BULK OPTICAL PROPERTIES OF POTATO FLESH IN THE 500-1900 nm RANGE

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Abstract

In this study the optical properties of potato flesh tissue were estimated using double integrating sphere (DIS) measurements combined with an inverse adding doubling (IAD) light propagation model. Total reflectance, total transmittance and unscattered transmittance were measured for the wavelength range 500–1900 nm with 5 nm resolution. From these measurements, the bulk optical properties (absorption coefficient, scattering coefficient and anisotropy factor) of 53 potato tubers of the Hermes cultivar were estimated. The estimated absorption coefficient spectra were dominated by water and starch absorption bands, the main chemical components of potato tissue. Comparison of these values to those reported in literature for similar products showed comparable absorption profiles. The obtained scattering coefficient spectra showed a smooth decrease from 166 cm^{-1} to 160 cm^{-1} in the NIR spectral range with increasing wavelength, which is common for biological tissues. The anisotropy factor spectra obtained for the full wavelength range studied, ranged between 0.949 and 0.959 with a maximum variability of 0.009 among the set of samples used. The information obtained in this study is essential to understand the effects of absorption and scattering on the propagation of light through the potato tubers in order to design more efficient sensors for non-destructive quality evaluation.

Keywords: Optical properties, double integrating spheres, scattering, absorption, inverse adding doubling, potatoes.

1. INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the main food products globally as it is considered a staple food in many developing countries (López et al., 2013). According to the Food and Agriculture Organization of the United Nations (FAOSTAT, 2013), potato was the fifth most produced crop worldwide in 2012, after sugar cane, maize, rice and wheat.

The consumption of potatoes was reported to provide several benefits for health since these tubers are a good source of potassium, protein, calcium and vitamin C (Lister & Munro, 2000). Both potato consumers and the retail sector prefer potatoes of high quality, which is determined by the tubers' chemical constituents and physical properties (microstructure). Moreover, besides the nutritional value of potatoes, also appearance, flavour and the presence of defects caused by mechanical damage are important.

The traditional methods employed to determine the main constituents of potatoes involve wet chemistry methods, such as high-performance liquid chromatography (HPLC) (Mehrubeoglu & Cote, 1997). However, in today's competitive market, where the importance of quality monitoring is increasing, it is becoming necessary to develop faster, more economical, safer and more versatile non-destructive techniques to efficiently measure these properties. Near-infrared spectroscopy (NIRS) is considered one of

the most advanced technologies for non-destructive quality assessments of fruits and vegetables (Cubeddu et al., 2001; Nicolaï et al., 2007; Cubero et al., 2011; Wang & Li, 2013).

NIRS technology was introduced in the industry in the 1970s, and has been used since then for moisture, protein, and fat content determination in many agricultural and food products (Davies & Grant, 1987; Gunasekaran & Irudayaraj, 2000; Nicolaï et al., 2014). The detection of these chemical components relies on the wavelength-specific NIR absorbance which is linearly proportional to the concentration of the absorbing constituents. Nevertheless, next to the sample's chemical composition, the absorbance of the photons also depends on the travelled path-length. In agricultural and food products, light is typically scattered by the numerous local non-uniformities (physical microstructure): cells, cell organelles, air pores, fibrous structures, fat globules, etc. (Wang & Li, 2013). Because these light deflections increase the photon's path-length, the NIR absorbance increases to an unknown extend. In practice, the measured NIR reflectance or transmittance spectra are often dominated by these scattering effects. Accordingly, a decrease in NIR reflectance or transmittance caused by a change in scattering might be misinterpreted as an increase of the sample's actual absorption coefficient, while the latter should be normalized for the photon's path-length to follow a linear relation with the sample composition.

