Postharvest Biology and Technology 86 (2013) 494–501

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Measurement of the light absorption and scattering properties of onion skin and flesh at 633 nm

Weilin Wang, Changying Li*

Bio-Sensing and Instrumentation Laboratory, College of Engineering, the University of Georgia, 712F Boyd Graduate Studies, Athens, GA 30602, USA

ARTICLE INFO

Article history:
Received 15 December 2012
Accepted 26 July 2013

Keywords:
Onion
Optical properties
Monte Carlo
Inverse adding-doubling
Scattering
Absorption

ABSTRACT

Understanding the optical properties of onion tissues is essential to applying optical methods for onion quality inspection. This study estimated the optical properties of dry skin, wet skin, and flesh of red, Vidalia sweet, white, and yellow onions at the wavelength of 633 nm. The total diffuse reflectance, total transmittance, and collimated transmittance of single-layer onion tissues were measured by spectroscopic systems. Based on the measured data, the absorption coefficient μ_a and the reduced scattering coefficient μ_s' of onion tissues were calculated using the inverse adding-doubling method. The results indicated that the dry and wet skins had significantly higher μ_a and μ_s' than the flesh at 633 nm. For both skins and flesh, the μ_a varied between cultivars, while the differences of the μ_s' between cultivars were less profound. All types of onion tissues were high-albedo materials at 633 nm. Using the calculated optical properties, Monte Carlo simulations were performed to model the light propagation in 25 different scenarios of multi-layer onion tissues for four cultivars, respectively. The results showed that the incident light at 633 nm would lose 99% of its energy within 6 layers in any of the simulated scenarios, and the light penetrated more layers in the sweet onions than in the other three cultivars. This work provided fundamental understanding of the optical properties of onion tissues and the light propagation in onion bulbs at 633 nm. The investigation of the onion optical properties will be extended to a broader spectrum in the future.

1. Introduction

Onion (*Allium cepa* L.) is an important fresh vegetable which had an approximate production value of $1 billion in the United States in 2012 (USDA, 2013). Mature onions are well ripened bulbs that have concentric layers of dry skins and flesh scales. The flesh scales of onions are edible parts while outer dry skins can be used to make natural food color additive (Block, 2010). Onion skins were also found to be good sources of other by-products such as flavonoids (Ko et al., 2011). The health benefits of onion consumption, such as antifungal, antibacterial, antitumor, antiinflammatory, and antithrombotic, have been widely recognized and advertised in recent decades (Griffiths et al., 2002; Corzo-martíez et al., 2007).

Nowadays, onion buyers have high expectation of quality. In addition to the nutritional values of onions, other factors such as the appearance, flavor, and existence of defects are also important to consumers (Griffiths et al., 2002). Thus, it is critical to monitor all key onion quality factors throughout the onion harvest and marketing chain. Currently, although some onion packing houses have employed automated systems to sort onions based on the size, other quality properties such as disease infection and surface blemishes have to be evaluated by trained human inspectors, which is often inefficient and subjective. Particularly, human visual inspection is not capable of evaluating the internal quality attributes of onions. Thus, it is necessary to develop more versatile nondestructive techniques to improve the efficacy and efficiency of onion quality inspection.

Among various sensing techniques, optical methods such as imaging, spectroscopy, and spectral imaging are attractive to food industry because they are fast, safe, nondestructive, and low-cost (Zude, 2008; Cubero et al., 2011). Several optical techniques were reported for onion quality inspection. Birth et al. (1985) applied near-infrared spectroscopy to predict dry matter content of intact onions, and Wang et al. (2012) employed spectral imaging technology to differentiate sour skin-infected onions from healthy ones. The main principle of these techniques was to observe the interaction between the incident light and the onion, which was determined collectively by the optical properties of onion tissues and the properties of the incident light.

* Corresponding author. Tel.: +1 706 542 4696; fax: +1 706 542 2475.
E-mail address: cyli@uga.edu (C. Li).

0925-5214
http://dx.doi.org/10.1016/j.postharvbio.2013.07.032

Onions are biological tissues and their key optical properties include: the absorption coefficient (μ_a), scattering anisotropy (g), scattering coefficient (μ_s), and the refractive index (n) (Tuchin, 2007). The g and μ_s could be combined into one parameter called reduced scattering coefficient ($\mu_s' = \mu_s(1-g)$). These optical properties are requisite parameters in the radiative transport equation to describe the light reflection, propagation, and attenuation on/in the onion tissue. If the optical properties of onion tissues are known, mathematical methods (such as Monte Carlo simulations) can be applied to numerically model the light-onion interaction, which could provide useful references for designing appropriate optical systems to monitor onion quality. In addition, the optical properties of onion tissues could be indicative to their physical/chemical properties. Therefore, to make optical measurements effectively, it is important to understand and quantify the optical properties of the onion tissues. However, although the physical and chemical attributes of onions have been widely investigated (Maw et al., 1996; Abhayawick et al., 2002; Rodríguez Galdón et al., 2009), the key optical properties (μ_a and μ_s) of onion tissues have never been reported.

Various methods have been reported for measuring the optical properties of biological tissues in the biomedical area (Cheong et al., 1990; Tuchin, 2007). These methods can be classified into "*ex vivo*" and "*in vivo*" categories in terms of the way of the sample preparation (Kim and Wilson, 2011). The *ex vivo* method, which requires samples of certain shapes and sizes, is often based on the transmittance and reflectance measurements. Then, the optical properties of the measured tissue can be estimated by using computational methods such as the adding-doubling (Prahl et al., 1993; Pickering et al.,1993) or Monte Carlo (Wang et al., 1995) in their inverse forms. The *in vivo* method is often based on the approximations of radiative transport theory by using the diffuse reflectance or backscattering measurements directly made on the sample in the spatial, temporal, or frequency domains (Kim and Wilson, 2011). In the past decade, the optical properties of a number of food products have been investigated by using *ex vivo* methods such as the apple (Saeys et al., 2008) and mandarin (Fraser et al., 2003), or by *in vivo* methods, such as milk and juice (Qin and Lu, 2007), apple (Qin and Lu, 2008, 2009), and beef (Xia et al., 2007). Generally, the *ex vivo* method is used to obtain the knowledge of the optical properties of a food product, while the *in vivo* method could be used for online inspection of food quality.

Despite the difference, both *ex vivo* and *in vivo* methods require a collimated incident light with a very small beam size (Tuchin, 2007). For this reason, the coherent laser has been considered as one of the standard light sources for measuring the optical properties of tissues. As a relatively inexpensive and reliable light source, laser has short emission time and emits monochrome light beam with high intensity. Due to these advantages, many laser-based optical systems have been reported to measure the optical properties of food products (Cubeddu et al., 2001; Fraser et al., 2003) or to predict certain quality parameters of food items by measuring their scattering profiles under the laser irradiation (Lu and Peng, 2007; Qing et al., 2008; Romano et al., 2011).

