oral paper

Early fungus infection detection in lemon fruits by means of spectral and colour analysis.

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ABSTRACT

Citrus are one of the major plants cultivated in the world. Current commercial systems classify the fruit based on quality parameters. Fungi of the genera Penicillium are responsible for substantial losses in citrus fruit during post harvest processes. It is imperative to detect the problem as early as possible, before it becomes visible, to allow the producer to take the corrective actions. Measurements of spectral reflectance and colour characteristics of healthy and inoculated lemons were taken with a PR715 Photo Research spectroradiometer in four experiments with controlled conditions. From the results, a wavelength (676 nm), and particularly its temporal variation were identified as carriers of useful information about the infection process. The increased gradient of the spectral reflectance value at 676 nm compared with the gradient corresponding to the healthy portion of the fruit could be an indicator of the presence of the infection. Other analyses were done taking into account the colour measurements. Results obtained suggest that consideration and analysis of "colour-change speed" (i.e. the colour displacement within a suitable colour space divided by the time it takes), seems to be a very efficient tool to diagnose the infection before it becomes visible.

1.- INTRODUCTION

Citrus are one of the major plants cultivated in the world. They are grown in more than 100 countries including the main producers such as China, Brazil, the USA, Spain, Mexico, Italy and Argentina. With respect to lemon, Argentina is the first producer (approx. 1.4 Mt). This number shows the economic importance of this activity (Sighicelli et al. 2003). Current commercial systems classify the fruit based on quality parameters. The main characteristic to attribute the quality of fresh fruits is the appearance, characterized by combination of size, shape, colour and absence of defects. The defects could be caused by biological, physiological or environmental factors in addition to mechanical damage. These defects could be originated in the cultivar or in the post harvest management. Among the later, fungi of the genera Penicillium are responsible for substantial losses in citrus fruit during post harvest processes. Due to citrus fruit infected with that fungus are not marketable, it is imperative to detect the problem as early as possible, before it becomes visible, to allow the producer to take the corrective actions. As was stated by Merzlyak et al. (1999) plant tissues undergo sometimes remarkable changes in spectral reflectance and colour as well, as a result of changes in the content and proportion of individual pigments. This fact makes reflectance spectroscopy a suitable technique for the assessment and monitoring of the physiological state of plants. Merzlyak examined changes in reflectance of plants related to mechanisms responsible for the senescence of their tissues, identifying some specially significant bands and wavelengths, like 500 nm and 670-680 nm and their relationships with respect to senescence and ripening. This

suggests to carefully examining the spectral response of the fruit searching for clues that indicate the presence of fungus infection. (Merzlyak et al. 1999, Sandoval et al. 2010)

2.- OBJECTIVES

The importance of spectroscopy in plant and post harvest monitoring becomes evident from the recent increase in research projects and publications dealing with the identification and measure of various quality attributes of fruit and vegetables (Nicolaï et al. 2007, Liew et al. 2008). In that context, the objectives of this research were (1) to acquire spectral reflectance of fungus attacked citrus peel conditions, (2) to identify the significant wavelengths that have the maximum discriminatory capability, and (3) to derive a methodology using these wavelengths that could allow the early detection of the infection. Besides, an approach based on colour description as an early detection methodology was tested.

3.- MATERIALS AND METHODS

In order to reach those objectives we analyse the process by means of spectral reflectance techniques, to detect the infection between 24 and 48 hours after the inoculation (Blasco et al. 2009). For this purpose, four sets of 10 lemons each, without visible skin defects were collected at random from a packing line. Five of them act as a control (healthy) group and the rest was inoculated with (*Penicillium digitatum*) by means of a standardized procedure. Figure 1 shows details of the situation.

Figure 1: left) healthy lemon sample; middle) infected sample after 72 hours from inoculation when rottenness is already clearly visible; right) zone of measurement.

Measurements of spectral reflectance and colour characteristics of healthy and inoculated lemons were taken with a PR715 Photo Research spectroradiometer, in the range of 380-1050 nm under diffuse lighting conditions, in four experiments with controlled conditions. In all the four series measurements were taken periodically on the inoculation point and on the opposite side of the fruit with intervals of 12 hours, over samples with different size and ripening.

4.- RESULTS

Figure 1 shows an example of the results obtained for the spectral reflectance of a measuring spot (1 cm diameter around the inoculation point). From the results, a wavelength (676 nm) was identified as a carrier of useful information about the infection process, particularly the temporal variation of the spectral reflectance at 676 nm. Values of reflectance at 676 nm from averaged curves corresponding to all inoculated samples (upper curve) and to healthy (control) samples plus healthy side of the inoculated samples are plotted in Figure 3.

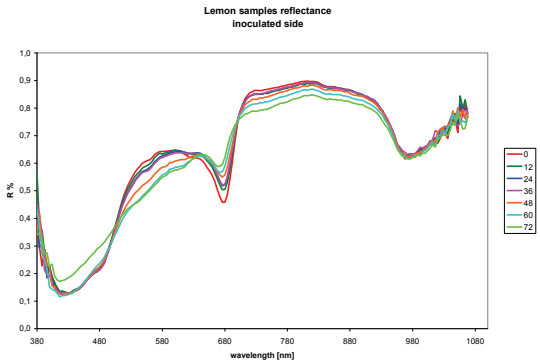

Figure 2: Temporary evolution of the spectral reflectance of the zone of measurement around the inoculation point.

