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Preliminary Results on the Non-Destructive Determination of Pear (*Pyrus communis* L.) cv. Rocha Ripeness by Visible/Near Infrared Reflectance Spectroscopy

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Abstract

Pear (*Pyrus communis* L.), cv. Rocha was rapidly adopted by consumers due to its inherent quality and currently has great acceptance in both national and international markets, being mainly produced in the west region of Portugal. We report here a first approach to the use of the non-intrusive method of Visible/Near Infrared Reflectance Spectroscopy (Vis/NIRS) to estimate the ripeness of pear cv. Rocha. Mature unripe pears obtained from Frutoeste (Mafra, Portugal) after a six-month cold-storage, were maintained in a dark room at circa 20°C during three weeks. They were followed using the Vis/NIRS in the wavelength band between 400 and 950 nm with two different configurations for the spectra acquisition, namely the Integrating Sphere (IS) and the Partial Transmittance (PT). The diffuse reflectance spectra obtained by the two configurations were compared with the respective fruit ripening parameters (colour, firmness, soluble solids content and % dry matter), determined through the standard techniques. Concerning the rough estimation of ripening parameters, data suggested an increase in both the intensity in the green to red band and pulp %dry matter, but a decreasing firmness. All other parameters remained constant. Relatively to the optical results, we have observed that the PT spectra exhibited clearer features than the IS spectra, especially from 700 nm onwards. This is probably due to the fact that the PT configuration probes more deeply into the fruit pulp. Three peaks at 600 (circa 30%), 725 and 812 nm (both at circa 50%) and a minimum at 675 nm, were identified in both IS and PT spectra. The values of reflectance peaks were approximately constant during ripening, but they moved to slightly lower wavelengths in the second week. A significant increase (circa 3-fold) in the minimal diffuse reflectance was observed in the second week, most probably associated partially, to a decrease in the fruit peel chlorophyll content.

INTRODUCTION

The pear cv. Rocha (*Pyrus communis* L.) had its origin approximately 150 years ago in Sintra, Portugal. Because of its fruit qualities (a slight flavoured white pulp, soft and juicy) it was rapidly adopted by consumers and currently has great acceptance in both national and international markets, being mainly produced in the west region of Portugal. The assessment of its quality and ripeness is essential for its pre-harvest and post-harvest long-term storage periods.

The application of optical diagnostics in medicine (Hebden and Delpy, 1997, for a

review) are now being followed by applications in the agricultural sciences. The most adapted method for use in fruit diagnostics is the Visible/Near Infrared Reflectance Spectroscopy (Vis/NIRS), and the first studies in this field may be traced back to the early 80's (Knee, 1980). The advantage of Vis/NIRS includes fast execution, limited sample pre-processing and easy use in process control and grading systems. Indeed, it has been used to assess quality attributes such as acidity, juiciness, sugar content, firmness, texture parameters, pigments content and starch degradation in a large variety of horticultural products, namely, peach, nectarine, kiwifruit, melon, mandarin, pineapple, grape, papaw and mango (Kawano et al., 1992; Guthrie and Walsh, 1997; Guthrie et al., 1998; Lammertyn et al., 1998; McGlone and Kwano, 1998; Ventura et al., 1998; Greensill and Newman, 1999; Zude-Sasse et al., 2002; Herrera et al., 2003; Solovchenko et al., 2005).

The aim of these preliminary studies was to test the application of two different Vis/NIRS configurations to estimate 'Rocha' pear ripeness.

MATERIALS AND METHODS

Mature unripe pears cv. Rocha stored in CA conditions and at 1ºC for six months were provided by Frutoeste (Mafra, Portugal). Then, they were maintained in a dark room at circa 20°C and were followed during three weeks using Vis/NIRS reflectance spectroscopy. Measurements were made approximately every three days, and the same group of ten pears was used for the optical measurements and followed during the whole study. Extra pears were further used to obtain a rough estimation of the respective fruit ripeness through the standard methods.