This study was aimed to quantitatively measure the optical properties of the tissues of four common types of onions (red, sweet, white, and yellow) using a coherent laser. Specific objectives of this research were to:

- Estimate the absorption coefficient μ_a, the reduced scattering coefficient μ_s', and the scattering anisotropy g of the onion tissues using laser and integrating shpere based systems.
- Compare the optical properties of the onion skin and flesh.
- Evaluate the differences of the optical properties between four cultivars of onion tissues.
- Model the light propagation in multi-layer onion tissues using Monte Carlo simulation.

2. Materials and methods

2.1. Sample preparation

Onions of four common types: red (cv. Salsa), Vidalia sweet (cv. Century), white (cv. White Cloud), and yellow (cv. Granero) were used in this study. The sweet onions (short-day onions) were harvested from the state of Georgia, USA in May, 2011 and others (long-day onions) were harvested in the state of Idaho, USA in October, 2011. Onions were stored 2–4 months in a cold storage room ($2 \pm 1\,^{\circ}C$) in the Vidalia Onion Research Lab at the University of Georgia. Onions were taken out from the cold room 2–3 h prior to the test and a box fan was used to remove the condensed moisture on the onion surface under room temperature ($22 \pm 1\,^{\circ}C$).

Three types of onion tissues were examined: dry outer skin, wet outer skin, and flesh. The dry outer skin is the onion outer scale(s) dried during harvesting and storage, which protects flesh scales from pathogens in the surrounding environment. It has to be noted that the drying of the outer scales is a slow process accompanied by the biochemical changes of pigments and carbohydrate compositions of cell walls of onion tissues (Brewster, 2008). Thus, in most cases, there is an onion scale whose status is at the intermediate stage between the dry skin and flesh scale, which is called wet outer skin. For the sake of brevity, we used dry skin to represent the onion dry outer skin, and wet skin to indicate the onion wet outer skin in the following sections of this paper.

In each cultivar, 20 large size onion bulbs (76–102 mm in diameter) were tested. All onions were manually selected and inspected to be disease-free and with intact dry skins. Onion bulbs were cut in half longitudinally (from neck to root). In each half of the onion, a set of onion tissues were cut from the equatorial area. At each set of onion tissues, the surface dry skin, the wet skin, the first layer of flesh scale, and the second layer of flesh scale were selected. Two flesh scales instead of one were examined so that the difference between the two layers of scales could be evaluated. Then, selected onion scales were cut into square pieces (30×30 mm) and the flesh samples were shaved to slabs using a razor blade. In total, 640 pieces of onion tissue samples (4 cultivars $\times$ 20 onions $\times$ 2 sample sets $\times$ 4 pieces of tissues) were measured.

2.2. Thickness and refractive index of the sample

The thickness (d) of each sample was measured using an electronic micrometer (model 35-025, iGaging, San Clemente, CA, USA) with an accuracy of ± 0.001 mm in the central area of the tissue. For onion flesh samples, two 1-mm thick glass slides were used to sandwich the sample so that the metal tip of the micrometer could not cut into the tissue, and the thickness of the glass slides were deducted from the measurement later. Three replicates were made for each sample and the average value was reported. The refractive indices (n) of samples were measured by a Leica Abbe refractometer (model Abbe Mark II, Reichert, Inc. Depew, NY, USA). For each cultivar, eight pieces of dry skins and eight pieces of flesh tissues were randomly selected and measured. The refractive index of dry skins were measured by directly placing the sample at the prism of the refractometer and using distilled water as the contact liquid. The refractive index of the onion flesh was estimated by testing the onion juice extracted from the tissue. For the dry skin and flesh samples, each of them was measured three times. Then, for each onion group, the mean refractive index was calculated and used in the later calculations.

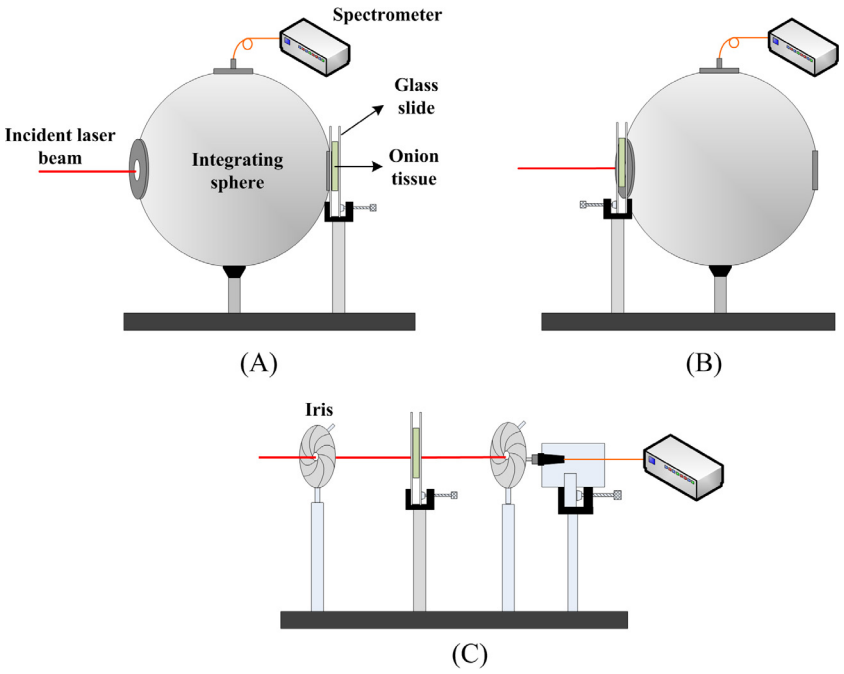

Fig. 1. The schematic of the system configurations for measuring the total reflectance (A), the total transmittance (B), and the collimated transmittance (C).

In practice, it is difficult to measure the refractive index for some wet skin samples by using the Abbe refractometer since they were too thick to be measured as a film but not fleshy enough to be measured by using its juice. It was also observed that, depending on its moisture content, the refractive index of the wet skin of an onion was either close to the one of its dry skin or close to that of its flesh. Thus, based on their thickness values, the refractive indices of onion wet skins were approximated individually to those of dry skins (thin as dry skin) or those of flesh (thick as flesh).

2.3. Reflectance and transmittance measurements

The spectroscopic system (Fig. 1A–C) was assembled to measure the total reflectance, total transmittance, and collimated transmittance spectra of onion samples at 633 nm. The system mainly consisted of an integrating-sphere (model 4P-GPS-060-SF, Labsphere, North Sutton, NH, USA) and a Vis-NIR spectrometer (model USB4000, Ocean Optics, Dunedin, FL, USA). Based on the availability of the device, a low power helium-neon (HeNe) laser (0.8 mW, 633 nm) (model HRP008-1, Thorlabs, Newton, NJ, USA) was used in this study. This type of laser has been widely used for studying the optical properties of biological tissues (Tuchin, 2007; Kim and Wilson, 2011).

