

# **A preliminary approach to assess peach fruit texture by Time-Resolved Spectroscopy (TRS)**

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## **Abstract**

**Most fruits can be modeled as a diffusive medium at visible and near-infrared wavelengths. The low absorption in this spectral range allows exploiting VIS/NIR spectroscopic techniques to probe non-destructively the internal food properties. Time-resolved Reflectance Spectroscopy (TRS), in particular, allows the separate but simultaneous estimation of absorption and scattering coefficients. Absorption and scattering properties give very different information about the medium investigated. The pigments and pulp constituents produce characteristic spectral features, so that absorption spectra determination gives information concerning the chemical composition. Light scattering inside the medium is due to local variation of the refractive index, and thus scattering spectrum provides information concerning the structural characteristics of the fruits. The possibility of applying TRS to assess the texture type of peach fruit was tested. To this purpose, an instrument for TRS, developed at Politecnico di Milano-Department of Physics, was exploited. At least two cultivars for each peach flesh phenotypes (melting, non melting, stony hard and slow melting) and a total of 30 fruits for each cultivar were analyzed over the spectral range 540-940 nm.**

**The absorption spectra exhibit high values around 550 nm, due to the anthocyanins' absorption features. Furthermore, an absorption peak is visible at 670 nm: this peak is linked the chlorophyll-a content and, then, gives an idea about the fruit ripeness (a higher chlorophyll content corresponds to less ripe fruit). With the exception of the cultivar 'Iride', particularly rich in anthocyanins, and 'Ghiaccio', a cultivar totally depigmented, the absorption spectra of all the samples are similar.**

**For what concerns the scattering properties of peaches, by considering the equivalent density and the scatter power Mie parameters, it is possible discriminates between three out of four texture type (melting, slow melting and stony hard). Further improvements may lead to a full discrimination in the future.**

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## **INTRODUCTION**

Until a few years ago, information on internal structure and properties of a fruit could be obtained only by destructive techniques, which were slow and prone to operational errors. These techniques could obviously be applied only to a limited number of samples, and the resulting information was extended to the whole batch of fruits, with

the inaccuracies that can easily be imagined, given the high levels of biological variability.

The need to assess the quality of each single fruit, in order to improve consumer satisfaction and thus industrial profitability, has pushed researchers to develop better non-invasive techniques (Lu and Peng, 2006).

Different techniques have been developed in connection with the quality parameter to be evaluated (Cen et al., 2012; Dahm and Dahm, 2001).

Both the absorption coefficient ( $\mu_a$ ) and the reduction of the scattering coefficient ( $\mu_s'$ ) may characterize adequately the optical properties of fruits and vegetables (Cen et al., 2012), but absorption and scattering properties of the fruit give very different information about the medium. Absorption is determined by the pigments and pulp constituents that produce characteristic spectral features in the visible and near infrared region, giving information on the chemical composition of the fruit. Scattering is due to local variation of the refractive index inside the medium. Microscopic changes in refractive index caused by membranes, air vacuoles or organelles deviate the photon paths and are ultimately responsible for light diffusion (Cubeddu et al., 2002). Scattering thus provides information concerning the structural characteristics of the fruits. In order to discriminate between the different flesh phenotypes of peaches, or more generally of fruit, it is essential to know the structural characteristics of the sample, and the scattering measurements could have a key role. For this reason scattering information has necessarily to be separated from the absorption information, which is possible with Time-resolved diffuse Reflectance Spectroscopy (TRS).

When a light beam (or streams of photons) impinges on a diffusive medium (the fruit), a small fraction is reflected from the surface (specular reflectance) while the majority of the light penetrates into the fruit. The penetrated light scatters and propagates in different directions in the fruit owing to the local changes in the index of refraction at the interfaces of different cellular structures. Some of the penetrated light is absorbed, some goes through the whole fruit and emerges from the opposite side (transmission), and some scatters back and re-emerges from a region close to the beam incident point: this is often called diffusive reflectance. In a diffusive medium such as a fruit, in the visible and near infrared spectral region, light scattering is stronger than light absorption, so that light can be scattered many times before being either absorbed or re-emitted from the medium. This phenomenon is called multiple scattering of light (Cubeddu et al., 2002). During this process photons are also absorbed, so that the scattering profile at the surface of the fruit is influenced by, or related to, both absorption and scattering properties of the fruit (Lu and Peng, 2006). The scattering probability per unit length for non-isotropic propagation of photons is described by the reduced scattering coefficient  $\mu_s' = (1 - g)\mu_s$ , where  $g$  is the anisotropy factor, that is the mean cosine of scattering angle and  $\mu_s = 1/l_s$ ,  $l_s$  being the photon mean free path between successive scattering events. A direct measure of the photon path length allows to know the effect of scattering, and, since the photon path length is directly related to the photon time-of-flight in the medium, the natural choice is to perform time-resolved measurements (Cubeddu et al., 2002).