Vis/NIRS Analysis

Vis/NIRS measurements were performed in the wavelength band between 400 and 950 nm with an optical spectrometer (USB4000, Ocean Optics, USA) in two different configurations, namely the Integrating Sphere (IS) and the Partial Transmittance (PT) as represented schematically in Figure 1. Light from a tungsten-halogen source (HL-2000-FHSA, Ocean Optics, USA) was sent to the fruit through an optical fibre and the re-emerging light was collected by a second fibre and sent to the spectrometer. The low radiation from the light source below 400 nm and the low sensitivity of the CCD camera in the lower and upper (<400 nm and >950 nm) parts of the spectrum produced a low signal to noise ratio and determined the useful wavelength range, between 400 and 950 nm. For the acquisition, processing and calibration, specific software was used (Spectra Suite, Ocean Optics, USA). The diffuse reflectance (R) spectra from each fruit was calculated automatically taking into account the raw sample spectrum (R_S), the dark spectrum (R_D) (effect of the detector's inherent dark current) and the reference spectrum (R_R) (taken from a Spectralon white surface, WS-1, Ocean Optics, USA), according to the mathematical expression $R=100x(R_S- R_D)/(R_R-R_D)$.

Destructive Assessment of Quality

Fruit ripening parameters were determined for one extra pear on each of the same days of the Vis/NIRS measurements, by using the standard methods. Fruit surface colour was determined in a non-destructive manner with a colorimeter (Minolta CR-200 Chroma meter, Japan). Results were expressed in the CIE L*a*b* color space. Firmness, was determined by puncture (without skin) with a TCD 200 Chatillon penetrometer (USA) fitted with an 8 mm diameter plunger at a depth of 7 mm. Soluble solids content in °Brix was determined with a digital Atago refractometer (Japan) from juice extracted from the fruit. Dry matter (%), was calculated for pulp and peel fruit, as the percentage of pulp or peel fresh weight, after drying these tissues for at least 6 days at 60ºC.

Statistical Analysis

The effects of time and Vis/NIRS configuration on each calculated R ratio were tested by two-way ANOVA. Statistical analysis was carried out with SigmaStat 2.0 (SPSS Science, Chicago, IL).

RESULTS AND DISCUSSION

Concerning ripening parameters given by the standard methods, each value presented in Table 1 refers to one extra fruit in the same ripening stage as the ten fruits used in the optical measurements. The correlation among these parameters and spectra is only possible when a large number of replicates are used, through standard multivariate data analysis. Thus, values in Table 1 have served merely as a rough timetable of the different stages in fruit shelf life ripening to help following the spectra evolution. Nevertheless, this data suggest that pear cv. Rocha, ripening in the shelf life comprehended tissue softening, increasing intensity in the green to red band of fruit surface, and a higher % dry matter increased only between the 10^{th} and the 16^{th} days, resulting from the reduction of fruit water content (Table 1). The latter was observed for instance in avocado in the post-harvesting ripening period (Ozdemir and Topuz, 2004). We did not investigate the composition of the total soluble solids content (SSC), but previous results reported by Elias et al. (2004a) suggest that the different components, such as sugars, increase during pear post-harvest ripening.

The Vis/NIRS configurations were previously tested in 'Golden Delicious' apple, which guaranteed us, by comparison of our spectra with those published in e.g. Ventura et al. (1998), their proper functioning and reliability (data not shown).

In respect to pear cv. Rocha, PT diffuse reflectance (R) spectra presented a higher dispersion among replicates than the IS (Figs. 2 and 3). However, PT produced clearer spectra features than the IS, especially from 700 nm onwards (Figs. 2 and 3), which is probably due to the fact that PT configuration probes more deeply into the fruit pulp.

