Characterization of citrus fruit quality using reflectance spectroscopy

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Abstract - The application of reflectance spectroscopy for citrus fruit quality monitoring is discussed. The reflectance measurements were performed on orange, mandarin, and grapefruit fruit surface in the spectral range from 385 nm to 1100 nm with the Perkin Elmer spectrometer equipped with the integrating sphere. The changes observed in reflectance spectra of oranges due to fruit maturation and post-harvest storage mainly are due to the chlorophyll loss. The difference in reflectance spectra for healthy orange fruits and fruit infested by fungi are shown. The potential of quantitative *in vivo* measurements of plant pigments like chlorophylls, carotenoids, and anthocyanins, in citrus fruit peel is discussed.

Keywords: reflectance spectroscopy, plant pigments, chlorophyll, citrus, orange, fruit

1. INTRODUCTION

Citrus are one of the major plants cultivated in the world. They are grown in more then 100 countries including the main producers such as Brazil, the USA, China, Spain, Mexico and Italy. Among citrus the oranges are the specie most cultivated following by mandarin, lemon and grapefruit. In particular the blood oranges are fruits characterized by presence of anthocyanins and phenolics compounds with therapeutic proprieties as anti- cancer, antioxidant and antimutagenic activities. It is also reported that they may reduce the risk of cardiovascular disease (Cook and Samman 1996) and stroke (Peterson, Dwyer, 1998). So, besides of known health benefits of citrus fruit there is an increasing interest in blood oranges for their positive effects on human health.

Most of the orange fruits go to the market as fresh fruits and the 30-35% of total production is transformed into juice. The principal component to attribute the quality of fresh fruits is the appearance, characterized by combination of size, shape, color and absence of defects (Shewfelt, 1999). Specifically the absence of visible defects on fruit is considered important for quality consideration. The defects could be caused by biological, physiological, environmental factors in addition to mechanical damage and genetic variation and aberration. In recent years different optical methods such as near infrared (NIR) and visible (VIS) spectroscopy techniques have been utilized to evaluate the fruit quality including the health monitoring, of fruit in post-harvest period. The advantages of non-intrusive optical techniques are represented by rapid measurements on a large number of samples, which thereafter remain intact and could be used for further analysis. Furthermore it is possible to apply biological methods to recover the analyzed fruit.

Reflectance spectroscopy is a non-invasive technique, which can be applied to study

- *pigment composition and content* in plant tissues using reflectance values in the visible range where pigments absorb light (Merzlyak, 1999, 2003; Sims, 2002);
- *pigment development or degradation as a response to biotic and abiotic stress* and during plant growth and aging. Reflectance spectra of plants undergo significant changes during tissue browning and necrotization, which is due to accumulation of polyphenol oxidation. Browning of leaves occurs during senescence (Merzlyak, 1997) and as a result of air pollution (Penuelas and Filella, 1998), whereas fruit browning represents a symptom of superficial scald (Chivkunova, 2001) and sunburn (Merzlyak, 2002);
- *water content* in thick tissues using the drop of reflectance at specific water absorption bands in the near infrared region.

The main objective of the work was a laboratory study of citrus peel reflectance spectra in VIS and NIR spectral range from 385 nm to 1100 nm to provide a scientific background for further elaboration of a non-invasive method for fruit quality *in vivo* monitoring.

2. MATERIALS AND METHODS

2.1 Techniques and instrumentation

In the experiments were used the following spectral techniques: reflectance spectroscopy for the samples of fruit peel with the spectrophotometer Perkin Elmer Lambda 25; absorption spectroscopy for methanol and acetone extracts of fruit peel with the spectrophotometer Perkin Elmer Lambda 25; fluorescence spectroscopy for methanol and acetone extracts of fruit peel with the PTI luminescence spectrometer.

The Perkin Elmer Lambda 25 spectrometer equipped with the integrating sphere and the certified reflectance standard (Spectralon, Labsphere) was used to measure reflectance and absorption spectra. The spectrometer covers the spectral range of 200 to 1100 nm But due to the reason that typically the fruit peel absorbs all incident light in the UV range, the measurements on plant samples were performed in the spectral range of interest from 385 nm to 1100 nm. The plant tissue samples were placed behind the integration sphere with a matt black paper as a background. The absorption spectra of solutions in were measured with the same instrumentation, but the samples were placed in the standard 1-cm quartz cuvette in front of the integrating

sphere, and the Spectralon reflectance standard was used behind the integrating sphere as a background.