Simulation of the light propagation through the tissues of interest could provide insight in the specific relations between scattering, absorption and measured spectroscopic signals, promoting a more efficient NIR sensor design, data processing and data interpretation (Zamora-Rojas et al., 2014; Steponavičius & Thennadil, 2009, 2011, 2013). Light propagation in homogeneous turbid media such as potatoes can be described with the radiative transport equation (RTE) which uses the bulk optical properties of the tissue: the bulk absorption coefficient (μ_a), bulk scattering coefficient (μ_s) and anisotropy factor (g) as input parameters. The g and μ_s can be combined to obtain a parameter called reduced scattering coefficient (μ_s') (Aernouts et al., 2013; Arimoto et al., 2005; Bashkatov et al., 2005a; Lino et al., 2003; Zamora-Rojas et al., 2013a). The bulk absorption coefficient relates linearly to the sample's composition, while the bulk scattering coefficient and scattering anisotropy factor define the level and the angular distribution of the light deflections (Xia et al., 2007). An anisotropy factor of 0 implies isotropic scattering, while a value of 1 denotes exclusive forward scattering. Knowledge of the potential range of the sample's bulk optical properties is essential to perform detailed light propagation studies (Arimoto et al., 2005; Lino et al., 2003; Zamora-Rojas et al., 2013a).

Double integrating sphere (DIS) measurements, either alone or in combination with unscattered transmittance measurements, are the "golden standard" method to obtain accurate estimates of the optical properties for thin slices of biological tissue (Pickering et al., 1993). A DIS setup allows to measure the total reflectance and total transmittance simultaneously, providing more robust and accurate estimates of the bulk optical properties compared to single integrating sphere measurements (Bashkatov et al., 2011; Pickering et al., 1993; Prahl, 2011). The theory and practicability of integrating spheres for measuring the reflectance of a sample have been widely studied (Pickering et al., 1993). However, the bulk optical properties cannot be estimated directly from DIS and unscattered transmittance measurements, but have to be estimated by performing an iterative inversion of a light propagation model (Zamora-Rojas et al., 2013a). The RTE describes photon propagation in scattering and absorbing media for single scattering events, based on the optical properties of the sample (Martelli, 2012). With the objective of solving the RTE for multiple scattering samples, the adding doubling (AD) method allows to calculate the total reflectance and transmittance very accurately, while maintaining a high degree of flexibility and time efficiency. Moreover,

this method uses the RTE to obtain the reflectance and transmittance for a single 'infinitesimally' thin sample layer. Accordingly, this layer is 'doubled' and the reflectance and transmittance of this doubled layer are calculated by combining the values from two single layers. Finally, this process is repeated until the desired thickness of the homogeneous sample is reached (Saeys et al., 2008; Zamora-Rojas et al., 2013a). As the AD method calculates the total reflectance and transmittance for a tissue layer with known bulk optical properties, it has to be inverted to allow extraction of the bulk optical properties (μ_a and μ_s') from DIS measurements. The inverse AD (IAD) algorithm, developed by Prahl et al. (1993), estimates the optical properties by iteratively changing their values in the AD simulations until the simulated reflectance and transmittance values match with the measured ones (Prahl, 2011; Saeys et al., 2008; Zamora-Rojas et al., 2013b). Moreover, by an accurate measurement of the unscattered transmittance, the bulk extinction coefficient (μ_t) is obtained and all bulk optical properties (μ_a, μ_s and g) can be derived (Prahl, 2011). The unscattered transmittance should be measured in a configuration especially optimized to minimize the collection of scattered photons (Aernouts et al., 2013; De Vries et al., 1999; Prahl, 2011). Otherwise, the unscattered transmittance could be overestimated, leading to an underestimation of μ_t (Prahl, 2011, Aernouts et al., 2013).