TRS consists in determining the delay, the attenuation and the time broadening suffered by a short near infrared laser impulse propagating in a diffusive medium such as fruit pulp. The delay of the collected impulse depends on the light propagation speed inside the medium between source and detector, and the time broadening is a consequence of the different paths of the light inside the medium because of its diffusion (multiple scattering). Finally, the attenuation depends on absorption, which causes loss of photons,

and on diffusion, which scatters photons along directions different from the direction under consideration (Torricelli et al., 2008).

Compared to conventional optical methods, TRS presents several advantages. First, it allows measurements of the scattering properties of the pulp independent of those of the absorption spectrum. Second, TRS is insensitive to skin color, being most sensitive to internal features. Moreover TRS measurements probe a depth of at least 2 cm within the pulp (Cubeddu et al., 2002).

## **MATERIALS AND METHODS**

### **Peach samples**

The experiment was carried out during the 2011 harvest season.

Peaches belonging to the four flesh phenotypes (melting, slow melting, non melting and stony hard) were picked in July 2011 from the Experimental Orchard of the “Agency for Technology Experimentation and Agroenvironmental Research” (ASTRA), Azienda Agricola “Zabina” of Castel San Pietro (BO), and from the Experimental Orchard of the University of Milan, Azienda Agricola “F. Dotti” of Arcagna (LO). Peach samples were visually inspected and only those free of visual defects were selected for the experiment. During the travel, fruits were placed in peach nest trays, in order to prevent any damage. Table 1 reports the list of accession analyzed.

### **Ripening parameters**

Immediately after harvest, a total of 30 fruits from each accession were preliminarily classified into three maturity stages (less mature, medium mature, more mature), basing on the index of absorbance difference ( $I_{AD}$ ). These measurements were performed using the DA-Meter (Sintéleia Srl, Bologna, Italy). A total of 330 fruits were analyzed in 2011. Each sample (fruit) was individually numbered.

### **TRS system**

A system for TRS measurements of food quality, developed at the Politecnico di Milano-Department of Physics, was used. The schematic of the TRS setup is shown in figure 1. The light source is a super continuum fiber laser (SC450-6W, Fianium, UK) providing white-light picosecond pulses, adjustable in power by a variable neutral-density attenuator. A filter wheel loaded with 14 band-pass interference filters is used for spectral selection in the range 540-940 nm. Light is delivered to the sample by means of a multimode graded-index fiber. Diffuse remitted light is collected by 1 mm fiber. The light then is detected with a photomultiplier (HPM-100-50, Becker&Hickl, Germany) and the photon distribution of time-of-flight is measured by a time-correlated single-photon counting board (SPC-130, Becker & Hickl, Germany).

### **Data analysis**

The temporal profile of the TRS curve was analyzed using a model for the light propagation in diffusive media based on the solution of the Diffusion Equation (Martelli

$$\text{et al., 2009): } R(\rho, t) = A t^{-5/2} S(\mu'_s; t) e^{-\mu_a \nu t}$$

where  $R(\rho, t)$  is the number of photons per unit time ( $t$ ) and area remitted from the tissue at a distance  $\rho$  from the injection point,  $\rho$  is the source-detector distance (or inter-fiber distance) and  $\nu$  is the speed of light in the medium. This allows for the simultaneous estimate of  $\mu_a$  and  $\mu'_s$ .

After having retrieved the absorption and scattering coefficients at different wavelengths, concentrations of fruit constituents and structural parameters can be obtained. In particular, absorption spectra can be interpreted as a function of constituent concentrations by the Beer's law:

$$\mu_a(\lambda) = \sum_i C_i \varepsilon_i(\lambda) = C_{H_2O} \varepsilon_{H_2O} + C_{CHL-a} \varepsilon_{CHL-a}$$

On the other hand, structural parameters can be obtained from the scattering spectra following Mie theory (Mourant et al., 1997; Nilsson et al., 1998):

$$\mu_s'(\lambda) \approx a \lambda^{-b},$$

Where  $a$  and  $b$  are two parameters linked to the structural properties of the fruit: in particular,  $a$  is proportional to the density of the scattering centers and  $b$  depends on their size.