Three peaks at 600 (circa 30%), 725, 812 nm (both at circa 50%) and a minimum at 675 nm, were identified in both IS and PT spectra (Figs. 2 and 3). An extra value was considered between 725 and 812 nm, namely at 750 nm. In order to have a better evaluation of spectra changes, ratios of R in the previous mentioned wavelengths were calculated (the ratios are more insensitive to variations in the intensity of detected light due to optical fibre misalignments). In general, they changed in a similar way in IS and PT during fruit ripening, although in PT the changes were more prominent and values could differ ($p<0.05$) (Fig. 4). Values of reflectance peaks were approximately constant during ripening, but a significant increase (circa 3-fold) in the minimum registered at 675 nm was observed on the 10^{th} day, remaining approximately constant afterwards (Figs. 2 and 3). Additionally, ratios of R_{600}/R_{675} and R_{725}/R_{675} decreased consistently up to the 10^{th} day ($p<0.05$) (Fig. 5). Overall, this resulted most probably from the decrease in the pear peel chlorophyll *a* (Chl *a*) content, as previously reported by Elias et al. (2004b), and fairly agrees with the apparent higher intensity in the green to red band observed in ripening pears (Table 1). Indeed, pear peel photosynthetic pigments (chlorophylls and carotenoids) (Ben and Baszczyk, 2002) are expected to contribute to spectra features in the wavelength between 400 and 700 nm (Heldt, 2005), since they are known to absorb at wavelengths below 480nm and between 550 and 700 nm. Particularly, Chl *a*, the constituent of the photosynthetic reaction centres, is regarded as the central photosynthetic pigment, with a peak of absorbance at 675 nm. All others make part of the antenna, which gathers light at other wavelengths (450, 650 and the "green window") and transfers it to Chl *a* at the reaction centres (Heldt, 2005). On the other hand, the fact that the intensity in the blue to yellow band colour did not change along fruit ripening agrees with previous data obtained by Elias et al. (unpublished), concerning the carotenoids content in pear cv. Rocha peel, which remained constant along post-harvesting ripening.

The effect of fruit peel was previously tested, using fruits without peel. We have observed higher R values in the "green window" band and below, as well as at 675 nm, these changes being more obvious in the IS configuration (data not shown). However, these non-peel pear spectra exhibited the same general features of normal fruits, suggesting that the mentioned ratios are also dependent on the pulp properties. Indeed both R_{600}/R_{675} and R_{725}/R_{675} decreased significantly ($p<0.05$) up to the 10^{th} day, following fruit firmness behaviour (Fig. 5; Table 1). Vis/NIRS spectra have been shown to correlate positively to apple firmness (Zude-Sasse et al., 2002), but not to that of kiwi (McGlone

and Kawano, 1998). Despite the significant differences between the two Vis/NIRS configurations ($p<0.05$) in respect to the ratio R_{812}/R_{750}, both exhibited a minimum on the 3rd day, increasing from this point onwards (Fig. 4). This ratio was not easily related to any of the ripening parameters determined (Table 1), although this region is expected to be affected by the SSC and % dry matter, due to water and the carbohydrates absorbance region (McGlone and Kawano, 1998). In contrast to other fruits such as apple and grapes (Ventura et al., 1998; Zude-Sasse et al., 2002; Herrera et al., 2003), the spectra obtained and the corresponding ratios could hardly be related to the °Brix, since this parameter was unaltered along ripening.

As a conclusion, Vis/NIRS was applied successfully to pear cv. Rocha, producing reliable and reproducible spectra, which can apparently be related to some of the typical ripening parameters determined by the standard methods. Despite the higher dispersion among replicates, PT produced spectra with clearer features than IS. The different light penetration distances in the fruit (higher in PT) provided us complementary information, which may be very useful for future applications, e.g., in the analysis of superficial fruit pathologies. The apparent relation of the diffuse reflectance spectra and the classic ripening parameters will be tested later in a larger and appropriate sampling. This will allow, through the application of a standard multivariate data analysis, to determine the exact correlation levels and produce prediction models, which will be used to estimate non-intrusively the ripeness of pear cv. Rocha.

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Tables

Table 1. Maturation parameters of pear (*Pyrus communis* L.), cv. Rocha determined during fruit ripening, by using the standard methods. Each value refers to one extra fruit, taken on the same day of the optical measurements.

Time	Colour			Firmness	°Brix	% Dry matter	
(days)	L*[1]	a*[2]	b*[3]	(N)	(%)	Peel	Pulp
0	67.49	-6.87	42.92	43.6	12.6	-	-
3	73.23	-4.92	43.06	26.0	13.0	7.19	3.97
7	70.70	2.93	41.50	14.2	12.7	4.99	3.76
10	75.40	1.44	39.12	9.81	11.2	4.44	3.64
16	68.17	4.95	39.66	2.4	12.4	20.46	14.31
18	65.84	5.43	40.76	3.5	13.5	15.54	21.27

[1]Dark to light band.
[2]Green to red band.
[3]Blue to yellow band.