The interior wall of the integrating sphere is coated by Spectraflect material that has a reflectivity of 98% at 633 nm. The internal diameter of the sphere is 152.4 mm and it has four ports (25.4 mm in diameter) at 0°, 90°, 180°, and the north pole. The aperture to surface area ratio of the sphere is less than 3%. The ports at 0° and 180° were used as the entrance and exit, respectively. An optical fiber with a diameter of 400 μm and numerical aperture of 0.37 (model M32L02, Thorlabs, Newton, NJ, USA) was used to deliver the light to the spectrometer from the port at 90° of the sphere. The laser head was placed on a kinematic V-clamp mount (model C1503, Thorlabs, Newton, NJ, USA) so that its position can be precisely adjusted at the horizontal and vertical axes. To align the laser and the sphere, two circular cross targets (25.4 mm in diameter) were printed on transparent films and placed on the entrance and the exit ports, respectively. Then, the positions and orientations of the laser head and the integrating sphere were adjusted until the incident laser beam can go through the center spots of both the entrance and the exit ports. The wavelength accuracy of the spectroscopic system was calibrated by using a pencil style krypton calibration lamp (model 6031, Oriel Instruments, Stratford, CT, USA).

To measure the total reflectance, the sample was placed behind the exit port while the entrance port was open (Fig. 1A). To measure the total transmittance, the sample was placed in front of the entrance port and the opposite exit port was blocked by a Spectraflect plug (Fig. 1B). The reference signal (*Ref*) was measured by using the setup in Fig. 2A, but replacing the onion sample with a certified 99% diffuse reflectance Spectralon target (model AS-00158-060, Labsphere, North Sutton, NH, USA). The dark signal (*D*) of the system was measured by covering all ports of the integrating sphere. Then, the total diffuse reflectance (R_{raw}) and the total transmittance (T_{raw}) were converted to the relative total reflectance (R) and relative total transmittance (T) by:

$$R = \frac{R_{raw} - D}{Ref - D} \quad (1)$$

$$T = \frac{T_{raw} - D}{Ref - D} \quad (2)$$

The collimated transmittance was measured following the setup recommended by Prahl (2011) (Fig. 1C). The laser beam was aligned with the center of the detecting optic fiber. An iris was placed between the laser head and the sample to adjust the intensity of the laser beam to avoid saturation of the spectrometer. Another iris in front of the detector was used to block the scattered light from entering the detector. The relative collimated transmittance (T_c) was obtained by:

$$T_c = \frac{Tc_{raw} - Dark}{Tc_{ref} - Dark} \quad (3)$$

where Tc_{ref} was the original light intensity of the incident laser beam and Tc_{raw} was the intensity of the laser beam transmitted through the onion tissue sample. The dark signal (*Dark*) of the spectrometer was measured when the probe of the spectrometer was covered by a black cap.

In all measurements, the onion tissue was sandwiched by two pieces of 70 × 70 × 2 mm (width × height × thick) borosilicate

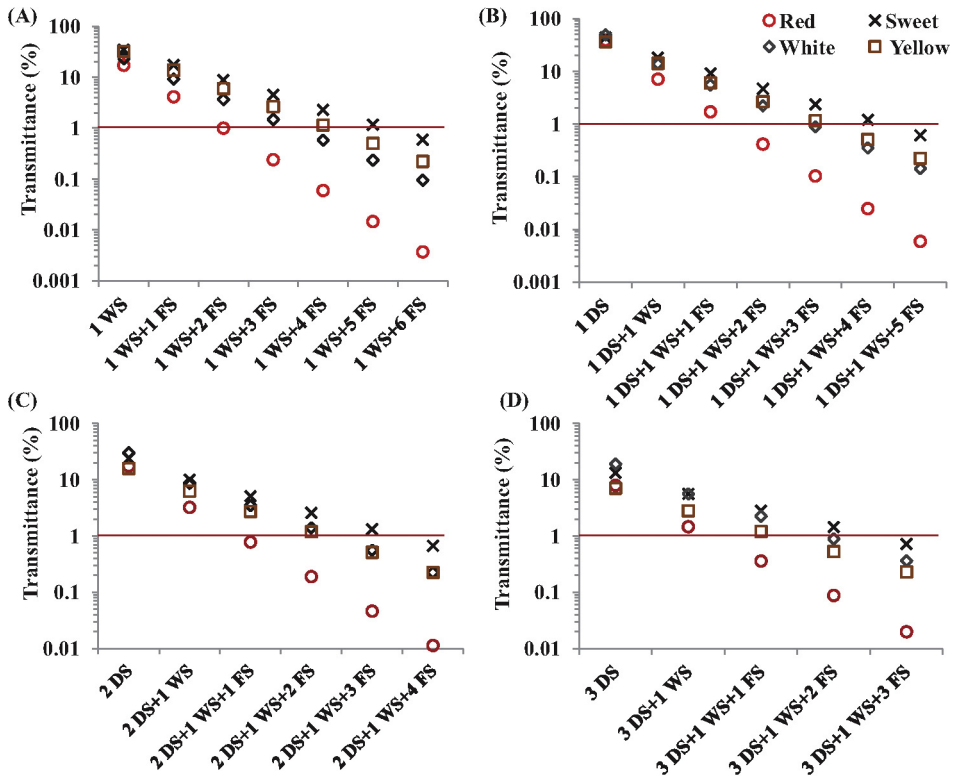

Fig. 2. The relative transmittance of the light propagation (at 633 nm) in multi-layer onion tissues in the scenarios of: no dry skin (A), 1 layer of dry skin (B), 2 layers of dry skins (C), and 3 layers of dry skins (D). The meaning of the labels on the horizontal axis: DS – outer dry skin, WS – wet skin, and FS – flesh scale.

glass slides (model BOROFLOAT 33, SCHOTT North America, Inc. Louisville, KY, USA). BOROFLOAT glass was used because of its good homogeneity and high transmittance (>90%) at the visible range. The refractive index of the BOROFLOAT 33 glass is 1.47 at 633 nm. A U-shape clamp and a C-shape clamp were used to apply slight pressure on the glass slides so that the onion tissue formed a flat slab. The whole system was enclosed in a dark chamber covered by a black cloth. All measurements were performed at room temperature ($22 \pm 1\,^{\circ}C$).