### Statistical data analysis

The data were tested for differences between flesh phenotypes using the one-way analysis of variance (ANOVA; general linear model) using IBM SPSS statistic software. The differences between flesh phenotypes were tested with Tukey's high significance difference (HSD) test at the 0.05 significance level.

## RESULTS AND DISCUSSION

### Absorption

The absorption spectra were constructed by plotting the value of  $\mu_a$  as a function of wavelength. Analysing the graphs obtained, it is evident that the absorption spectrum of the peaches between the wavelengths 540 and 940 nm is dominated, with few exceptions, by the anthocyanin peak around 540-580 nm (Fig. 2). In particular cultivar 'Iride' scored the highest values in this range (Fig. 2). This is due to the large amount of anthocyanin in the pulp of this cultivar, which is, in fact, particularly red colored.

The water peak, around 970 nm, is not visible in the measurements being outside the sensitivity range of the photomultiplier used; however, it's possible to see an increasing of absorption around 940 nm in every cultivar.

A significant absorption peak at 675 nm, corresponding to chlorophyll-a, is found in all the cultivars, with the exception of 'Ghiaccio' (literally 'Ice'), which is characterized by being completely devoid of chlorophyll pigment and by having skin and flesh totally white cream-colored.

### Scattering

A summary of the values of the reduced scattering coefficients based on the average of the 30 fruits tested for every cultivar is showed in figure 3. In general, a greater slope in the reduced scattering profile (like in 'Big Top') indicates the presence of smaller structures (related to parameter  $b$ ). 'Glohaven' shows a very high dispersion of the value of scattering, from 4 to 20  $\text{cm}^{-1}$ . This is due to the fact that some peaches were softer to the touch (thus riper) than others. Soft fruits in fact tend to have a broader scattering profile than firmer fruits (Peng and Lu, 2004).

Figure 4 shows parameter  $a$  (on the x-axis), proportional to the density of scattering centers, and parameter  $b$  (on the y-axis), proportional to the size of the scattering centres. Analysis of this graph reveals that the scattering technique has limited capabilities to discriminate between the peach flesh phenotypes. As a matter of fact, melting, slow

melting and stony hard peaches are indeed clustered in quite distinct groups, but the other non-melting peach varieties overlap.

This application therefore, although very interesting because it can cluster three flesh phenotypes out of four, cannot be considered decisive for the discrimination of the different flesh phenotypes in peach.

Indeed, by analyzing the two parameters separately it results that, if the “flesh type” is used as the discriminating parameter for statistical analysis, the “a” parameter discriminates three flesh phenotypes out of four (tab 2), but it does not discriminate melting ( $16.27 \text{ cm}^{-1}$ ) from non-melting ( $16.35 \text{ cm}^{-1}$ ) phenotypes. If the cultivar is used as discriminating parameter, cultivars of the same flesh type did not fall in the same subset, apart for the stony hard (tab. 3). For “b” parameter, with flesh type as discriminating parameter, it is possible to discriminate again three phenotypes out of four (tab. 4); but “b” does not discriminate slow melting (0.36) from non-melting (0.37). Again if the cultivar is the discriminating parameter, cultivars of the same flesh type did not fall in the same subset apart for the stony hard (tab. 5).

## CONCLUSIONS

The possibility of applying the TRS technique to assess the texture phenotype of peach fruit has been tested.

The TRS technique allowed separating the effects of absorption and scattering properties. The absorption spectrum is dominated by peaks around 550 nm, which is close to the anthocyanins' peak. A second peak is visible at 675 nm, which corresponds to the peak of chlorophyll-a. This peak gives an idea about the fruit ripeness (a higher peak corresponds to less ripe fruit). With the exception of the cultivar ‘Iride’, particularly rich in anthocyanins, and ‘Ghiaccio’, a cultivar totally depigmented, the absorption spectra of all the samples are similar.