Since 2000, the bulk optical properties of many food products have been characterized in the visible (Vis) and near infrared (NIR) spectral ranges. Cubeddu et al. (2001), measured the internal optical properties of apples by Time-resolved reflectance spectroscopy, while Fraser et al. (2003) studied the light distribution inside mandarins using a custom-made probe connected to a spectrometer. Wang & Li (2013) and Saeys et al. (2008) used integrating sphere techniques to determine the bulk optical properties of respectively onions and apples. Regarding potatoes, Karagiannes et al., (1989) studied the bulk absorption and reduced scattering coefficients of Idaho potatoes in the 340-1360 nm spectral range, assuming a wavelength-independent value for g. Nevertheless, both the bulk scattering coefficient and the anisotropy factor are essential inputs for simulation studies to develop and evaluate novel optical quality sensors for the non-destructive analysis of tubers. Additionally, the NIR range above 1400 nm include important absorption bands related to potato constituents (e.g. water, starch, …). Therefore, the aim of this study was to measure all three bulk optical properties (μ_a, μ_s and g) of potato flesh in the 500 - 1900 nm wavelength range.

2. MATERIALS AND METHODS

2.1- Sample set

Potato (*Solanum tuberosum* L.) samples were provided by The Basque Institute for Agricultural Research and Development (Neiker Tecnalia) in Spain, and were sent to KU Leuven Department of Biosystems, MeBioS, Leuven, Belgium, for the measurements. A total of 53 different tubers belonging to the Hermes cultivar were used in this study and 2-3 replicates of each tuber were analysed accounting for a total of 143 samples of flesh. Potato tubers were kept in a refrigerator at 4 °C before analysis. Then, tubers were washed, weighted and peeled. Potato flesh samples were sliced into 550 µm thickness portions of 30 mm diameter using a meat slicer (Junior sup 19 mod 30-595A, CAD, ITALY). The thin portions were sandwiched between two optical borosilicate glass plates after adding distilled water to remove air and consequently reduce the refractive index (RI) mismatch at the glass-sample boundary. The thickness of the sample slides was measured with a calliper with an accuracy of 5 µm. The thickness of the cuvette, together with the thickness of the sample slab and glass plates (1100 µm) was used to calculate the thickness of the present water layer.

124 2.2- DIS and unscattered transmittance measurements

125 To acquire total reflectance (M_R), total transmittance (M_T) and unscattered transmittance (M_U), a
126 wavelength-tuneable DIS and unscattered transmittance measuring system was used. Detailed information
127 about the used system can be found in Aernouts et al. (2013). The measurements were taken in the
128 wavelength range 500 – 1900 nm with an interval step of 5 nm.

129 In this setup, illumination of the samples is provided by a high power (4 W) supercontinuum laser source
130 with a 460–2400 nm range (SC450-4, Fianium Ltd., Southampton, UK) coupled into a high precision
131 monochromator (Oriel Cornerstone™ 260×, Newport, Irvine, US) covering the 450-2800 nm wavelength
132 range in order to obtain a high signal to noise ratio (SNR) for DIS measurements on biological tissues. A
133 flip mirror, located behind the monochromator, reflected or passed the collimated light towards either the
134 DIS or the unscattered transmittance measurement path.

135 The thin potato tissue samples were placed between two optical borosilicate glass slides (1.1 mm wall
136 thickness), separated by a spacer of 550 μm (path-length). For DIS measurements, the sample (tissue +
137 glass plates) was positioned between the two Infragold® coated integrating spheres (RT-060-IG,
138 Labsphere, Inc., North Sutton, US. 700–20,000 nm wavelength range; 6 inch inner diameter). The sample
139 port has a diameter of 1 inch, while all detector ports were 0.5 inch in diameter (Aernouts et al., 2013;
140 Zamora-Rojas et al., 2013a). Two detectors are located on the wall of each sphere: a large-area Silica (Si)
141 detector (PDA 100A, Thorlabs Inc., New Jersey, USA) for the 500-1050 nm range and a one-stage Peltier-
142 cooled extended-InGaAs detector (PDA10DT-EC, Thorlabs Inc., New Jersey, USA) for measurements in
143 the 1050-2250 nm range. To avoid detection of reflected or transmitted light coming directly from the
144 sample, baffles are located on the inner sphere wall between the sample and the detectors (Aernouts et al.,
145 2013).