2.4. Estimating the absorption and scattering coefficients

The inverse adding-doubling (IAD) program provided by Prahl (2011) was used to calculate the absorption and scattering coefficients for each onion tissue sample. The IAD method has been widely used for estimating the optical properties of biological tissues due to its flexibility and reliability in measuring tissues with various albedos and optical depths (Tuchin, 2007). The fundamental principle of the IAD method is to iteratively solve the stationary (steady-state) radiative transfer equation based on the measured reflection and transmittance values of the sample (Prahl et al., 1993). As the mathematical description of continuous wave light propagation in a medium, the stationary radiative transfer equation (Tuchin, 2007) can be described as:

$$\frac{\partial I(r,\hat{s})}{\partial s} = -(\mu_a + \mu_s)I(r,\hat{s}) + \frac{\mu_s}{4\pi}\int_{4\pi} p(s,\hat{s})I(r,\hat{s})d\Omega \quad (4)$$

where the μ_a is the absorption coefficient, μ_s is the scattering coefficient, $I(r,\hat{s})$ is the average flux density at the point r along the direction $\hat{s}$, $d\Omega$ is the unit solid angle for the direction $\hat{s}$, $p(s,\hat{s})$ is the phase function of the angular distribution of the scattered light, which describes the probability of the polar deflection angle of the photon after each scattering event. The phase function used in all IAD calculations was Henyey-Greenstein (HG) phase function (Prahl et al., 1993), described as $\rho(\theta) = (1/4\pi)(1 - g^2)/(1 + g^2 - 2g\cos\theta)^{3/2}$. The HG phase function approximates the angular distribution of scattering in biological tissues depending on the mean cosine of scattering angle (the scattering anisotropy coefficient $g = \langle\cos\theta\rangle$) (Tuchin, 2007).

Using the IAD program, μ_a and μ_s' can be calculated from the measured R and T values, with a fixed g value. The g value can also be estimated by the IAD method when the collimated transmittance T_c is included. In our work, since the anisotropy of onion tissues was unknown before this study, it was first estimated by using the IAD program with three inputs: T, R, and T_c. A small part of data sets (42 out of 640) were removed because of the invalidity of T_c (either T_c was greater than T or the sum of T_c and R was equal to or greater than 1). When the g had been estimated, the IAD program with two inputs of T and R were used to calculate μ_a and μ_s' again. Once g, μ_a, and μ_s' were estimated, μ_s was calculated by $\mu_s = (\mu_s'/(1 - g))$.

It should be noted that the IAD program is often prone to error in estimating the anisotropy due to the difficulty of measuring T_c accurately (Prahl, 2011). In this study, to compensate the individual bias in the estimated g values, the mean g of each group of onion tissues was calculated and then was used for all samples in the group in later calculations. The more accurate quantifications of anisotropies of onion tissues could be made by using other specific measuring approaches in the future, such as measuring the angular distribution of the scattered light by rotating a collimated detector around the sample enclosed in a cylinder tank with index-matching liquid (Kim and Wilson, 2011).

Analysis of variance (ANOVA) was used to evaluate the differences between four types of onion tissues and between different onion cultivars. The significance of the differences between the means of the measured optical properties of different groups were evaluated by using Tukey's HSD (honest significant difference) tests at the significance level of 0.05. All ANOVA tests were conducted

Table 1
The thickness and refractive indices (mean ± SD) of onion tissues in four cultivars.

	Tissue type	Red onion	Sweet onion	White onion	Yellow onion
Thickness (mm)	Dry skin	0.071 ± 0.019	0.087 ± 0.033	0.083 ± 0.025	0.083 ± 0.023
	Wet skin	0.827 ± 0.566	0.468 ± 0.517	0.638 ± 0.802	0.391 ± 0.489
	First flesh	2.833 ± 0.554	3.941 ± 0.734	3.708 ± 0.984	2.962 ± 0.684
	Second flesh	3.317 ± 0.398	3.995 ± 1.109	4.049 ± 0.745	3.392 ± 0.833
Refractive index (n)	Dry skin	1.3345 ± 0.0006	1.3371 ± 0.0074	1.3345 ± 0.0004	1.3337 ± 0.0005
	Flesh	1.3450 ± 0.0007	1.3410 ± 0.0011	1.3413 ± 0.0003	1.3413 ± 0.0015

by using the GLM (general linear models) procedure in SAS (v9.2, SAS Institute Inc. Cary, NC, USA).

2.5. Validation procedure

The system was verified by using the phantoms made from a standard scattering material (Intralipid-20%, Sigma Aldrich, St. Louis, Missouri, USA) and two absorption dyes (Nigrosin and Naphthol Green B, Sigma Aldrich, St. Louis, Missouri, USA). In the validation test, the Intralipid-20% was first diluted to one percent by distilled water, and then the absorbers were added to make three different types of liquid solutions. Liquid solutions were added into a quartz glass cuvette with a 10 mm light path to make the phantoms. The cuvette had 40 × 40 mm side walls which can cover the entire sampling port of the integrating sphere. The T and R of the phantom were measured by the system respectively (5 replicates for each type of phantom). Then, the μ_a and μ_s' of the phantoms were estimated by the IAD program using $g = 0.73$, in which the g was calculated by using the empirical equation ($g(\lambda) = 1.1 - 0.58\lambda$) established by Van Staveren et al. (1991). The estimated values were compared with each other and with the values reported by Ninni et al. (2011).

2.6. Monte Carlo simulation of light propagation in multi-layer onion tissues

Monte Carlo simulations were performed to model the light propagation in multi-layer onion tissues. The "MCML" program provided by Wang et al. (1995) was used for the simulation. Typically, a mature onion bulb has 1–3 layers of dry skins, one layer of wet skin, and a number of flesh scales. Thus, onion models with multiple layers were simulated with the presence of 0–3 layers of dry skins. All examined scenarios had one layer of wet skin and 1–6 flesh scales, which were gradually included in the model. The simulations were stopped at the seventh layer since light rarely can penetrate deeper than that. In each Monte Carlo simulation, 500,000 photons were launched, and the spatial resolution of radial distance and tissue depth was 0.01 mm. The number of grids for the radial and tissue depth was 1000. To simplify the simulation, the onion scales were assumed to be slabs with the mean thickness value listed in Table 1 and the tiny gaps between the inside scales of onions were ignored.

3. Results

3.1. Thickness and refractive indices of onion scales

The thickness of most onion dry skins tested in this study was 0.07–0.09 mm and the thickness of onion flesh tissue was 3–5 mm (Table 1). As expected, the average thickness values of onion wet skins (0.391–0.827 mm) were in-between those of the dry skin (0.071–0.087 mm) and the flesh (2.833–4.049 mm). On average, the dry skins of the tested red onions were about 15% thinner than those of other cultivars, and the flesh scales of red and yellow onions were about 15–20% thinner than those of sweet and white onions.