Figures

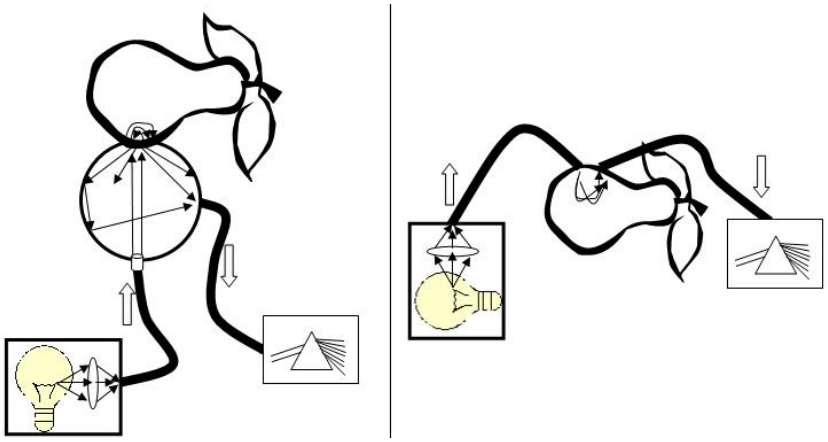

Fig. 1. Left: IS configuration - an integrating sphere is used to collect all the diffusively reflected photons from the fruit surface. Optical fibres guide the light from the source to the integrating sphere and from here to the spectrometer. Right: PT configuration - in partial transmittance measurements the collection fibre gathers photons at a specific distance from the input fibre. The collected photons have travelled more deeply inside the fruit pulp than in the IS configuration.

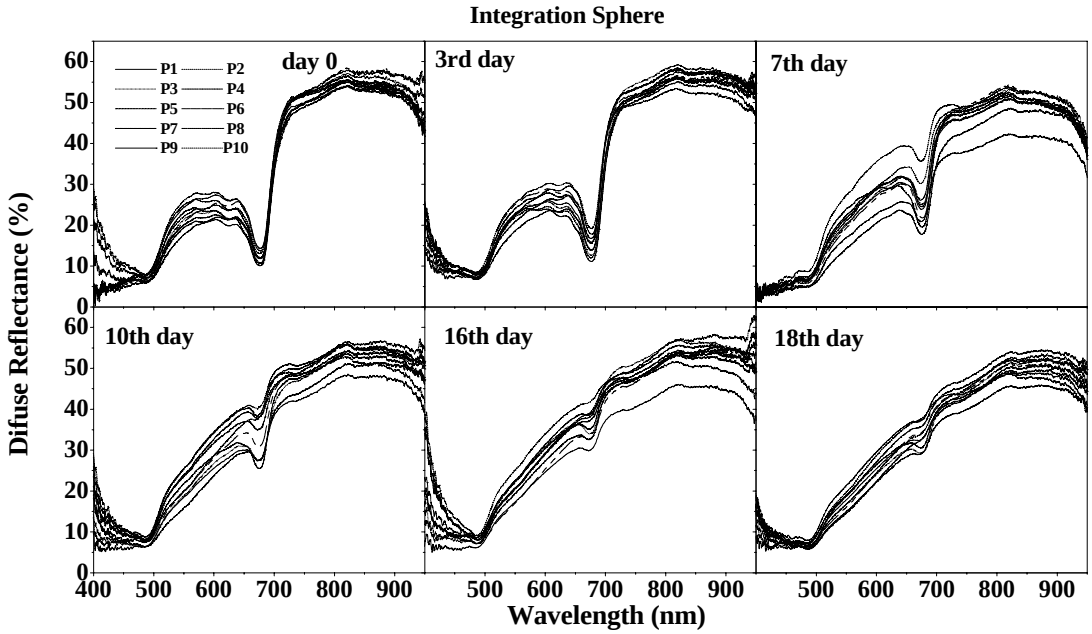

Fig. 2. Diffuse Reflectance (%) spectra of pear (*Pyrus communis* L.), cv. Rocha during fruit ripening, obtained by Vis/NIRS in an integration sphere between 400 and 950 nm. For each day, the spectra of ten fruits are represented.