146 For the unscattered transmittance measurements, 3 round slits are optically aligned: one immediately before
147 and after the sample and a third one at a 1.5 m distance behind the sample. This design ensures a maximum
148 collection angle of 5 mrad in order to minimize the number of scattered photons captured. The sample is
149 placed in a sample holder, perpendicular to the incident collimated light beam. An automated flip mirror,
150 located behind the third slit, reflects or passes the light respectively towards a Si or an extended-InGaAs
151 detector with the specifications mentioned above.Instrumental calibration and sample analysis were
152 performed following the methodology described by Prahl (2011). To prevent detector saturation during
153 calibration of the unscattered transmittance setup, a neutral density filter (NDF, Qioptiq Limited,
154 Luxembourg) of optical density 3.0 was located in the sample holder (Zamora-Rojas et al., 2013a). The
155 system (laser, monochromator, flip mirror and detectors) and data collection were automated and controlled
156 with LabView 8.5 software (National Instruments Corporation, Austin, TX) (Aernouts et al., 2013).

157 2.3- Optical properties estimation

158 M_R, M_T and M_U were calculated from the DIS and unscattered transmittance measurements in Matlab (ver
159 7.10, The Mathworks Inc., Massachusetts, USA), as described by Aernouts et al. (2013). From these values,
160 the absorption coefficient (μ_a), reduced scattering coefficient (μ_s') and anisotropy factor (g) were calculated
161 using the IAD software provided by Prahl (2011) and implemented in Matlab. To correct for reflections at
162 the glass-sample boundaries, the refractive index (RI) of potato tissue samples has to be supplied to the
163 algorithm in order to solve the inverse problem. The wavelength-dependent RI values were estimated by
164 adding the difference between the RI of potato and water at 589.29 nm to the wavelength-dependent RI

values for water reported by Hale and Querry (1973) and Segelstein (1981). According to Birth (1978), the RI value for potato at 589.29 nm is 1.4, while the RI of water is 1.33 (Hale & Querry, 1973; Segelstein, 1981). The scattering coefficient (μ_s) was calculated from μ_s' and g using the similarity relation (Tuchin, 2007):

$$\mu_s = \frac{\mu_s'}{(1-g)} \quad (1)$$

Additionally, through the unscattered transmittance measurement of 1 mm water, the μ_a spectrum of pure water was derived. Taking into account this water spectrum, as well as the thickness of the water layer and the sample slab in the cuvette, the μ_a spectrum of the sample slab alone was obtained. Furthermore, the μ_s and μ_s' spectra were rescaled with the thickness of the sample slab relative to the total thickness (sample slab + water layer).

3. RESULTS AND DISCUSSION

In Fig. 1 the mean and standard deviation (vertical lines) of the total reflectance (M_R), total transmittance (M_T) and unscattered transmittance spectra (M_U) acquired for 143 potato tissue samples in the 500-1900 nm wavelength range are illustrated.

All the measured spectra (M_R, M_T and M_U) show clear valleys around 970, 1210, 1440-1490 and at 1900 nm, all associated with water absorption bands related to the second and first overtones of OH stretching and OH combination bands. As raw tubers usually have a water content of approximately 80% (Büning-Pfaue, 2003), it is not surprising that the acquired spectra are dominated by the absorption signature of water. At 1765 nm another valley can be observed which can be related to the first overtone of the CH stretching. This can be attributed to starch which represents approximately 60-80% of the dry matter of raw potato tubers and contains both CH and CH_2 groups. Due to the high water absorption, the measurement values were close to zero in the 1900-2250 nm wavelength range (data not shown). As a result, the system failed to estimate the bulk optical properties at these wavelengths. In the 500-1900 nm range M_U values varied between 0% and 0.04% ± 0.01%. These low values indicate that only a very small fraction of the photons passes through the tissue without being scattered. This limits the SNR of the obtained M_U (Zamora-Rojas et al., 2013a).