Generally, the measured refractive indices of onion flesh (1.341–1.345) were in agreement with the values (1.343–1.351) reported by Foskett and Peterson (1950). For either onion dry skin or onion flesh, the ratio of SD to mean of the refractive index was very small (<0.5%). No statistical difference was observed in flesh or dry skin between cultivars in their refractive indices with the exception of red onion flesh. Since the difference was minute (≈0.003), it was ignored. The averaged values (1.335 for dry skin and 1.342 for flesh) were used for all samples. A threshold value 0.5 mm was arbitrarily selected based on the histogram of the thickness values of the tested wet skins. If a wet skin was thicker than 0.5 mm, the refractive index value for flesh (1.342) was used for it; otherwise, the value of dry skin (1.335) was used.

3.2. System validation

On average, the estimated μ_s' of the phantom showed 3.1% difference to the μ_s' value of Intralipid 20% reported by Ninni et al. (2011). The results of the validation tests showed that the system successfully separated the μ_a and the μ_s' for the three types of phantoms. The estimations of the μ_a and μ_s' for each type of phantom had standard deviations of 1.6–6.7% (on average 3.2%). Based on the two tailed t-tests at the significance level of 0.05, the mean μ_s' of three types of phantoms were not significantly different from each other, while their mean μ_a were significantly different ($p < 0.001$). Overall, the accuracy of the system in estimating the μ_a and μ_s' was comparable to other reported studies (Pickering et al., 1993). As discussed by Pickering et al. (1993), the error of the IAD estimation could be caused by the light loss at the rim of the sample and by the interference between the glass slides.

3.3. Scattering anisotropy

The estimated anisotropies of onion tissues at 633 nm (Table 2) varied from 0.35 to 0.73 in different groups, which were smaller than those of regular human tissues (0.8–1) (Tuchin, 2007) and close to those of apples (0.6–0.8) (Saeys et al., 2008). The anisotropies of onion dry skins were lower than those of flesh, except for yellow onions. No significant difference was found between the first and the second flesh scales. The onion wet skins did not show consistent patterns in different cultivars. For red and white onions, the anisotropies of their wet skins were significantly higher than those of dry skins, while not significantly different from those of flesh scales. For sweet and yellow onions, however, the differences between the anisotropies of the wet skins and the dry skins and flesh were not distinctive. This inconsistency can be explained by the data shown in Table 1: the wet skins of red and white onions were thicker than those of sweet and yellow onions, which indicated that the physical characteristics of their wet skins could be more similar to those of flesh scales than those of sweet and yellow onions. These results indicated that the anisotropies of onion tissues could be significantly different between cultivars and between different types of tissues. Therefore, the mean anisotropy of each type of onion tissue in each cultivar was computed and used in later calculations. Since the anisotropies of the first and second flesh

Table 2
The means and standard deviations of the estimated anisotropies of onion tissues at 633 nm.

	Tissue type	Red onion	Sweet onion	White onion	Yellow onion
g	Dry skin	$0.589 \pm 0.023^{A,b}$	$0.474 \pm 0.034^{B,b}$	$0.371 \pm 0.035^{B,b}$	$0.545 \pm 0.046^{AB,a}$
	Wet skin	$0.720 \pm 0.025^{A,a}$	$0.589 \pm 0.045^{AB,ab}$	$0.509 \pm 0.032^{BC,a}$	$0.421 \pm 0.047^{C,ab}$
	First flesh	$0.686 \pm 0.015^{A,a}$	$0.727 \pm 0.036^{A,a}$	$0.511 \pm 0.025^{B,a}$	$0.499 \pm 0.045^{B,ab}$
	Second flesh	$0.691 \pm 0.016^{A,a}$	$0.599 \pm 0.042^{AB,ab}$	$0.560 \pm 0.028^{B,a}$	$0.354 \pm 0.037^{C,b}$

Upper case letters in the superscripts indicate the significant differences between the four onion cultivars ($p < 0.05$), and lower case letters indicate the significant differences between the four types of onion tissues ($p < 0.05$).

scales were not significantly different, they were averaged and used in later computations.

3.4. Absorption and reduced scattering coefficients

Table 3 shows the means and standard deviations of the estimated μ_a and μ_s' values of onion tissues. Onion dry skins had strongest light absorption at 633 nm in red onions ($\mu_a = 1.97 \times 10^3\ m^{-1}$), which was significantly higher than those of the other cultivars. The colorless dry skins of white onions showed the lowest μ_a value ($0.5 \times 10^3\ m^{-1}$), which was significantly lower than that of yellow onions. The difference between the μ_a of sweet and white onions was not statistically significant. The average μ_s' of dry skins were from $18.53 \times 10^3\ m^{-1}$ (white onion) to $22.47 \times 10^3\ m^{-1}$ (yellow onion) at 633 nm. The μ_s' of the dry skin of red, sweet, white, and yellow onions were about 10, 25, 37, and 16 times of their corresponding μ_a, respectively. No significant difference was observed between the μ_s' of different cultivars.

As illustrated in Table 3, the μ_a and μ_s' of the onion wet skins were in-between those of dry skins and flesh, respectively. The average μ_a of wet skins were about 20–60% of those of the corresponding dry skins, and 5–10 times higher than those of flesh. The average μ_s' of wet skins was about 10–25% of that of dry skins, and 2–18 times higher than that of flesh. Statistical data suggested that the onion wet skin had distinct optical properties from the dry skin and the flesh scale in all cultivars. Thus, it should be considered as a separate type of medium in optical models of onions.

The average estimated μ_s' values of onion flesh were from $0.032 \times 10^3\ m^{-1}$ to $0.117 \times 10^3\ m^{-1}$, which were significantly smaller (13–41 times) than those of the dry skin. The average μ_a of the flesh scale of red onions was significantly higher than that of the other three cultivars, while no significant difference was found between the other three cultivars. Statistical tests did not reveal any significant difference between the first and the second flesh scales of the μ_a in all examined cultivars. The average μ_s' values of onion flesh were from $0.15 \times 10^3\ m^{-1}$ to $0.66 \times 10^3\ m^{-1}$. The μ_s' of the flesh of red, white, and yellow onions were quite close, which were significantly higher than those of sweet onions. The mean μ_s' of the flesh of red, white, and yellow onions were about 30 times smaller than those of their dry skins, and the mean μ_s' of sweet onions was about 120 times smaller than that of their dry skins. Similar to the μ_a, no significant difference was presented between the mean μ_s' of the first and the second flesh scales of onions.

4. Discussion

4.1. Optical properties of single layer onion tissues at 633 nm

It is well known that for fruits and vegetables, their scattering coefficients (μ_s or μ_s') are mainly determined by the cellular structures of their tissues, and the absorption coefficients are related to their chemical compounds (Zude, 2008). In this study, the tissues of the four tested onion cultivars did not statistically differ from each other in terms of the μ_s' of their dry skins, but had significant differences between the dry skins of red, yellow, and other onions. In this regard, the results suggested that the dry skins of four onion cultivars had similar cellular structures but had different contents of chemical compounds. As for the onion flesh, it is observed that red onions showed higher μ_a than other cultivars and the μ_s' of sweet onions illustrated significant difference from those of other cultivars at 633 nm. In sum, the optical properties of onion dry skin and flesh could be different between certain cultivars.