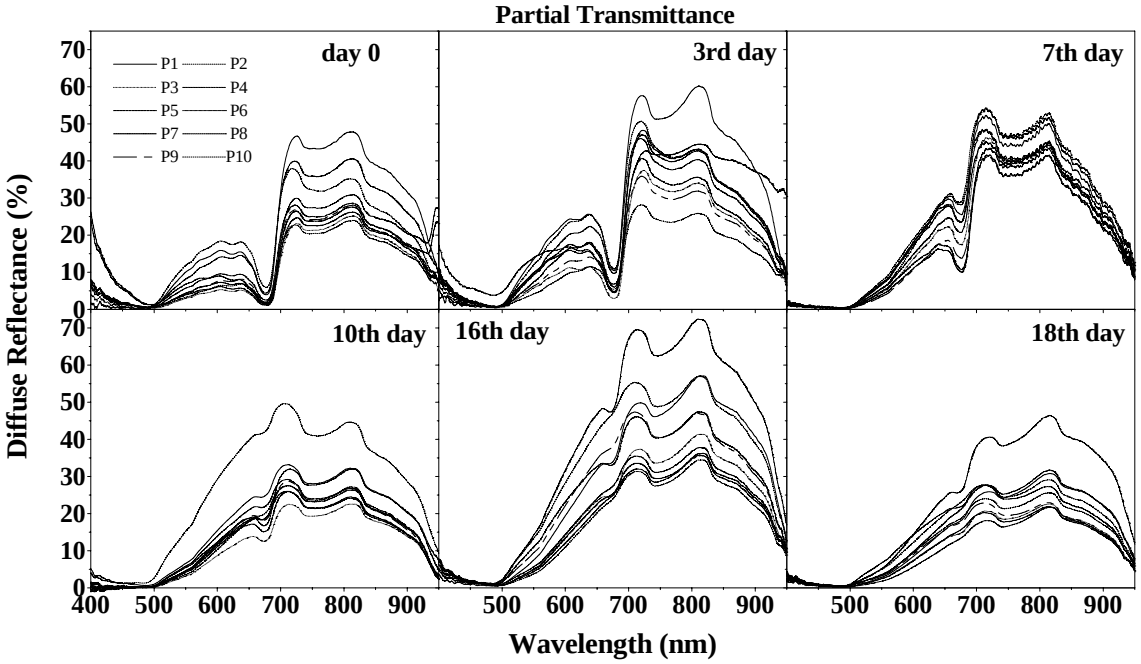

Fig. 3. Diffuse Reflectance (%) spectra of pear (*Pyrus communis* L.), cv. Rocha during fruit ripening, obtained by Vis/NIRS in the configuration of partial transmittance, between 400 and 950 nm. For each day, the spectra of ten fruits are represented.

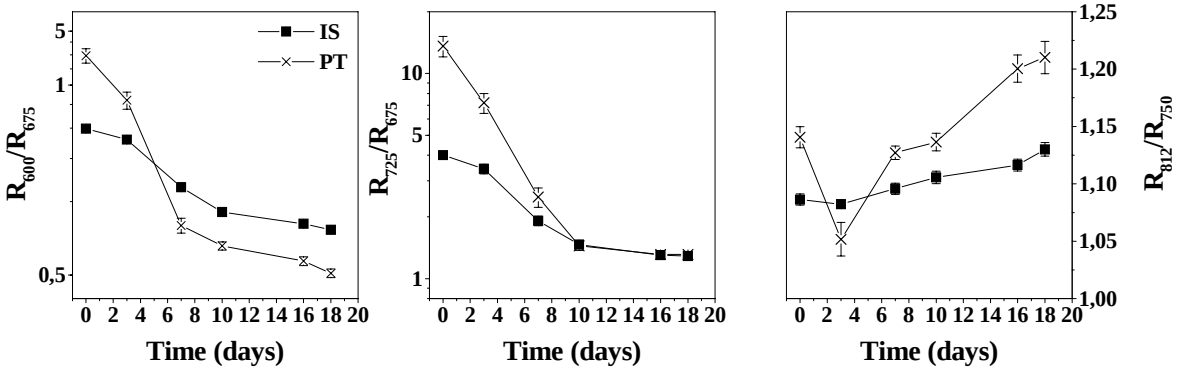

Fig. 4. Ratios of R_{600}/R_{675}, R_{725}/R_{675} and R_{812}/R_{750} of pear (*Pyrus communis* L.), cv. Rocha**,** during fruit ripening. R stands for diffuse reflectance (%). Each value represents the mean ± SE of 10 fruits.