters characterizing crops in a defined range. A research group took into account the focused light of 1.5 mm diameter and left out the plateau of profiles (from the center till 1.6 mm). Cutting out of plateau was used because it makes easier the regression of Lorentz functions. In my opinion with this method the physical content of measured values is distort because the origin (0 mm) of the distance is considered from the remaining part of the profile. Unfortunately based on the published studies the choice of the eliminated plateau range is not clear.

### ***3. Research aims***

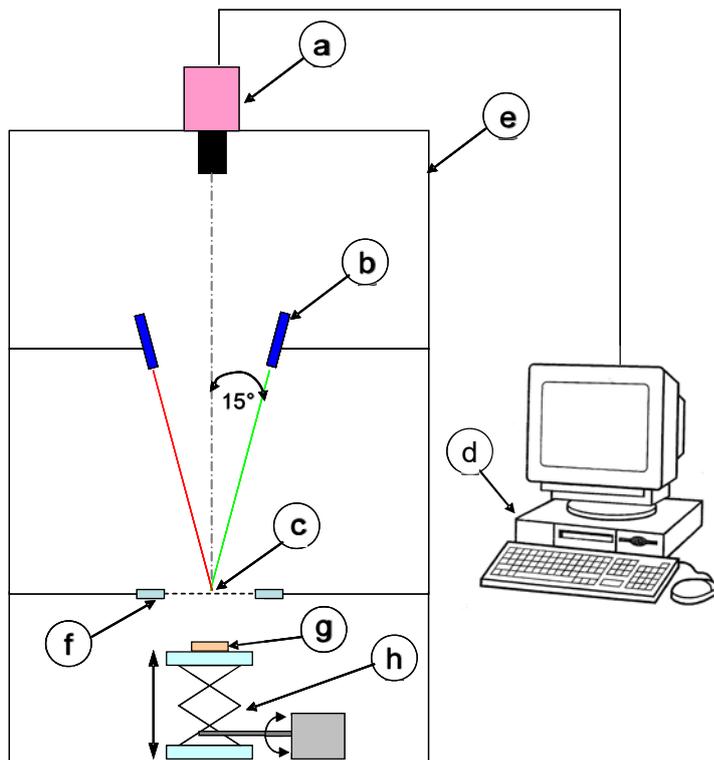
The aim of my PhD work was to monitor and simulate the typical changes during storage and processing of fruits and vegetables. With these measurements and obtained results I would like to establish the bases of in vivo diffuse reflection method.

Based on the scientific literature I designed and constructed the measurement system. I developed a diffuse reflectance measurement and evaluation method based on computer vision system. Measurements were performed on several different types of food and crops, including experiments, such as apple drying, potato and pear storage. For measurement system, testing the applicability and optimal settings are of great importance. I compared the results with those of reference methods (dynamic and acoustic stiffness, dry matter based and wet matter based water content, chlorophyll fluorescence, NIR). I created profiles with known optical properties by the simulation of photon propagation, I performed predictions (MLR and SVM) by parameters of modified Gompertz functions fitted on profiles. Therefore, predictability of several optical parameters was determined.

## 4. Materials and methods

### 4.1. Development of measuring system based on diffuse reflectance method

During my experiments I worked with a self-developed and composed diffuse reflection device (Figure 1.). The automated measuring equipment's main part is a monochrome CMOS IP camera with 12 bit resolution (Photon Focus MV1–D1312), its maximal capture size is  $1312 \times 1082$  pixel and its spectral sensitivity is in the range of 320 – 1080 nm. Seven solid laser diode modules were built in ( $532 \pm 5$ ,  $635 \pm 5$ ,  $650 \pm 5$ ,  $780 \pm 5$ ,  $808 \pm 5$ ,  $850 \pm 5$  and  $1064 \pm 5$  nm) for light sources. Laser light sources were placed symmetrically in round shape above the measured sample at  $15^\circ$  angle of incidence. This adjustment made it possible that a majority of the radiated photons penetrate the tissue and their focal point could be in the same circle about 2 mm in diameter, and eliminate the problems resulting from direct reflections.



**1. Figure** Theoretical composition of self-developed diffuse reflectance imaging device: CMOS IP camera (a); laser source (b); lasers' focal point on camera focal plane (c); computer with gigabit Ethernet card (d); darkened chamber (e); optical gate (f); sample holder (g); lifting device (h).

## Intensity profile and its parameters

The illuminated area was scanned and intensity values were calculated with radial averaging relative to the incident point.