In Fig. 2 the mean and standard deviation of the bulk optical properties (μ_a, μ_s and g) calculated with the IAD method are illustrated. As mentioned above, no reliable values could be obtained in the 1900-2250 nm wavelength range (not shown) as the signal-to-noise ratios of the DIS and unscattered transmittance measurements were too low. As can be seen in the plot, the absorption coefficient (μ_a) spectrum shows absorption peaks at 970, 1210 nm and 1440-1490 nm due to the high water content in raw potatoes. A small peak around 1765-1800 nm is observed which can be related to the starch content. Additionally, an increase in the absorption of visible light can be noticed towards 500 nm. This is more clearly presented in the detail plot in Fig. 3. The absorption at these wavelengths corresponds to the yellow colour of potato flesh and is probably related to the presence of beta-carotene (Penner, 2010; Du et al., 1998). The bulk scattering coefficient (μ_s) spectrum changes only gently with increasing wavelength, decreasing from 166 to 160 cm^{-1} ± 7.35 cm^{-1} for wavelengths below 1900 nm. This decreasing tendency with increasing NIR wavelength is typical for the scattering characteristics of biological tissues (Bashkatov et al., 2005b). The estimated anisotropy factor values were quite stable ranging from 0.959 to 0.949. It should be noted that the accuracy of the estimated anisotropy factor values was negatively influenced by the strong water absorption at 1440-1490 nm, as can be seen from the larger standard deviations.

In Fig. 3 and 4, the bulk optical properties of potato flesh of this study are plotted together with the values reported by Karagiannes et al. (1989) for Idaho potato tubers. In the latter study, potato tuber samples of 3.5 mm were measured in air in the 340-1360 nm spectral range with a two-channel integrating sphere spectrophotometer. The optical properties were estimated from these data with two formulations of the 1D diffusion approximation differing in the utilized phase function, assuming a refractive index for potato equal to 1.36 (Karagiannes & Grossweiner, 1988) and a fixed anisotropy factor g of 0.

In Fig. 3, the average absorption coefficient spectrum obtained in this study and the one reported by Karagiannes et al. (1989) are plotted together with the absorption coefficient spectrum of water from 500 to 1900 nm (Segelstein et al., 1981). Although the same absorption bands for water appear in both potato spectra, the values at the absorption peaks are on a different level. The estimated values at 970 and 1200 nm reported by Karagiannes et al. (1989) considerably exceed the absorption coefficient values of pure water. These values are highly questionable as water is the most important NIR absorbing component in potatoes. Therefore, it is hypothesized here that these values are overestimated due to an inferior separation between scattering and absorption. This can additionally be noticed at 800 nm, where a baseline is clearly present in the μ_a reported by Karagiannes et al. (1989), while nearly no absorption is expected as potato constituents do not absorb significant amounts of light at this wavelength. In the 600-1400 nm range, the μ_a spectrum obtained in this study is very close to the absorption coefficient spectrum of water (Fig. 3), suggesting that the sample consists mainly of water. As explained before, some water was added to the samples to reduce the RI mismatch at the boundaries, probably causing an increase in water content of the samples. In the 1500-1790 nm range, the average μ_a spectrum of the samples is higher compared to the water spectrum. This can be attributed to the first overtone absorption bands of the CH and CH_2 bands in the starch molecules. The μ_a values in the 1400-1500 nm range are probably slightly overestimated due to imperfect separation of the scattering and absorption coefficient values in this region, induced by the low signal levels (Fig. 1). Although the results in this study are not perfect, they are much closer to reality than those reported by Karagiannes et al. (1989).