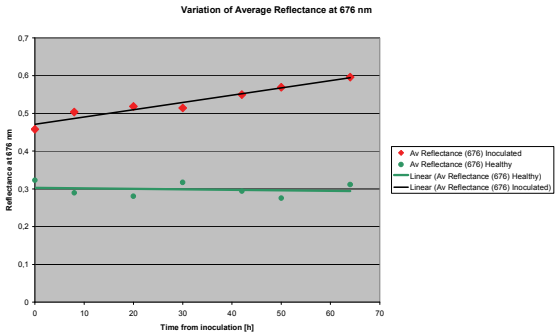

Figure 3: Values of reflectance at 676 nm for inoculated samples (upper curve) and healthy samples (lower curve) as function of time from inoculation

The increased gradient of the spectral reflectance value at 676 nm compared with the gradient corresponding to the healthy portion of the fruit seems to be an indicator of the presence of the infection. Measuring the spectral reflectance of samples at regular time intervals, extracting from them the reflectance value at 676 nm and calculating the gradient for reflectance change arise as a useful tool for early detection of infection by *Penicillium digitatum* in a packing line or storage stage of the fruit process.

Table 1: Colour points (u', v') for Sample set 3, measurements of inoculated and healthy side at different times.

S3-inoc.	u'	v'	S3-healthy	u'	v'
0	0,2699	0,5511	**0**	0,2855	0,5469
12	0,2702	0,5510	**12**	0,2911	0,5459
24	0,2721	0,5507	**24**	0,2950	0,5454
36	0,2708	0,5509	**36**	0,2930	0,5457
48	0,2728	0,5506	**48**	0,2983	0,5442
60	0,2718	0,5510	**60**	0,2990	0,5436
72	0,2759	0,5503	**72**	0,2933	0,5403

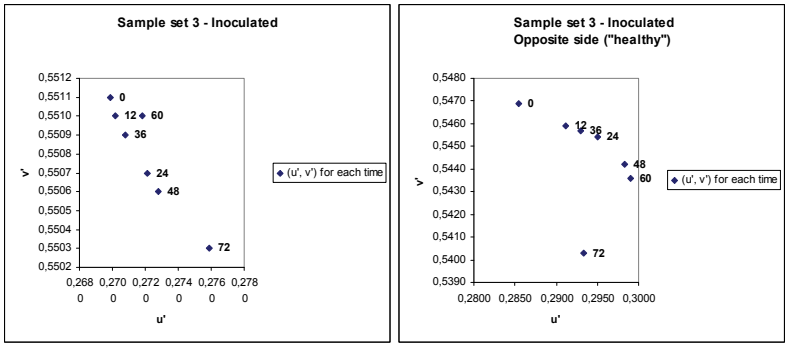

Figure 4: Points (u', v') averaged over sample set 3 as function of time from inoculation.

A different approach in analyzing reflectance characteristics employs a colour description system that models colour perception over the visible range. Since many plant stress factors impact on leaf biochemistry and morphology and consequently on reflectance spectral characteristics in the visible range, it follows that these changes can be related to leaf/fruit colour (Liew et al. 2008). Similar analyses were then done, taking into account the colour measurements. Results obtained suggest that consideration and analysis of "colour-change speed" (i.e. the colour displacement within a suitable colour space divided by the time it takes) seems to be a very efficient tool to diagnose the infection before it becomes visible. From

the measurements, the colour points (u', v') corresponding to inoculated and healthy samples were determined and plotted in a u', v' space, as shows Figure 4. As an example, we calculate the distance between points corresponding to 0 hours (inoculation time) and 36 hours after, for "inoculated" and "healthy" side of the samples by: $\mathbf{D_{36} = \sqrt{((u'_0 - u'_{36})^2 + (v'_0 - v'_{36})^2)}}$, resulting $D_{36\text{-healthy}} \cong 0.0076$ and $D_{36\text{-inoculated}} \cong 0.0009$. Then, we define a Colour Speed value (distance the colour point (u', v') shifts from 0 to 36 hours divided by 36 hours) as: $\mathbf{CS_{36} = D_{36} / 36}$ (or 24, 48 or the time interval more suitable for the situation we deal with). In our case, we choose 36 hours because this time interval appears to be enough to take preventive and corrective actions before the infection becomes visible, avoiding major damage. Calculating CS_{36} we obtain 0.000210 unit/h for healthy and 0.000025 unit/h for inoculated case. These results suggest that the presence of fungus infection slows down the colour speed of the lemon with respect to natural ripening process.

5.- CONCLUSIONS

The determination at regular time intervals of reflectance values at 676 nm and calculus of the gradient for reflectance change compared with the gradient corresponding to natural ripening process arise as a very useful tool for early detection of infection by *Penicillium digitatum* in a packing line or storage stage of the fruit process. Besides, taking into account the colour measurements and applying a very simple analysis of what we name "colour-change speed" (i.e. the colour displacement within a suitable colour space divided by the time it takes), seems to be also a very efficient tool to diagnose the infection before it becomes visible. Although much work has to be put on it and refinement has to be done, this approach appears as a very promising way, which allows producers to take preventive and corrective actions in the lemon process, before the infection becomes visible, avoiding major damage over the whole lemon production.

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