For what concerns scattering properties, by considering the two Mie parameters, *a* and *b*, it is possible to assert that this technique is not able to distinguish different cultivars on the basis of the scattering parameter. These preliminary results suggest that TRS is not useful for our purpose. TRS could be used on a population of cultivars to study the nature of their flesh, but further studies need to be done on this topic.

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## **Tables**

Table1. List of all the accessions analyzed

<b>CULTIVAR</b>	<b>HARVEST DATE</b>	<b>TEXTURE TYPE</b>
Alice Col	13 July 2011	NM
BO000200006	28 July 2011	NM
Iride	8 July 2011	NM
Dixired	8 July 2011	M
Glohaven	22 July 2011	M
Redhaven	15 July 2011	M
Ghiaccio	21 July 2011	SH
IFF 331	30 July 2011	SH
Big Top	15 July 2011	SM
Rich Lady	15 July 2011	SM
Vistarich	19 July 2011	SM

Tab. 2.a parameter ( $\text{cm}^{-1}$ ) and flesh type

HSD di Tukey, flesh type	N	subset		
		1	2	3
melting	90	16.27	17.60	18.56
non melting	90	16.35		
slow melting	90			
stony hard	60			

Tab. 3. a parameter ( $\text{cm}^{-1}$ ) and cultivar

HSD di Tukey cv (flesh type)	N	subset				
		1	2	3	4	5

Glohaven (M)	30	13.04				
Iride (NM)	30	13.72	13.72			
Rich Lady (SM)	30		15.02			
BO00020006 (NM)	30			16.92		
Redhaven (M)	30			17.07	17.07	
Vistarich (SM)	30			17.42	17.42	
Alice Col (NM)	30			18.39	18.39	
IFF 331 (SH)	30			18.45	18.45	
Ghiaccio (SH)	30			18.67	18.67	18.67
Dixired (M)	30				18.72	18.72
Big Top (SM)	30					20.38

Tab. 4. *b* parameter and flesh type

HSD di Tukey flesh type	N	subset		
		1	2	3
melting	90	.27		
slow melting	90		.36	
non melting	90		.37	
stony hard	60			.54

Tab. 5. *b* parameter and cultivar

HSD di Tukey cv (flesh type)	N	subset					
		1	2	3	4	5	6
Iride (NM)	30	.18					
Glohaven (M)	30	.21	.21				
Dixired (M)	30		.28	.28			
Rich Lady (SM)	30		.30	.30	.30		
Redhaven (M)	30			.31	.31	.31	
Vistarich (SM)	30				.37	.37	
Alice Col (NM)	30				.38	.38	
Big Top (SM)	30					.40	
Ghiaccio (SH)	30						.53
BO00020006 (NM)	30						.53
IFF331 (SH)	30						.55

## **Figures**

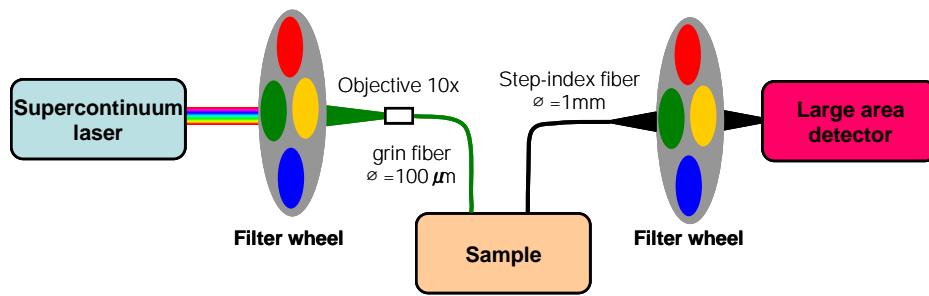


Fig. 1. Scheme of the TRS instrumental setup.