In Fig. 4, the scattering coefficient μ_s' values in the 500-1900 nm spectral range estimated in both studies are shown. It can be observed that the μ_s' spectrum obtained in this study is relatively steady along the entire spectral range with values between 7.5 and 8.3 cm^{-1} except at the 1440-1490 nm range where it is influenced by water and values decrease down to 6.6 cm^{-1}. The reduced scattering coefficient values obtained by Karagiannes et al. (1989) are smaller, which may be attributed to a difference in the microstructure properties of the cultivars and samples considered in both studies. However, as no microstructure information on the samples is available, this hypothesis cannot be verified. On the other hand, as their absorption coefficient values were most likely overestimated, it is likely that they underestimated the reduced scattering coefficient μ_s' values due to imperfect separation of the absorption and scattering properties (Zamora-Rojas et al., 2013a).

Recently, Wang & Li (2013) measured the bulk absorption coefficients, reduced scattering coefficients and scattering anisotropy of skin and flesh of four different onion cultivars at 633 nm. They obtained absorption coefficient values between 0.32-1.2 cm^{-1} for the flesh of the different cultivars and bulk scattering coefficients in the 5.8-6.6 cm^{-1} range for white and yellow onions. These values are similar to those obtained in this study for potato flesh. The estimated anisotropy values at 633 nm for onion flesh tissues varied from 0.35-0.73, which is considerably smaller than those obtained for potato tissue in our study.

Saeys et al. (2008) studied the optical properties of both skin and flesh for three different apple cultivars at 1450 nm by using a single integrating sphere measurements in combination with IAD. The reduced scattering coefficient values for apple flesh obtained in their study ranged from 10 to 15 cm^{-1} in the 350-1900 nm range, while those for apple skin ranged from 30 to 60 cm^{-1} in the same wavelength range. This suggests that both apple flesh and skin tissue are more optically dense than potato flesh tissue.

The fact that the bulk optical properties estimated in this study are more reliable than those obtained by Karagiannes et al. (1989) can most likely be attributed to the use of a DIS system in combination with a high power supercontinuum laser, rather than the use of a single integrating sphere in combination with a halogen light source. The DIS system allows for simultaneous and robust measurement of M_T and M_R without replacing the sample or the sphere. The use of a supercontinuum laser light source makes it possible to focus a high power beam on a very small spot on the sample, reducing the lateral light losses. Another possible explanation for the difference between the bulk optical properties obtained in both studies may be related to the use of different light propagation models, the instrument calibration and the heterogeneity of the samples (Tuchin, 2007).

4. CONCLUSIONS

In this study, the bulk optical properties of potato flesh were measured in the 500-1900 nm wavelength range by means of double integrating spheres and unscattered transmittance measurements. This resulted for the first time in a wavelength-dependent estimate of the anisotropy factor for potato flesh over that wavelength range. The obtained results show that the bulk absorption coefficient spectra are mainly dominated by water, especially in the NIR region, while the starch absorption bands are less pronounced, but still clearly visible. The bulk scattering coefficient spectra slightly decreases with increasing wavelength. The estimated values for the anisotropy factor were high (>0.94) over the considered spectral range, which indicates that potato tissue is highly forward scattering. Compared to the results obtained by other authors, the absorption and reduced scattering coefficient spectra for potato tissue retrieved in this study were likely more reliable. Moreover, the optical properties were obtained in the visible and extended NIR range from 500 to 1900 nm, compared to 340-1360 nm. The added value of the extended range is significant as the relevant absorption peaks for water and starch are located in the NIR region of the spectrum. Additionally, the obtained optical properties were comparable to the values obtained for other products (apple and onion flesh and skin) reported in literature.

The optical characterization of potato flesh elaborated in this study provides information about the absorption, scattering and the angular scattering distribution of light propagating through the tissue. This information is essential to understand the effects of absorption and scattering on the reflectance and transmittance spectra measured with Vis-NIR spectroscopy.

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