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Short communication

Rapid spectroscopic method to assess moisture content in free and packaged oregano (*Origanum vulgare* L.)

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ABSTRACT

A spectroscopic study on oregano has been performed in order to find optical parameters that could be related to moisture content. Reflectance spectra have been obtained varying excitation wavelengths from the visible to the near infrared (NIR) regions of the electromagnetic spectrum. The latter region was very sensitive to water content displaying good correlations between reflectance values and moisture. Reflectance measurements were also performed by interposing a film of a commercial packing material (composed of polyethylene terephthalate and polyethylene) between the excitation source and the oregano sample. We managed to infer the moisture content of the samples through the packaging from the measurement of the diffuse reflected light. The results show the applicability of this spectroscopic technique to the non-destructive sensing of moisture for free or packaged oregano, whenever the wrapping film does not show absorption at 1440 or 1920 nm.

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1. Introduction

Non-destructive methodologies to infer quality parameters in samples are relevant because they allow easy and a fast quality control even in in-line production of foods and other materials (Talo and Peng, 2014; Huke et al., 2013). Counting on the ability to assess both packaging and storage conditions to preserve the good quality of a given sample is also important. Usually, quality controls are performed on the free product before being packed and no additional checkings are carried out during storage. Massive monitoring during warehousing, without destroying either the sample or the packaging would be obviously highly convenient and contributive.

Moisture content is regulated for a large quantity of dry foods, herbs and spices. In particular, for oregano, which is the most consumed herb in Europe and USA (Heperkan, 2006), moisture content should be kept below a maximum of 12% (Heperkan, 2006). Oregano is an aromatic herb not only important for flavoring foods but also for medicinal uses (Bruneton, 1999; Fleming, 2000; Salgueiro et al., 2003; Kulišic et al., 2007; Kaurinovic et al., 2011). This commercial product is a mixture of dry leaves and inflorescences of *Origanum vulgare* plant. The measurement of the amount of moisture is relevant because it determines weight, pricing and it has a direct relation with the microbial growth (US Food and Drugs Administration, 2001).

It is well known that water shows strong absorption bands in the near infra-red, especially in the region 1408–1470 nm (where absorption is due to the first overtone of O—H stretching) and in the region 1887–1960 nm (due to a combination of O—H stretching and bending) (Corredor et al., 2011).

The official method to determine moisture content in commercial oregano consists in determining the quantity of water entrained by azeotropic distillation (ISO 939, 1980). Another methodology is the oven method (drying at 100 °C until constant weight) (García et al., 2007). Both of these methods are time consuming and they involve manipulation and alteration of the studied sample. Faster methods for water estimation in solids have been developed, using contact or non-contact measurements (Wernecke and Wernecke, 2014; Chen et al., 2014). Sensors based on electrical determinations (voltage, impedance, current, resistance) usually require a contact interface between the sample and the sensor. On the other hand, sensors based on optical signals allow

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distance monitoring, avoid sensor contamination of the sample (or vice versa) and enable real-time control. In a recent publication (Mendes Novo et al., 2013), fluorescence of *Origanum vulgare* L. leaves, inflorescences and stems were studied and a dependence of fluorescence spectra with water content was found. In fact, it was shown there that the green/far-red and blue/far-red fluorescence ratios displayed a linear correlation with moisture content in oregano leaves.

In this study, our working hypothesis was based on the fact that if water absorbed radiation in the near IR, its presence in oregano would cause a decrease of reflectance in this region of the electromagnetic spectrum which could be used for sensing moisture in this herb. Moreover, when oregano was packaged in a commercial film transparent in the NIR region, moisture could be directly and non-destructively sensed by analyzing the signal through the film. With the aim of setting basis for the potential development of optical sensors, it was important to test this hypothesis by studying spectroscopically both oregano and the packaging film and by quantifying the observed signals as a function of the moisture content of the samples.

2. Materials and methods

2.1. Samples

Experiments were performed on leaves of *Origanum vulgare* ssp. *vulgare* grown under full sunlight in a land located in the surroundings of Buenos Aires. Intact leaves detached from the plant, with different water content, were prepared by drying about 20 g of fresh leaves in an oven at 50 °C for different time intervals in order to obtain values ranging from 90 (fresh leaf) to 10% of wet basis moisture content. Each set of leaves with a defined moisture content was divided into three portions: two of them were used for the duplicate of moisture determination (by the oven method) and the third portion was used for spectroscopic measurements as described below in Section 2.2. To determine moisture, oregano samples were dried in an oven at 100–105 °C for 24 h and moisture content (MC) was determined by the difference between the weight of the leaves before and after drying. Then, moisture content per wet basis (MC) was calculated according equation (1).

$$MC = \frac{m_1 - m_2}{m_1} \cdot 100 \quad (1)$$

where m_1 and m_2 represent the mass of leaves before and after oven drying, respectively (García et al., 2007). The packing film employed in the experiments was the one commonly used at the commercial level and it consisted of a sheet composed of two attached layers of polyester (polyethylene terephthalate) (12 μm) + polyethylene (90 μm).