Application of well defined, objective parameters that give profile characterising results is a very important point during profile analysis. One of them is Full Width at Half Maximum (FWHM). In this case it is important because FWHM is proportional with penetration depth. Inflection point (IP) of the profile (minimum of first derivative) and slope of logarithmic profile can be determined.

## 4.2. Methodological studies and tools

The aim of methodological examinations was to find the optimal settings of tools and the analysis of system stability (reproducibility). For the examination of reproducibility a PLC (Zelio Logic SR3) was used and a stepper motor (Robotron SPA 42/100-558) in addition to the diffuse reflectance imaging device. The applied stepper motor was able to make 100 steps in whole round, thus  $3.6^\circ$  rotation by one step.

## 4.3. Apple drying experiment

The apple discs were dried in a convective hot air drying chamber (Venticell 222 flow-through laboratory dryer). Drying experiments were carried out at air temperature of  $65^\circ\text{C}$ . The air ventilation was adjusted to maximum value. At the start of the experiment and in every consecutive hour twelve slices were taken out from the dryer and used for further analysis. Altogether eight different drying levels were generated (0 to 7 h drying time). All samples were weighed before ( $m_{\text{initial}}$ ) and after ( $m_{\text{actual}}$ ) the drying process. After the tests the samples' drying was continued until 24 h, using the same drying parameters. With this technique the final mass ( $m_{\text{final}}$ ) was determined. Optical method of laser light diffuse reflection (laser scattering) method and NIR technique (1000-1700 nm with 2 nm step) were applied. SNV normalisation was performed on NIR data.

## 4.4. Potato storage experiment

The amount of 110 pieces of commercial potato were stored at room temperature for one month. Randomly selected tubers were withdrawn for measurement twice or three times per week.

Non-destructive texture measurements were performed: acoustic impulse response and impact method. Optical method of laser light diffuse reflection (laser scattering) method was applied.

#### 4.5. Pear storage experiment

As a member of a research team, I examined the sample of 120 pieces of pear (*Pyrus communis* cv. Bosc kobak) which were randomly divided into four groups. Samples were stored at optimum temperature (0-2 °C), one group was withdrawn in every second week and stored for other two weeks at room temperature (22±2 °C). The whole storage procedure was made until 60 days. On the withdrawn samples non-destructive measurements (diffuse reflexion, chlorophyll fluorescence, acoustic and impact methods) were performed every second day (except weekends).

#### 4.6. Monte Carlo simulation

During Monte Carlo (MC) simulation, the path and direction of the photon are determined by probability variables, which are influenced by the tissue properties. For the simulation profiles presented in the dissertation, I used a customized Monte Carlo simulation program (Baranyai and Zude 2009). During the simulation, I determined fixed parameters that create similar conditions to the measurement results. In the MC simulation, three parameters (absorption =  $\mu_a$  [ $\text{cm}^{-1}$ ], scattering factor =  $\mu_s$  [ $\text{cm}^{-1}$ ] and anisotropy =  $g$  [1]) characteristic of tissue structure and material quality largely affect the entire path of every photon. During the simulation I have changed these three factors and computed the diffusion profiles. For each profile, these values are known and can be counted in addition to the penetration depth equivalent to  $1/\mu_{\text{eff}}$  (Wilson and Jacques 1990) and diffusion factor  $1/\mu_t$  (Wilson and Jacques 1990). In the course of profiling, the measured  $\mu_a$  and  $\mu_s'$  values of horticultural crops were selected in the following interval (Qin and Lu 2008): the  $\mu_s'$  value ranged from 5 to 15  $\text{cm}^{-1}$ , while  $\mu_a$  was in the range of 0.02-1.2  $\text{cm}^{-1}$ .

#### 4.7. Applied softwares and statistical methods

Self prepared programs were applied in R project (version 3.2.2, R Foundation for Statistical Computing, Vienna, Austria) for the evaluation of images, calculation of intensity profiles and statistical data analysis. During statistical evaluation PLS, SVM and MLR packages were used.

For data treatment and diagrams I applied Excel (Microsoft® Office Excel 2010). PLS estimation of NIR spectra was realized in Unscrambler statistical program (v9.1, CAMO Process AS).