Results of this study (Table 4) showed that onion dry skins have greater μ_a and μ_s' than flesh. Onion flesh consists of high percentage of moisture (>90%), glucose, fructose, sucrose, protein, ash, *etc.* (Brewster, 2008). All these major chemical compounds in onion flesh do not have strong absorption at 633 nm. Also, it is known that the absolute content of pigments decreases from outer scales to inner scales in onions (Takahama and Hirota, 2000; Pérez-Gregorio et al., 2010). During the dehydration process of an onion flesh scale to dry skin, with respect to the decrease of its moisture content, the concentration of the pigment in the scale increases (Takahama and Hirota, 2000) and other physical properties such as the density of the scale also change (Hole et al., 2000). Thus, it is reasonable that the dry skin has higher μ_a and μ_s' than the flesh, since it has greater content of pigments and denser cell structure than the flesh scale.

Compared to the onion dry skin and onion flesh, the onion wet skin showed higher standard errors in their μ_a and μ_s' values. The relative standard error of the μ_a and μ_s' of onion wet skins were 30–100% higher than those of onion dry skins or flesh. This can be explained by the physiological process of onion wet skins in drying.

Table 3
The means and standard deviations of the absorption coefficients μ_a and the reduced scattering coefficients μ_s' of onion tissues at 633 nm.

	Tissue type	Red onion	Sweet onion	White onion	Yellow onion
μ_a ($\times 10^3\ m^{-1}$)	Dry skin	$1.974 \pm 0.819^{A,a}$	$0.760 \pm 0.460^{C,a}$	$0.501 \pm 0.485^{C,a}$	$1.371 \pm 1.107^{B,a}$
	Wet skin	$0.599 \pm 0.841^{A,b}$	$0.305 \pm 0.316^{AB,b}$	$0.284 \pm 0.378^{B,b}$	$0.325 \pm 0.375^{AB,b}$
	First flesh	$0.117 \pm 0.051^{A,c}$	$0.043 \pm 0.020^{B,c}$	$0.036 \pm 0.018^{B,c}$	$0.033 \pm 0.022^{B,b}$
	Second flesh	$0.093 \pm 0.028^{A,c}$	$0.044 \pm 0.023^{B,c}$	$0.032 \pm 0.010^{B,c}$	$0.033 \pm 0.023^{B,b}$
μ_s' ($\times 10^3\ m^{-1}$)	Dry skin	$19.733 \pm 8.396^{A,a}$	$19.075 \pm 8.326^{A,a}$	$18.480 \pm 7.524^{A,a}$	$22.476 \pm 15.81^{A,a}$
	Wet skin	$1.988 \pm 2.829^{B,b}$	$3.756 \pm 3.994^{AB,b}$	$4.424 \pm 4.39^{A,b}$	$5.573 \pm 4.044^{A,b}$
	First flesh	$0.616 \pm 0.194^{A,b}$	$0.152 \pm 0.155^{B,c}$	$0.579 \pm 0.319^{A,c}$	$0.659 \pm 0.270^{A,c}$
	Second flesh	$0.600 \pm 0.194^{AB,b}$	$0.253 \pm 0.164^{C,c}$	$0.479 \pm 0.247^{B,c}$	$0.657 \pm 0.300^{A,c}$

Upper case letters in the superscripts indicate the significant differences between the four onion cultivars ($p < 0.05$), and lower case letters indicate the significant differences between the four types of onion tissues ($p < 0.05$).

Table 4
The average absorption coefficient (μ_a), scattering coefficient (μ_s), anisotropy (g), thickness (d), refractive index (n), and optical depth (τ) of the single-layer onion tissues of red, Vidalia sweet, white, and yellow onions.

Cultivar	Tissue type	μ_a ($\times 10^3$ m^{-1})	μ_s ($\times 10^3$ m^{-1})	g	d (mm)	n	τ
Red	Dry skin	1.974	47.987	0.589	0.071	1.335	3.547
	Wet skin	0.599	7.104	0.72	0.827	1.335	6.370
	Flesh	0.105	1.9505	0.688	3.075	1.342	6.321
Sweet	Dry skin	0.76	36.243	0.474	0.087	1.335	3.219
	Wet skin	0.305	9.137	0.589	0.468	1.335	4.419
	Flesh	0.044	0.593	0.663	3.968	1.342	2.526
White	Dry skin	0.501	29.385	0.371	0.083	1.335	2.481
	Wet skin	0.284	9.014	0.509	0.638	1.335	5.932
	Flesh	0.034	1.137	0.536	3.879	1.342	4.542
Yellow	Dry skin	1.371	49.355	0.545	0.083	1.335	4.210
	Wet skin	0.325	9.617	0.421	0.391	1.335	3.887
	Flesh	0.033	1.166	0.427	3.177	1.342	3.809

The formation of an onion skin is a slow process affected by many conditions such as the storage time and the relative humidity of the environment. As a result, the physical and chemical properties of onion wet skin could be very different at different stages of drying. Therefore, the optical properties of onion wet skins, intermediate between those of onion dry skins and flesh scales, could have large variations. For this reason, although in this study the μ_a and μ_s' were significantly different between certain tested cultivars, it might not be conclusive that the optical properties of the wet skins of these cultivars are significantly different. The μ_a and μ_s' of onion wet skins reported in this article could only represent the situations of the onions tested in this study.

Overall, the estimated μ_a (0.5×10^3–1.97×10^3 m^{-1}) of onion dry skins were at the same magnitude as those of apple skins (<2×10^3 m^{-1}) reported by Saeys et al. (2008) at 633 nm. The estimated mean μ_s' of onion dry skins (18.48×10^3–22.48×10^3 m^{-1}) were several times higher than those of apple skins (about 3.5×10^3–4×10^3 m^{-1}). The μ_a and μ_s' of onion flesh at 633 nm were at the same order of magnitude with those of other fruits and vegetables (kiwifruit, peach, pear, plum) presented by Qin and Lu (2008).

Similar to other biological tissues, onion dry skins and flesh are high-albedo biological media (albedo is defined as $\mu_s'/(\mu_a + \mu_s')$) at 633 nm. It is known that the biological materials must be scattering dominated to apply the diffusion theory to estimate their optical properties nondestructively (Zude, 2008). Thus, our results supported the feasibility of nondestructively measuring the optical properties of onions at 633 nm by using the diffusion theory, such as the diffuse reflectance or backscattering.