2.2. Reflectance and transmittance

Reflectance spectra have been recorded by means of a spectrophotometer (UV3101PC, Shimadzu, Tokyo, Japan) equipped with an integrating sphere (ISR-3100, Shimadzu, Tokyo, Japan), which is 60 mm in inner diameter and it is equipped with a photomultiplier and a PbS cell. The 100% reflectance value was adjusted by using a white reference of powdered barium sulphate. To record the baseline, barium sulphate was placed in both positions (sample and reference) of the integrating sphere. To record sample reflectance, the sample-side white barium sulphate was replaced by a thick layer of oregano leaves (null-transmittance) with a given moisture content. Five reflectance measurements were performed and averaged on each sample. Diffuse reflectance percentage ($\%R_\lambda$) was recorded as a function of wavelength from 400 to 2500 nm. An integrating sphere was used to measure only diffuse reflectance

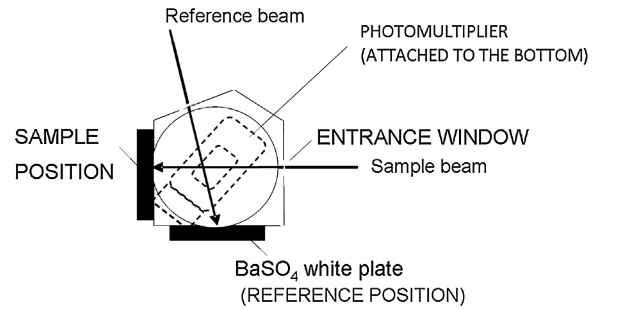

Fig. 1. Schematic representation of measurement setup using the integrating sphere.

(Fig. 1). The records were repeated by covering the oregano surface exposed to light with the commercial packaging film mimicking a surface of packaged oregano. The transmittance of the film was also recorded by using the integrating sphere from 400 to 2500 nm. Both diffuse reflectance of oregano samples and diffuse transmittance spectra of the film were collected on the spectrophotometer with the integrating sphere by using a spectral bandwidth of 3 nm both in the visible and NIR and employing a wavelength scanning speed of 50 nm/min (very slow). Data were manipulated by means of Shimadzu UVPC software.

2.3. Statistical analysis and sensitivity calculation

Each group of n data points (%R, MC) obtained from the measurements was fitted to a linear model $\hat{y} = \hat{m}x + \hat{b}$ by the least square method. In this equation, x stands for the MC, $\hat{m}$ is the least square estimator of the slope, $\hat{b}$ is the least square estimator of the intercept and $\hat{y}$ is the predicted value for%R based on the linear proposed model. The value for $\hat{m}$ gives the sensitivity of the calibration function (how much the %R changes for a unit change in MC). The standard deviation of the slope ($s\hat{m}$), the standard deviation of the intercept ($s\hat{b}$) and the coefficient of determination r^2 were also estimated (Montgomery and Runger, 2011).

Linear regression analysis was performed by using Sigma Plot 10.0 (Systat Software Inc., San Jose, CA).

The sensitivity of the method was also related to the measure of its ability to show significant differences in the signals for a given difference in moisture content (Harvey, 2009). Considering a linear correlation between the signal (%R) and the MC, if Δ(%R) was the smallest difference that might be recorded between two signals, it resulted that the minimum difference in water concentration that might be discriminated was $\Delta(MC) = \Delta(\%R)/\hat{m}$. This sensitivity concept was used to discuss our results.

3. Results and discussion

3.1. Reflectance spectroscopy

Reflectance spectra for dry and wet oregano leaves are shown in Fig. 2 where important differences are observed at 1440 nm and 1920 nm. The low reflectance values in these regions for fresh samples are due to water absorption in the NIR (Corredor et al., 2011). In Fig. 2, spectra are the obtained averages for a group of five samples for each moisture value.

When plotting the reflectance percentage as a function of the moisture content (%w/w) per wet basis of oregano leaves, linear dependencies were found for measurements at both 1440 and 1920 nm (Fig. 3). The corresponding fitted equations were: $\%R_{(1440nm)} = 82 - 0.72^*MC$ and $\%R_{(1920nm)} = 84 - 0.97^*$ MC, respectively.