## ***5. Results and discussion***

### 5.1. Results of methodical studies

#### Evaluation of repeatability

Using the intensity profiles from the pictures, I examined the sample properties on the same point. The FWHM parameter was used to characterize the profiles. The seven repetition curves are nearly the same. In order to quantify the differences, I calculated the percentage deviation at each rotation angle. At a wavelength of 532 nm, the variation coefficient (CV%) is less than 5% ( $2.37 \pm 0.67\%$  for total repetition), which is acceptable, considering that this measurement is also loaded with the positioning error of the stepper motor. At the 1064 nm wavelength the observed variation coefficient varied between 14 and 39.8 CV%, averaging  $22.6 \pm 4.9$  CV%. The measurement results support the assumption that repeatability at the sensitivity boundary of the camera (1080 nm) deteriorates significantly. At 1064 nm wavelength the camera has worked with significantly different error compared to other wavelengths.

#### Standardization of diffuse reflection profiles

The results of the repeatability and the measurements made during the research confirmed that the method can be used to characterize horticultural crops. At the same time, the standardization of measured profiles is indispensable for objective evaluation independently of the measurement conditions. As a first step, I used transformations that allow diffusion profiles with different integration times to be treated identically. It has been observed that the lower integration time decreased the profile plateau size and profile width as well. In this series of measurements, the main goal was to transform the intensity profiles obtained with different integration time to free the measurements from the effect of shutter speed, so I developed an equation to transform the profiles. Using the equation, it became apparent that low shutter speeds were subject to significant noise. Due to the large dark noise, I do not recommend capturing reflective images at 10 ms shutter speed or faster. After transformation, the profiles fit almost one single curve. For statistical confirmation of this, I calculated variation coefficient

(CV%) per distance. The  $CV\% \leq 1\%$  of the evaluable parts confirms that diffusion profiles with different integration time can be standardized with ignoring the initial saturated part.

### 5.2. Results of apple drying experiment

The 'Idared' apple slices were dried at 65 °C for 7 hours and analyzed by NIR spectrophotometer and laser-induced diffusion visualization system. At 740-1700 nm I have successfully applied the  $\log_{10} 1 / R$  spectrum in the PLS model and estimated the moisture content on the dry basis with  $R = 0.988$ ,  $RPD = 6.63$  and  $DW = 1.94$  and the moisture content of the wet basis  $R = 0.98$ ,  $RPD = 5.09$  and  $DW = 1.51$ . Similar correlations and Durbin-Watson (DW) statistics were observed for standardized spectra. In typical PLS models, typical water absorption peaks (970, 1200 and 1470 nm) have clearly appeared. I observed a monotonous change in laser-induced diffusion profiles. The parameters of the profiles, such as IP, SLOPE and FWHM, were measured and I could estimate the moisture content of dry basis ( $R = 0.969$ ,  $RPD = 4.014$ ,  $DW = 1.72$ ) using the FWHM value with the highest accuracy.

### 5.3. Results of potato storage

Comparing the diffuse reflection parameters and the firmness factors, it became apparent that the results measured by the two methods indicate a similar change. The average FWHM values measured with 635 nm wavelength laser had significant correlation with acoustic and dynamic firmness values. In all cases, there was an exponential relationship between the dynamic firmness and the optical parameter ( $r^2 = 0.96$ ;  $RMSEP = 6.136\%$ ;  $RPD = 5.48$ ). The closer correlation with impact firmness is explained by the fact that acoustic firmness refers to global feature (Muha 2008), but the impact firmness to the surface (which is the same as measured by the diffuse reflection method). Among firmness and diffuse reflection factors, like the results obtained with the 635 nm wavelength laser, I found a close correlation similar to that of Lu and Peng (2006).

### 5.4. Results of pear storage experiment

During the experiment we simulated the ideal and non-ideal storage with different storage conditions. The physical parameters of the samples stored at two temperatures (refrigerator and

room) changed at different speed depending on the storage time. Mass loss shown a change in water content that affects the structure. After withdrawal, linear relationship was found between mass loss and storage time in each group, and pear samples lost their mass differently. The variable speed refers to the change in the slope values of straight lines fitted to the measurement points (slope = 0.648 - 0.412). After the first withdrawal, the most intense weight loss change (ramp = 0.648) can be observed. From the pear samples, the FWHM values of the 635 nm wavelength laser profiles shown a similar trend compared to the change in mass loss as a function of storage time. Based on the results, the FWHM values of the diffuse reflection profiles show a similar trend as the loss of mass.

#### Introduction of equivalent storage time

In order to make model about physical changes, it was necessary to normalize the physical characteristics of samples stored at different temperatures. Weight loss as a parameter that is specific to the given storage condition was based on the calculation of the Storage Time Equivalent Value (STEV) value. The transformation is described in more detail in our published study (Zsom et al., 2014). By introducing the equivalent storage time, the measured characteristics of the samples stored at different temperatures could be analyzed uniformly, despite their originally different kinetics. Changes in physical characteristics (such as softening) can be approximated by the exponential equation as a function of storage time. With the introduction of the equivalent storage time, the four storage groups moved to single trend from separate ones, all points are aligned to a linear model.