The variances shown in the results of this study were most probably caused by the biological variations (moisture content, pigment content, firmness, *etc.*) of the samples. There were a few other factors that could affect the accuracy of this study. First is the systematic error introduced by the spectroscopic system and the IAD algorithm. In addition, based on our validation tests, the intensity of the laser beam presented a 2.1% deviation in 100 measurements, which could be another error source. Moreover, real onion tissues, particularly onion dry skins, are heterogeneous media with distinguishable textures. However, as a general rule in tissue optics, in this study the onion tissues were assumed being homogeneous to apply the IAD estimation.

Based on the measured optical properties of onion tissues, the light loss caused by absorption and scattering in single layer onion tissues could be estimated. For instance, assuming onion dry skins are pure absorption material (ignoring any scattering effect), based on the definition of μ_a, the probability for photons at 633 nm to survive after traveling 1 mm in the dry skin of red onions and white onions would be 13.81% ($e^{-\mu_a * d} = e^{-1.98*1}$) and 60% ($e^{-0.5*1}$), respectively. Onion bulbs often have 1–3 layers of 0.02–0.1 mm thick dry skins (Brewster, 2008). Thus, for μ_a at this level (0.5×10^3–2×10^3 m^{-1}), the light loss in the dry skins of an onion could approximately be about 1% ($1 - e^{-0.5*0.02}$) to 45% ($1 - e^{-1.98*0.3}$) depending on its cultivar and the number and thickness of its dry skins. Similarly, if only considering the scattering, using an anisotropy value of 0.5, even in a thin (0.02 mm) dry skin, the approximate light loss caused by the scattering at 633 nm could be higher than 50% ($1 - e^{-(\mu_s'/(1-g))*d} = 1 - e^{-(18.53/(1-0.5))*0.02} = 0.52$). Considering the thickness of the onion, the light absorption and scattering effects at 633 nm in single layer onion tissues can be indicated by their optical depths ($\tau = (\mu_a + \mu_s) \times d$) (Table 4).

4.2. Light propagation in multi-layer onion tissue at 633 nm

Results of the Monte Carlo simulations revealed that the laser light had different transmission rates in different onion cultivars at 633 nm. Based on the definition of the penetration depth of 1% light transmittance (Fraser et al., 2001), the laser light beam generally penetrated the deepest in Vidalia sweet onions and the least in red onions, while the light transmission in white and yellow onions were in-between (Fig. 2). Overall, the laser beam was not able to penetrate more than 6 onion layers regardless of onion cultivar and layer type. It should be noted that the transmission rate was not only determined by the attenuation factor (combination of absorption and scattering coefficients), but also affected by the thickness of each layer in each cultivar.

The onion dry skin greatly attenuated the propagation of the light in onion tissues in all cultivars. Although onion dry skins were much thinner than flesh scales, the attenuation effect of light from the dry skin was comparable to that from a flesh scale since the dry skin has greater absorption and scattering coefficients than the flesh, which were in accordance with our experimental observations in the total transmittance and collimated transmittance measurements. According to the results of simulations, by adding one, two, and three layers of dry skin(s) to the baseline scenario (one wet skin and one flesh scale) in red onions, the transmission rate of light was reduced from 4.1% to 1.7%, 0.8%, and 0.4%, respectively. After passing three dry skin layers and one wet skin layer of red onions, the light lost 99% of its energy and only a few photons could reach the flesh scales. Thus, in the optical measurements of onions, the onion dry skin should be considered and handled appropriately.

It should be noted that no air gap between onion scales was considered in our simulations. In practice, for some onions, there could be thin air gaps between onion scales. For instance, if the outermost dry skin of the onion bulb is loose, there could be a layer of air between the outermost dry skin and the scale beneath it. The

additional air layer could reduce the penetration depth. For example, in the last multi-layer onion model shown in Fig. 2D, when a thin layer of air ($d = 0.036$, $\mu_s = 10 \times 10^{-6}\ m^{-1}$, $\mu_a = 0.5 \times 10^{-6}\ m^{-1}$) was included between the first and second dry skins, the total diffuse reflectance increased 2.37%, the total absorption and transmission decreased 2.37% and 0.003% (of the overall energy of the initial light), respectively. Therefore, the results of our models could have overestimated the light penetration depth for those onions with air gaps.

5. Conclusions

Our study revealed that onion dry skin and wet skin had significantly higher absorption and reduced scattering coefficients than onion flesh at 633 nm. Different cultivars of onions could be significantly different in terms of the μ_s' of the flesh and the μ_a of both the flesh and dry skin. Our data also indicated that onion tissues are scattering dominated (high-albedo) biological materials. Monte Carlo simulations of light propagation in multi-layer onion tissues showed that the low power laser at 633 nm would lose 99% of its energy within 6 layers of onion tissues in any of the 25 simulated scenarios because of the substantial light absorption and scattering in onion tissues. The results suggested that the transmittance measurement at 633 nm of an intact onion is not likely and the reflectance or interactance measurements could be more suitable. For the applications that intend to measure the characteristics of onion flesh by using optical methods, it is important to handle onion outer skins properly.

This study is the first effort to measure the light absorption and scattering characteristics of onion tissues, which provides quantitative evidences to better design optical systems for onion quality evaluation. However, this study only investigated the optical properties of onion tissues at one wavelength and future research is needed to expand our understanding to a wider spectral range.

Acknowledgements

This work was funded by the USDA NIFA Specialty Crop Research Initiative (Award No. 2009-51181-06010). The authors gratefully acknowledge Dr. Ron Gitaitis, Dr. Howard Schwartz, and Dr. Krishna S. Mohan for offering onion samples, Dr. Elizabeth L. Andress for providing the Abbe Refractometer to this study. The assistance from Mr. Anthony M. Dean during data collection was also sincerely appreciated.

References

Abhayawick, L., Laguerre, J.C., Tauzin, V., Duquenoy, A., 2002. Physical properties of three onion varieties as affected by the moisture content. Journal of Food Engineering 55 (3), 253–262.

Block, E., 2010. Garlic and Other Alliums: The Lore and the Science. Royal Society of Chemistry, Burlington House. Piccadilly, London, UK.

Birth, G.S., Dull, G.G., Renfroe, W.T., Kays, S.J., 1985. Nondestructive spectrophotometric determination of dry matter in onions. Journal of the American Society for Horticultural Science 110 (2), 297–303.

Brewster, J.L., 2008. Onions and Other Vegetable Alliums. Cabi Series. CABI, Cambridge, MA, USA.

Cheong, W.F., Prahl, S.A., Welch, A.J., 1990. A review of the optical properties of biological tissues. IEEE Journal of Quantum Electronics 26 (12), 2166–2185.

Corzo-martíez, M., Corzo, N., Villamiel, M., 2007. Biological properties of onions and garlic. Trends in Food Science & Technology 18 (12), 609–625.