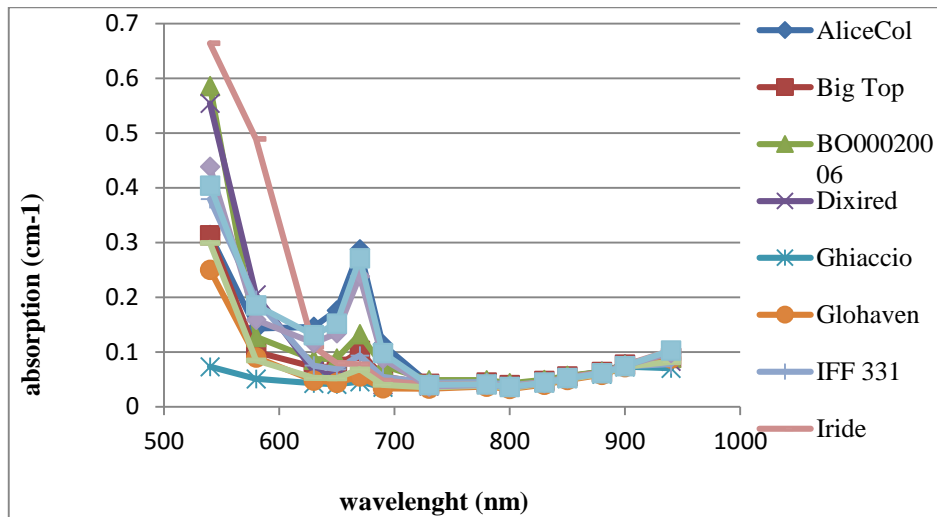


Fig. 2. Absorption spectra for different flesh phenotypes (average of 30 samples per each cultivar).

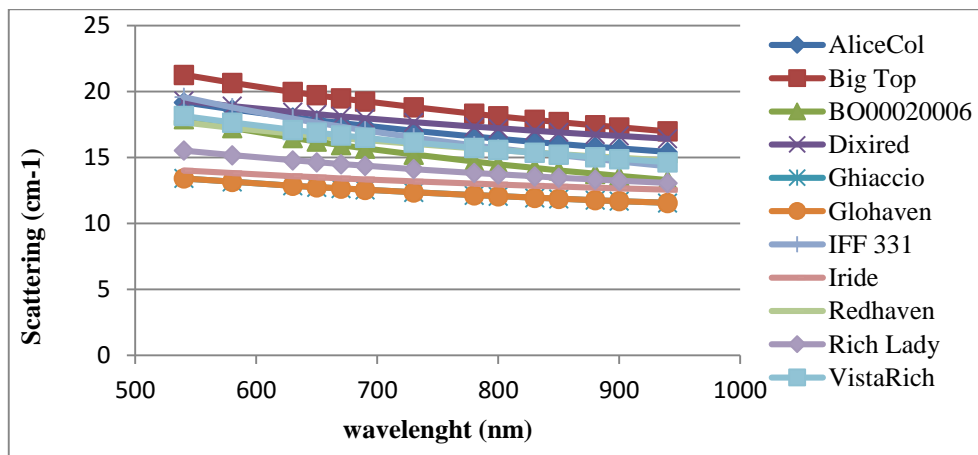


Fig. 3. Reduced scattering spectra for different flesh phenotypes (average of 30 samples per each cultivar).



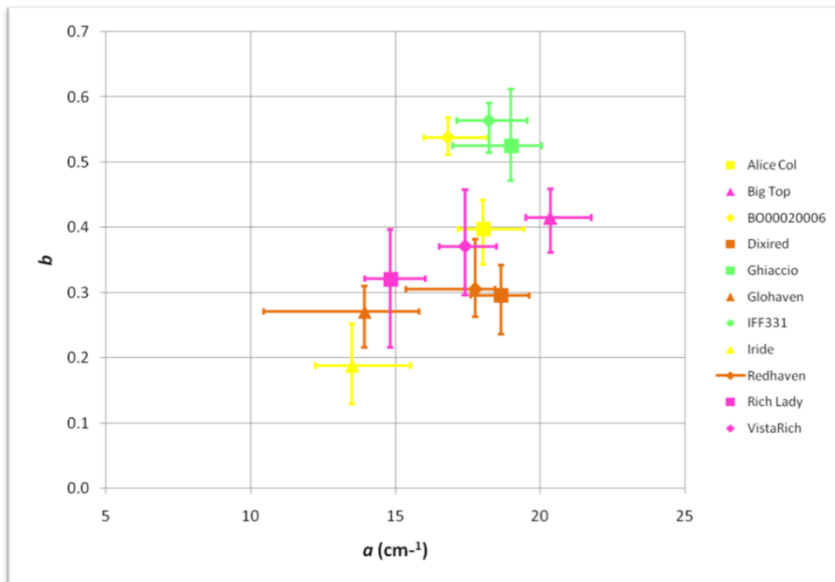


Fig. 4. Scatter plot of the equivalent Mie parameters for the different flesh phenotypes