#### Relationship between chlorophyll fluorescence and FWHM (635 nm) values

During the chlorophyll fluorescence and diffuse reflection measurements I analyzed the pear samples at the same measuring points. There were significant linear correlations between the three factors of the chlorophyll fluorescence ( $F_v / F_m$ ,  $F_0$  and  $F_m$ ) and the diffuse reflection parameter FWHM. The correlation between the two methods was the most closely related to the  $F_m$  value ( $R^2 = 0.842$ ).

#### Relationship of stiffness and diffuse reflection parameters

The firmness and the diffuse reflection parameters follow similar tendency. By comparing the diffuse reflection parameters and firmness factors, it was observed that the results measured by the two different methods indicate similar change. I compared the changes in the FWHM values of the 635 nm wavelength laser profiles with the acoustic and dynamic firmness values. For acoustic firmness, exponential relationship was found ( $R^2 \geq 0.89$ ), while linear relationship was stronger ( $R^2 = 0.957$ ) for the dynamic one.

### 5.5. Monte Carlo simulation

Monte Carlo simulation was performed to find the correlation between the modified Gompertz function (MGF) and the optical properties of the biological tissue ( $\mu_a$ ,  $\mu_s$ ,  $g$ ). The aligned prediction models (linear as MLR and non-linear as SVM with radial kernel) were used to estimate different parameters. When estimating the penetration depth ( $1 / \mu_{\text{eff}}$ ), a non-linear relationship between the dependent and independent variables was observed. When estimating this parameter, the SVM method yielded the best results with RMSEP = 8.27% and  $R^2 = 0.79$  determination coefficient. No models were loaded with systematic errors. In the case of the estimation of the diffusion coefficient ( $1 / \mu_t$ ) with the MLR method, there was a kind of rotation between measured and estimated parameters which was not observed with the SVM method. With the SVM estimate, it was possible to get  $1 / \mu_t$  values close to a line with RMSEP = 5.71%, autocorrelation-free ( $DW = 2.2$ ) and well-fitting determination coefficient ( $R^2 = 0.95$ ). All in all, the SVM method proved to be the best for estimating all four parameters ( $\mu_a$ ,  $\mu_s'$ ,  $\mu_{\text{eff}}^{-1}$ , and  $\mu_t^{-1}$ ). The best independent estimation was performed for the diffusion coefficient ( $1 / \mu_t$ ), where  $R^2 = 0.948$  and the estimation error was low as RMSEP = 5.71%. In either case, the estimate was not loaded with autocorrelation ( $1.7 < DW < 2.3$ ).

### 5.6. Optical parameters predicted by SVM models

I used pear test results to estimate the optical parameters ( $\mu_a$ ,  $\mu_s'$ ,  $1 / \mu_{\text{eff}}$  and  $1 / \mu_t$ ) using the best models (SVM) obtained with MC simulation. The estimated optical parameters of pear storage and the FWHM values measured at 635 nm wavelength with the absorption coefficient ( $\mu_a$ ) shown the closest linear relationship ( $R^2 = 0.878$ ).

## 6. *New scientific results*

On the basis of the research and the results achieved I have concluded the following new scientific results.