2.2 Pigment extraction

In order to determine pigments concentration in the fruit peel two types of extractions were prepared. Anthocyanins extraction was performed by methanol 90% acidified with HCl 1N (1:1:6 v/v) while chlorophyll and carotenoids were extracted in 90% of acetone. The pieces of peel of 1 cm^2 area were put in solvent and kept at 4°C overnight (24 hours). Then the samples were centrifuged at 4.000 rpm at 4°C for 20 minutes.

Investigation on orange acetone extracts of peel samples with different pigmentation of using absorption and fluorescence spectra was performed for the cultivar Navel. The same type of experiments was made for the cultivar Moro with methanol extracts.

2.3 Fruits material

The oranges of cultivar (cv) Navel (umbelicate), Moro and Tarocco (pigmented; blood oranges) were received at the same stage of ripe from Sicily. The plants were cultivated following organic methods which exclude any kind of treatment. The fruits were not treated both on pre- and post-harvest. The experiments were performed three days after harvest if other is not mentioned in the text. Other citrus fruit (mandarin, and grapefruit) were purchased at the market.

3. ORANGE FRUITS INOCULATION

Fruits of cv Navel, Moro and Tarocco were inoculated with both pathogens *Penicillium italicum* or *Phytophtora citrophtora*. These fungi are major pathogens causing orange disease in post-harvest period.

3.1 *P. citrophthora* inoculation

In order to induce sporulation, *P. citrophthora* was cultured on V8 juice agar medium and transfer on fresh medium every 20 days. Sporangia suspension was prepared by taking 30 day-old from a Petri dish. The concentration of sporangia/ml was determined by counting in a Burker chamber (Fortuna, Germany). For each experiment six fruits were inoculated by piercing the peel (six pick of 500 µl for each fruit) with a suspension of $2x10^4$.sporangia/ml. Visible symptoms were evaluated from 2 Day After Infestation (DAI) to 3 DAI.

3.2 *P. italicum* inoculation

P. italicum was cultured on Potato Dextrose Agar (PDA) medium. In order to stimulate spores development 10 days before inoculation the fungi was kept in the dark. The inoculum was prepared the same way as *P. citrophthora.* The spores concentration was $2x10^6$ spores/ml. Symptoms were evaluate from 1 to 3 DAI

4. RESULTS

4.1 Visible and NIR reflectance spectra of citrus fruits

Typical reflectance spectra of citrus peel samples with different coloration are shown in Fig. 1- 2.

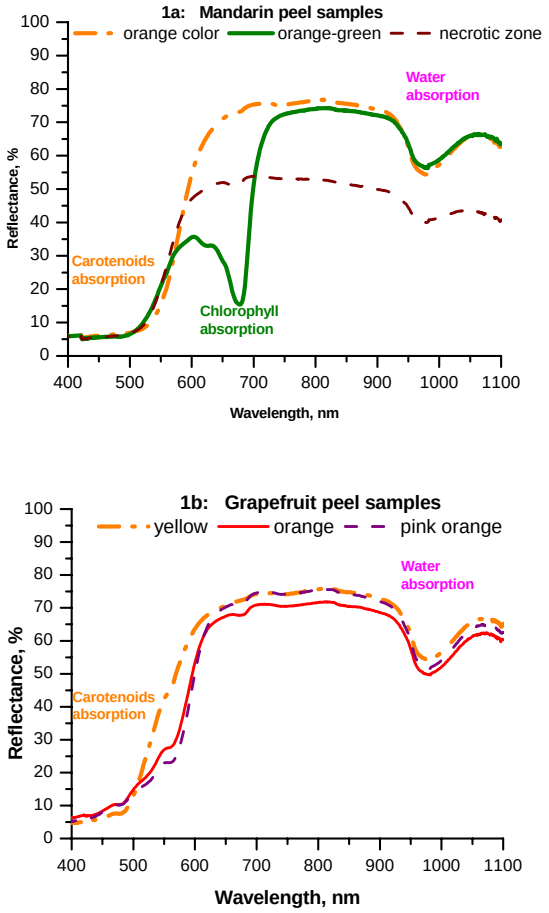

Figure 1. Reflectance spectra for mandarin peel (1a) and grapefruit peel (1b) samples of different coloration.

Typically absorption of pigments in the UV and in visible spectral range up to 500 nm is very high in the citrus peel, and low reflectance values are mainly originating from scattering in this range. Starting at 720 nm up to water absorption band centred at 980 nm citrus peel has very high reflectance values around 70-80%. From Fig.1a one can notice decrease of the NIR reflectance values due to softening (pre-necrosis stage) of the peel sample.

Reflectance features from 500 to 720 nm are due to plant pigments absorption. The difference between reflectance spectra for the peel samples with different coloration is due to changes in the region of chlorophylls (Chls) and carotenoids absorption. Although absorption of the main photosynthetic pigment - chlorophyll *a* - is centred on 675 nm, due to absorption of chlorophyll *b* and carotenoids linked to those chlorophylls we have reduction of reflectance values in the spectral region from 570 up to 720 nm for the green-coloured samples (see Fig. 2).