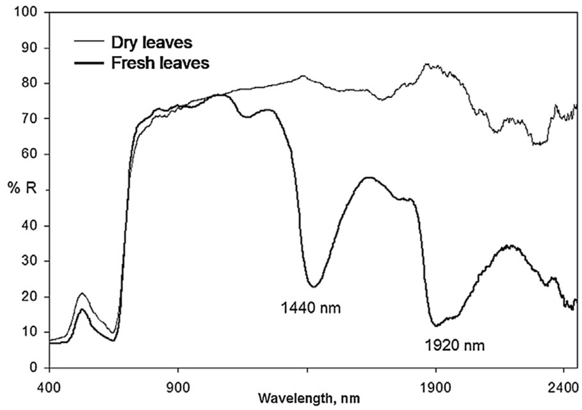

Fig. 2. Visible NIR Reflectance spectra of dry (MC = 4.0 ± 0.5%) (––) and fresh (MC = 82.0 ± 0.5%) (––) *Origanum vulgare* leaves.

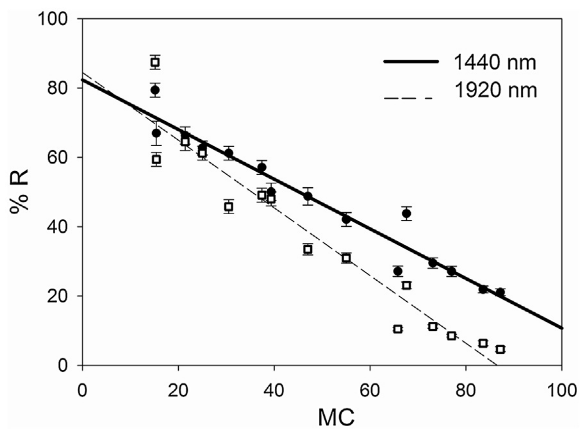

Fig. 3. Diffuse reflectance of *Origanum* leaves measured at 1440 nm (––) and at 1920 nm (- - -) as a function of moisture content on a wet basis expressed as percentage. The corresponding fitted equations are: $\%R_{(1440nm)} = 82 - 0.72*MC$ and $\%R_{(1920nm)} = 84 - 0.97*MC$, respectively.

As sensitivity is defined as the slope of the calibration curve at the concentration of interest (Inczedy et al., 1997), it arises that measurements at 1920 nm were more sensitive than those at 1440 nm. However, the second band is more symmetric and measuring at 1440 nm should be considered also as a good option despite its slight lower sensitivity.

In Fig. 4, the visible and NIR transmittance spectrum of the packing film is presented. It may be observed that almost 90% of the incident light was transmitted at both 1440 and 1920 nm, acting the packing film as a good transparent material for these radiation wavelengths and allowing the sensing of moisture through it.

In fact, it is well known that polyethylene typically shows absorption bands at 1200 nm (weak absorption due to C—H second overtone), 1400 nm (very weak absorption due to the first overtone of C—H combinations), 1730 nm (strong absorption due to C—H first overtone) and 2300–2500 nm (very strong absorption due to C—H and C—C combination) but non important absorption is detected for thin films of polyethylene (thickness lower than 100 μm) neither at 1440 nm nor at 1920 nm (Haanstra et al., 1998). Additionally, polyethylene terephthalate, which is also present in the packing film, does not present absorption in the region from 700 to 2000 nm except at 1656 nm (Masoumi et al., 2012).

Repeating the reflectance measurements on the oregano samples with different moisture content, interposing the packing film between the sample and the irradiation beam, a linear dependency was also found (Fig. 5). In these cases, the corresponding fitted equations were: $\%R_{(1440nm)} = 86 - 0.73*MC$ and $\%R_{(1920nm)} = 94 - 1.0*MC$, respectively.

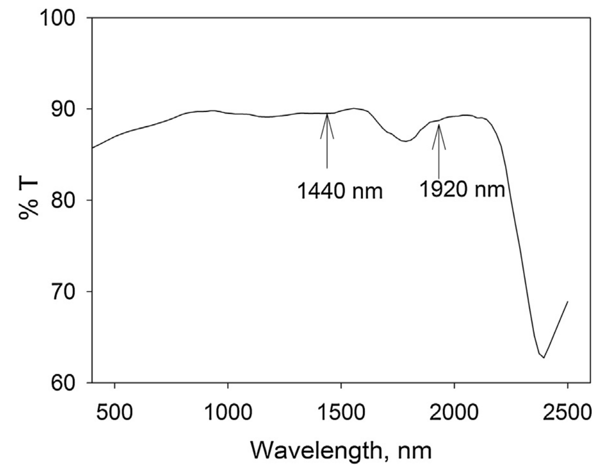

Fig. 4. Visible and NIR transmittance spectra of packing film made of polyester (12 μm) + polyethylene (90 μm).