1. I have elaborated a diffuse reflection measurement and evaluation method based on a computerized vision system and a research tool to estimate the physical and nutritional characteristics of certain vegetable and fruit that allow nondestructive assessment of the physical status of these crops. I have developed a generally applicable method for determining the system's usability constraints and standardizing device-dependent settings. The minimum recommended integration time of the given system is 10 ms based on the signal to noise ratio of the standardized data.
2. I demonstrated a clear correlation between the diffuse reflection parameters of the examined samples and their storage and drying time. I determined the exponential relationship between FWHM parameter of the diffuse reflection profiles and the storage time of potato (635 nm;  $r^2 = 0.976$ ; RMSEP = 5.1 %). When storing pears, I found linear relationship between the diffuse reflection profile parameter FWHM and the phenomenon representing the processes in the experimental sample (equivalent storage time) (635 nm;  $r^2 = 0.836$ ; RMSEP = 4.88%).
3. During the drying of apple slices, I prepared a prediction model approaching the efficiency of the reference method using the FWHM parameter of the diffuse reflection method to determine the dry-based moisture content ( $r^2 = 0.939$ , RMSEP = 7.63%, RPD = 4.04).
4. I have found that the diffuse reflection method is able to estimate the state of examined horticultural crops without touching. With the measurements of pear and potato samples, I proved that there is strong exponential relationship between the dynamic firmness factor and the FWHM values measured during storage with the 635 nm wavelength laser (potato  $R^2 = 0.963$ ; pear  $R^2 = 0.952$ ).
5. During the pear storage experiment, I demonstrated close linear relationship between diffuse reflection parameters and fluorescence characteristics (FWHM ~ Fm; 635 nm;  $r^2 = 0.843$ ; ~ Fv / Fm; 635 nm;  $r^2 = 0.668$ ; ~ F0; 635 nm;  $r^2 = 0.827$ ).
6. Using the Monte Carlo simulation I proved that the value of the penetration depth ( $1 / \mu_{\text{eff}}$ ) and the diffusion coefficient ( $1 / \mu_t$ ) can be estimated using the SVM model built with the modified Gompertz function coefficients from the calculated diffuse reflection profiles. ( $1 / \mu_{\text{eff}}$ :  $r^2 = 0.783$ ; RMSEP = 8.04%; DW = 1.992;  $1 / \mu_t$ :  $r^2 = 0.944$ ; RMSEP = 6.12%; DW = 2).

## ***7. Suggestions***

Based on my research results, the diffuse reflection method is suitable for non-destructive measurement of the local firmness of horticultural crops. Due to the size of the measuring system I built it is not suitable for a routine procedure. For a handheld instrument to be used in industry, typical wavelengths should be selected depending on the characteristic to be measured (such as chlorophyll, anthocyanin, moisture content, dynamic firmness). The portable handheld light sources need easy and quick replacement. In order to quickly determine the properties of the examined tissue sample, it would be expedient to apply contact measurement on the sample. This reduces the size of the target instrument. Another solution of feasibility may be the use of optical fibers to transmit light from the built in light source to sample and back to the sensor. A manual instrument can greatly facilitate the work of producers or commercial units by providing relevant information about the condition of the crop.

## ***8. Summary***

Healthy diet is very important in our everyday life. In case of seasonal crops, generally fresh, domestic goods are consumed that reach their desired nutritional characteristics in the optimal harvesting state.

In the trade, consumers' perception of fruit and vegetable is highly influenced by the quality of the product, that is always controlled by visual inspection and mechanical (by touching) examination. Shelf life of fruit or vegetable influences the time of their trading. In supply chain the changes of quality in time is a key point, including logistics, because the knowledge is essential for the post harvest technology and the prediction of features connected to the state. Due to the recent rapid development of technology, new methods have been introduced for testing food ingredients. Advanced equipments are able to provide fast and reliable prediction of crop quality. Compared to conventional methods, new procedures do not require large amount of chemicals, if any.

The aim of my PhD work was to develop a diffuse reflectance measurement and evaluation method based on computer vision system. Based on the scientific literature I designed and constructed the measurement system. Measurements were performed on several different types of food and crops, including apple drying, potato and pear storage experiments. For measurement system, testing the applicability and optimal settings are of great importance. The

main part of the currently used instrument is a 12 bit/pixel resolution CMOS camera. It was found in the literature that researchers are using different camera types (hyperspectral, monochromatic and RGB) even with diverse resolution. In my point of view, the standardization is key point to be able to compare diffuse reflection images captured by different cameras. Exposure time is very important attribute because it influences the size of diffusively illuminated area and the number of oversaturated pixels.

A formula was established for the decoupling of resolution and exposure time, to calculate the standardized profiles. In order to characterize diffusion profiles, slope, inflection point, full width at half maximum (FWHM) value and the parameters of modified Gompertz function (MGF) were analyzed. According to the experiences and measurement results, FWHM value was the best to characterize profiles. Strong correlation was found between FWHM and delta parameter of MGF fitted on profiles. Changes of the measured stiffness values during the two independent storage experiment (pear, potato) were in strong correlation with FWHM value measured by 635 nm laser, that is in agreement with the result of Lu and Peng (2006) for apricots. Harvesting is followed by post-harvest treatment of crops. These changes were modelled by storage experiments. During maturation, chlorophyll content was reduced which was confirmed by changes measured close to chlorophyll absorption wavelength. I hope my work will contribute to the adaptation of a less known non-contact analysis method in Hungary, as a basis of an in-vivo technique. The handheld adaptation of the technique can help farmers to predict the optimal harvesting state.

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## ***10. Publications related to the dissertation***

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