During fruit maturation and post-harvest storage different processes of pigment development and degradation take place in the fruit peel. This can be monitored using reflectance spectra (see Fig.2).

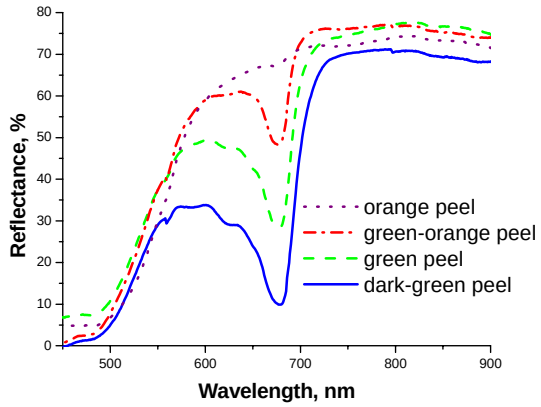

Figure 2. Reflectance spectra of orange peel samples for the cultivar Navel with different coloration of the peel.

The loss of chlorophyll is the most easily measured parameter for describing the yellowing of color changes in ripening fruits. The yellowing of peel samples during post-harvest storage is mainly due to unmasking and partial retention of carotenoids rather than to the new biosynthesis of yellow pigments (see Fig. 3).

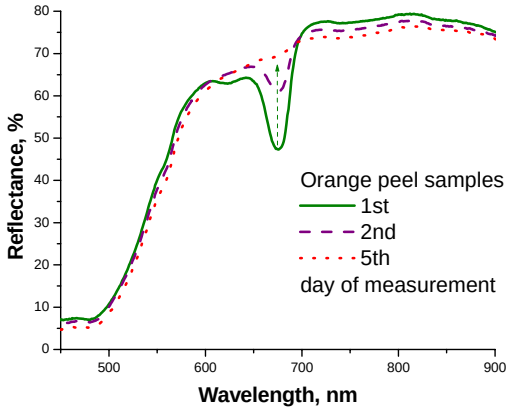

Figure 3. Reflectance spectra of orange peel samples for the cultivar Navel during storage at laboratory conditions (medium light and room temperature).

4.2 Quantification of pigments in the orange peel

Absorption and fluorescence spectra were measured from acetone and methanol pigment extracts of orange peel. From absorption measurements it appeared that absorbance values for extracts are very low to estimate chlorophyll concentration.

For pigment quantification the new technique was suggested to estimate concentration of pigments (Chl *a*, Chl *b*, total flavonoids and total phenolics) in orange peel using fluorescence spectra of acetone extracts. Fluorescence spectra of chlorophylls standard and typical spectra of orange peel extract are shown in Fig. 4 for two excitation wavelengths – 410 nm (optimal for excitation of Chl *a* emission) and 460 nm (optimal for excitation of Chl *b* emission). Fluorescence peaks for chlorophylls *a* and *b* are positioned at 670 and 650 nm in acetone correspondingly.

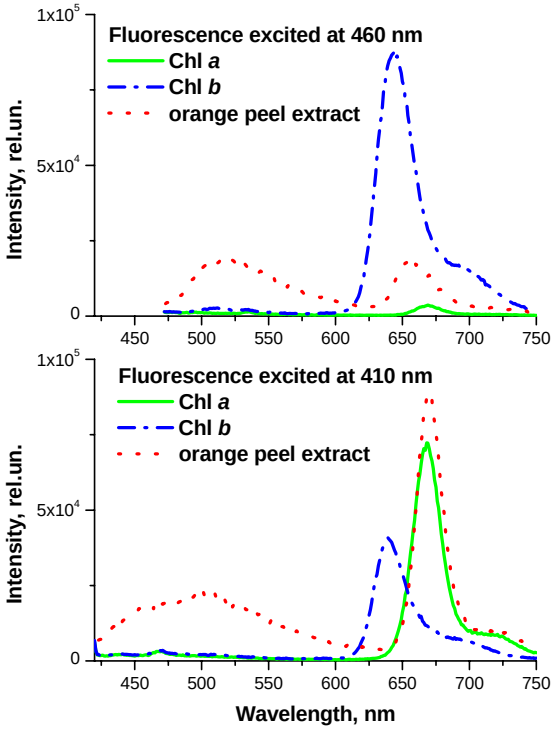

Figure 4. Fluorescence spectra of acetone extract of the orange peel and acetone solutions of chlorophylls *a* and *b*.