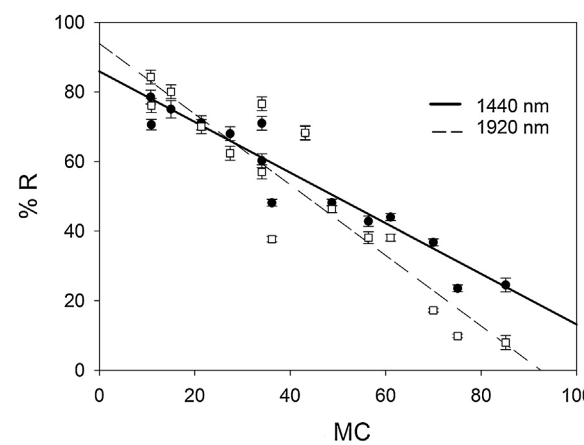

Fig. 5. Diffuse reflectance measured at 1440 nm (––) and at 1920 nm (- - -) of *Origanum* leaves through the packing film as a function of moisture content on a wet basis expressed as percentage. The corresponding fitted equations are: $\%R_{(1440nm)} = 86 - 0.73*MC$ and $\%R_{(1920nm)} = \mathbf{94} - 1.0*MC$, respectively.

Table 1
Parameters for the linear fitting ($\%R_\lambda = \hat{b} + \hat{m}*(MC)$) of the obtained data by Least Squares method.

Measurement Conditions	$\hat{b} \pm s\hat{b}$	$\hat{m} \pm s\hat{m}$	r^2
1440 nm without film	82 ± 3	−0.72 ± 0.05	0.9439
1440 nm with film	86 ± 3	−0.73 ± 0.07	0.8820
1920 nm without film	84 ± 4	−0.97 ± 0.08	0.9246
1920 nm with film	94 ± 5	−1.0 ± 0.1	0.8687

In Table 1, the least square estimators of the slope and the intercept, their standard deviation and the coefficient of determination r^2, for each set of data fitted to the linear model $\hat{y} = \hat{m}x + \hat{b}$, were summarized to allow an easy comparison.

From Table 1 it may be derived that the sensitivity for this methodology was higher for measurements at wavelength 1920 nm than for 1440 nm but the corresponding coefficients of determination resulted higher for data at 1440 nm, probably due to more important signal noise at 1920 nm. When comparing measurements with and without the packing film, it is observed that method sensitivity (given by the slope in the fitting) is kept almost invariant for free and packaged oregano considering the experimental error.

Regarding the minimum difference in moisture that could be discriminated with the NIR reflectance measurements described in the present work, Δ%R was about 1% and ΔMC varied around 1–1.4% taking into account the different slopes obtained. Hence, the sensitivity for the NIR reflectance method allowed discrimination of moisture content differing about 1%.

Additionally, in this work it was also proved (results not shown) that NIR signals only depended on moisture content regardless of the percentage of leaves and inflorescences in the sample.

Similar works on NIR spectroscopy on other substrates have been previously published. They have ranged from the detection of plant water stress by remote sensing using reflectance indices in the near and middle infra-red regions (Hunt and Rock, 1989) to the determination of water and other constituents in foods and pharmaceutics. Water content determination by using the NIR band placed at around 1920–1950 nm has been successfully used for grains, snacks, bread, sweets, cheese, and milk powder among others (Büning-Pfaue, 2003). A fibre-type NIR spectrophotometer was used for on-line sensing of soil moisture (Mouazen et al., 2005). In addition to this, measurements of water content and wet granulation in pharmaceutics via NIR spectroscopy have been reported (Rantanena et al., 2000). The present work provides thus a new contribution to the available list of determinations by using NIR spectroscopy, proving that it is a suitable technique for packaged oregano in the usual commercial plastic film.

4. Conclusions

Moisture content of oregano samples may be non-destructively performed by using NIR reflectance with good sensitivity.

Wavelengths of 1440 and 1920 nm are both convenient for these NIR reflectance measurements. The first one has the advantage of higher correlation coefficients and the second one of a slightly higher sensitivity.

The determinations may be performed on the intact packaged oregano through the polymeric film. In the present work, this methodology was proved to be suitable when the packing material was a polyester-polyethylene film, but in principle it may be adequate for any packing film transparent to wavelengths 1440 and/or 1920 nm.

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