Cubeddu, R., D'Andrea, C., Pifferi, A., Taroni, P., Torricelli, A., Valentini, G., Ruiz-Altisent, M., Valero, C., Ortiz, C., Dover, C., et al., 2001. Time-resolved reflectance spectroscopy applied to the nondestructive monitoring of the internal optical properties in apples. Applied Spectroscopy 55, 1368–1374.

Cubero, S., Aleixos, N., Moltó, E., 2011. Advances in machine vision applications for automatic inspection and quality evaluation of fruits and vegetables. Food and Bioprocess Technology 4, 487–504.

Foskett, R.L., Peterson, C.E., 1950. Relation of dry matter content to storage quality in some onion varieties and hybrids. Proceedings of the American Society for Horticultural Science 55, 314–318.

Fraser, D.G., Jordan, R.B., Künnemeyer, R., McGlone, V.A., 2003. Light distribution inside mandarin fruit during internal quality assessment by NIR spectroscopy. Postharvest Biology and Technology 27 (2), 185–196.

Fraser, D.G., Künnemeyer, R., McGlone, V.A., Jordan, R.B., 2001. Near infrared (NIR) light penetration into an apple. Postharvest Biology and Technology 22 (3), 191–194.

Griffiths, G., Trueman, L., Crowther, T., Thomas, B., Smith, B., 2002. Onions – a global benefit to health. Phytotherapy Research: PTR 16 (7), 603–615.

Hole, C., Drew, R., Gray, D., 2000. Humidity and mechanical properties of onion skins. Postharvest Biology and Technology 19 (3), 229–237.

Kim, A., Wilson, B.C., 2011. Measurement of Ex Vivo and In Vivo Tissue Optical Properties: Methods and Theories, 2nd Ed. Springer, Netherlands, pp. 267–319 (Chapter 8).

Ko, M.-J., Cheigh, C.-I., Cho, S.-W., Chung, M.-S., 2011. Subcritical water extraction of flavonol quercetin from onion skin. Journal of Food Engineering 102 (4), 327–333.

Lu, R., Peng, Y., 2007. Development of a multispectral imaging prototype for real-time detection of apple fruit firmness. Optical Engineering 46 (12), 123201.

Maw, B.W., Hung, Y.C., Tollner, E.W., Smittle, D.A., Mullinix, B.G., 1996. Physical and mechanical properties of fresh and stored sweet onions. Transactions of the ASAE 39 (2), 633–637.

Ninni, P., Di Martelli, F., Zaccanti, G., 2011. Intralipid: towards a diffusive reference standard for optical tissue phantoms. Physics in Medicine and Biology 56 (2), 21–28.

Pérez-Gregorio, R.M., Garca-Falcón, M.S., Simal-Gándara, J., Rodrigues, A.S., Almeida, D.P., 2010. Identification and quantification of flavonoids in traditional cultivars of red and white onions at harvest. Journal of Food Composition and Analysis 23 (6), 592–598.

Pickering, J.W., Prahl, S.A., van Wieringen, N., Beek, J.F., Sterenborg, H.J.C.M., van Gemert, M.J.C., 1993. Double-integrating-sphere system for measuring the optical properties of tissue. Applied Optics 32 (4), 399.

Prahl, S.A., van Gemert, M.J.C., Welch, A.J., 1993. Determining the optical properties of turbid media by using the adding-doubling method. Applied Optics 32 (4), 559–568.

Prahl, S., 2011. Everything I think you should know about Inverse Adding-Doubling. Oregon Medical Laser Center, Manual of the Inverse Adding-Doubling program, http://omlc.ogi.edu/software/iad/ (retrieved 01.10.12).

Qing, Z., Ji, B., Zude, M., 2008. Non destructive analyses of apple quality parameters by means of laser-induced backscattering imaging. Postharvest Biology and Technology 48 (2), 215–222.

Qin, J., Lu, R., 2007. Measurement of the absorption and scattering properties of turbid liquid foods using hyperspectral imaging. Applied Spectroscopy 61, 388–396.

Qin, J., Lu, R., 2008. Measurement of the optical properties of fruits and vegetables using spatially resolved hyperspectral diffuse reflectance imaging technique. Postharvest Biology and Technology 49 (3), 355–365.

Qin, J., Lu, R., 2009. Monte Carlo simulation for quantification of light transport features in apples. Computers and Electronics in Agriculture 68, 44–51.

Rodríguez Galdón, B., Tascón Rodríguez, C., Rodríguez Rodríguez, E.M., Díaz Romero, C., 2009. Fructans and major compounds in onion cultivars (*Allium cepa*). Journal of Food Composition and Analysis 22 (1), 25–32.

Romano, G., Nagle, M., Argyropoulos, D., Müller, J., 2011. Laser light backscattering to monitor moisture content, soluble solid content and hardness of apple tissue during drying. Journal of Food Engineering 104 (4), 657–662.

Saeys, W., Velazco-Roa, M.A., Thennadil, S.N., Ramon, H., Nicolaï, B.M., 2008. Optical properties of apple skin and flesh in the wavelength range from 350 to 2200 nm. Applied Optics 47, 908–919.

Takahama, U., Hirota, S., 2000. Deglucosidation of quercetin glucosides to the aglycone and formation of antifungal agents by peroxidase-dependent oxidation of quercetin on browning of onion scales. Plant and Cell Physiology 41 (9), 1021–1029.

Tuchin, V.V., 2007. Tissue Optics: Light Scattering Methods and Instruments for Medical Diagnosis, 2nd ed. SPIE Press, Bellingham, WA, USA.

USDA, 2013. U.S. Onion Statistics United States Department of Agriculture, http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1396 (retrieved 28.03.13).

Van Staveren, H.J., Moes, C.J., van Marie, J., Prahl, S.A., van Gemert, M.J.C., 1991. Light scattering in Intralipid-10% in the wavelength range of 400–1100 nm. Applied Optics 30 (31), 4507–4514.

Wang, L., Jacques, S.L., Zheng, L., 1995. MCML–Monte Carlo modeling of light transport in multi-layered tissues. Computer Methods and Programs in Biomedicine 47 (2), 131–146.

Wang, W., Li, C., Tollner, E.W., Gitaitis, R.D., Rains, G.C., 2012. Shortwave infrared hyperspectral imaging for detecting sour skin (*Burkholderia cepacia*)-infected onions. Journal of Food Engineering 109 (1), 38–48.

Xia, J.J., Berg, E.P., Lee, J.W., Yao, G., 2007. Characterizing beef muscles with optical scattering and absorption coefficients in Vis-NIR region. Meat Science 75 (1), 78–83.

Zude, M., 2008. Optical Monitoring of Fresh and Processed Agricultural Crops. CRC Press, Boca Raton, FL, USA.