For estimation of chlorophylls concentration C(Chl *a*) and C(Chl *b*) we used the formulas

$$C(\text{Chl } a) = A \times (FL(670/410) - 0.145 \times FL(650/460));$$
$$C(\text{Chl } b) = B \times FL(650/460),$$

where FL(670/410) and FL(650/460) are fluorescence intensities for Chls *a* and *b* at given emission/excitation wavelengths with subtracted fluorescence intensity of the blank solution with the same emission/excitation. Coefficients A and B can be derived from the fluorescence spectra of chlorophyll standards in acetone with known concentration. We estimated concentration of chlorophylls *a* and *b* in different orange peel samples using this newly developed technique and found that concentration of Chl *a* was varying for the mature fruits of the cultivar Navel from 10 to 100 μg for cm^2. Ratio of Chl *a* to Chl *b* concentration was 7 ± 2.

Other pigments like phenolics and flavonoids can be quantified in relative units from fluorescence spectra of acetone extracts using fluorescence intensities at 540 nm with excitation at 410 nm for flavonoids and at 450 nm with excitation at 355 nm for phenolics.

Methanol extracts fluorescence peaks of Chl *a* and *b* are too close to be used for selective quantification of chlorophylls. Methanol extracts can be used for estimation of total concentration of chlorophylls. Additionally methanol

extracts contain anthocyanins and its fluorescence can be used for their quantification.

This technique of quantification of plant pigments using fluorescence spectra of extracts works also at low concentration of pigments when it is not possible to quantify those using absorption spectra.

4.3 Response of orange fruits to pathogenic fungi infestation

Damages of the peel can be seen as browning of the tissue and as a reduction of the NIR reflectance values. Fig. 5 shows the response of orange fruits of the cv Tarocco infested with *P. italicum* and *P. citrophtora.* Changes in reflectance spectra can be seen already after 1-2 days after inoculation. Reflectance spectra is different for different pathogens (fig 5). Similar results were obtained from cv Navel and Moro.

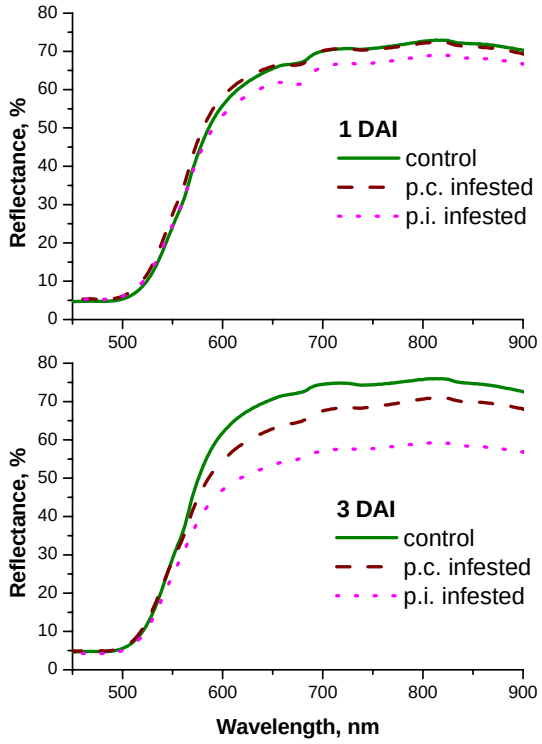

Fig. 5 Averaged reflectance spectra for orange peel samples for the cultivar Tarocco infested with pathogenic fungi *Penicillium italicum* (p.i.) and *Phytophtora citrophtora* (p.c.) for different DAI (Days After Inoculation).

5. CONCLUSIONS

The new technique was suggested to estimate concentration of pigments (Chl *a* and *b*, total flavonoids and total phenolics) in orange peel using fluorescence spectra of acetone extracts. This technique works also at low concentration of pigments when it is not possible to quantify those using absorption spectra. We estimated concentration of Chl *a* and *b b* in different orange peel samples using this technique and found that concentration of Chl *a* was varying in the peel of mature fruits of the cultivar Navel from 10 to 100 μg for cm^2. Ratio of Chl *a* to Chl *b* concentration was 7 ± 2.

- Ageing of fruits or degradation of pigments with time during post-harvesting period peel is manifested in reflectance spectra as loss of chlorophyll absorption.
- Degradation of pigments and cell damages in response of infestation can be monitored using reflectance measurements from the fruit peel. Changes in reflectance spectra of the orange peel caused by *P. italicum* and *P. citrophtora* pathogens can be seen already 1-2 days after inoculation. Reflectance spectra of fruits infested by *P. italicum* differ from that of *P. citrophtora.*

6. ACKNOWLEDGEMENTS

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