

## Preliminary results on the non-invasive diagnosis of superficial scald in 'Rocha' pear by fluorescence imaging

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**Abstract:** Superficial scald, a major physiological disorder of pome fruit, is a long-lasting issue waiting for a definitive non-chemical dependent solution. This is our first report on the use of a non-invasive method to detect scald in the valuable Portuguese variety 'Rocha' pear (*Pyrus communis* L.). Mature unripe fruits without any preventive treatment for scald and stored for about 2.5 months under cold normal atmosphere (NA: 0°C, rH 90–95%) and under cold controlled atmosphere (CA: 0°C, rH 90–95%, 1.5 kPaO<sub>2</sub> + 0.5 kPa CO<sub>2</sub>), were obtained from Frutoeste (Mafra, Portugal). After 6 h at 15°C, during transportation, and one additional cold-storage week (NA: 1°C, RH 90–95%) at University of Algarve (Faro, Portugal), fruit was transferred to shelf-life (circa 20°C and 70% RH). Scald Index (SI), severity (SS) and surface scald distribution (SSD) were determined in the beginning of the shelf-life (0 d) and periodically up to the 6<sup>th</sup> d. These parameters remained constant for each pear along that period: SI was 0-4 in NA and 0-3 in CA pears; SS was 0-3 in NA and 0-1 in CA and SSD was 0-100% in NA and 0-75% of the fruit in CA.

Fluorescence imaging photos of all fruits were taken periodically with a system made at the physics lab of CEOT, consisting of six commercial blue light LED lamps, two Ar-ion lasers emitting at 475 nm ( $\sim 1000 \mu\text{mol m}^{-2}\text{s}^{-1}$ ), lenses for beam expansion, a CCD camera, an optical neutral density filter (ONDF) and an optical band pass filter (OBF) centred at 690 nm. Images were stored and processed by the appropriate software. Two types of images were obtained: blue reflectance images, taken with the ONDF, and fluorescence images, taken with the OBF. Both images suggested a non-uniform field illumination effect. In order to eliminate this effect, images were divided, creating a new image of the ratio Fluorescence/Reflectance, which is expected to be independent on the variations of the illumination field.

All scald typical patches were detected easily by the fluorescence imaging system, as low fluorescence intensity areas, even those very small that were not visible in a normal digital photo. Yet, spots of other origin, such as peel scars were also detected by this system. Additionally, the fluorescence imaging system detected low fluorescence inten-

sity areas, though apparently normal to the naked eye. It is not yet known if these areas would be further expressed as a future scald patch with a longer cold storage.

## 1 Introduction

Superficial scald is a chilling induced oxidative major disorder of pome fruit, including the Portuguese exclusive and valuable POD 'Rocha' pear (*Pyrus communis* L.). It involves the synthesis of the sesquiterpene (E, E)- $\alpha$ -farnesene and the accumulation of its oxidation primary products, the conjugated trienols (CTols), on the epidermis and hypodermis layers of the fruit, disrupting the cells on the fruit skin (INGLE 2001). Scalded fruits exhibit brown patches on the peel that may cover them totally and attain 6 mm deep. Fruit prices are lowered as soon as the disorder is expressed, and later they won't be sold on the market as fresh commodities anymore. The fruit tendency to develop this disorder relates to nutritional, environmental as well as storage conditions (CHAPON & BONY 1997). Since low temperature is a key and unavoidable factor in 'Rocha' pear storage, superficial scald remains a pressing and economical issue, demanding for strict preventive methods, some of them include critical chemicals for human health, such as diphenylamine (DPA).

Measurements of fluorescence in fruits are omnipresent but, to our knowledge, only (DELL *et al.* 1996) has used fluorescence (Fv parameter) as a predictor of scald in apples, although with limited success. Nevertheless, photosynthetic pigments, particularly, chlorophylls are quite responsive and sensitive under oxidative conditions, their fluorescence signal, changing according to the effect on photosystems and photosynthetic metabolism (SCHREIBER *et al.* 1994). Chlorophyll fluorescence imaging (CFI) gives an image of fluorescence at each point of the fruit or plant tissue, being quite promising as a fast and accurate method to detect stressful conditions for plants (SCHLÜTER *et al.* 2009).

The aim of this study was to test the use of CFI to detect superficial scald in 'Rocha' pear, in order to obtain an early diagnosis of this physiological disorder, and therefore, avoid chemical treatments and economical losses at the initial grading steps after fruit collection.

## 2 Materials and methods

### ***Fruit and storage conditions***

Mature unripe fruits without any preventive treatment for superficial scald and stored for about 2.5 months under cold normal atmosphere (NA: 0°C, rH 90–95%) and under cold and controlled atmosphere (CA: 1.5 kPa O<sub>2</sub> + 0.5 kPa CO<sub>2</sub>, 0°C, rH 90–95%), were

obtained from Frutoeste (Mafra, Portugal). After 6 h at 15°C, during transportation, and one additional cold-storage week (NA: 1°C, rH 90–95%) at University of Algarve (Faro, Portugal), fruit was transferred to shelf-life (approx. 20°C and 70% RH).

### ***Scald parameters***

Scald Index (SI): Sum of all scald patches surface per fruit (0: 0%; 1: >0≤5%; 2: 5-10%; 3: 10-25%; 4: 25-50%; 5: >50%), scald severity (SS) (0: no scald patches; 1: superficial patches; 2: superficial and dry scald patches; 3: deep and dry scald patches) and surface scald distribution (SSD) (% of fruit surface where scald patches appear: 0-100%) were determined in the beginning of shelf-life (0 d) and periodically up to the 6<sup>th</sup> d. Scald Intensity stands for the % of scalded fruits among the whole fruit set, which in this case was about 300 for both NA and CA. For the following CFI measurements, sub-sets of NA (n = 11) and CA (n = 8) were chosen.

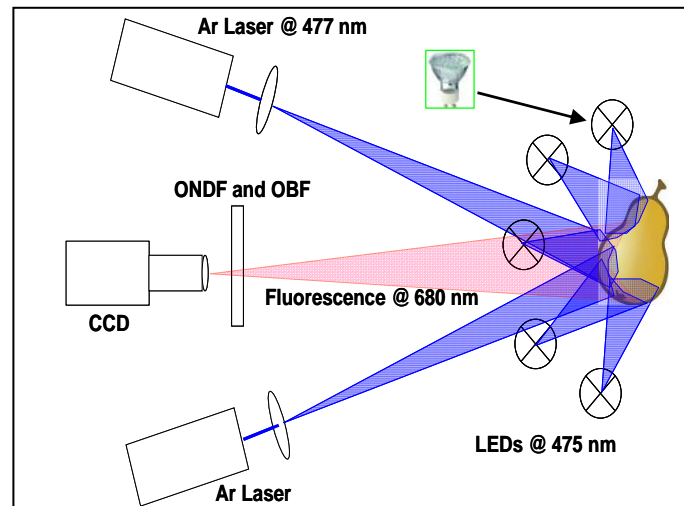
### ***Chlorophyll fluorescence imaging measurements***

Chlorophyll fluorescence imaging photos of all fruits were taken periodically along the shelf-life period with a homemade system, consisting of six commercial blue light LED lamps (Phillips, Netherlands; 1W @ 475 nm), two Ar-ion lasers emitting at 50 mW @ 477 nm (total PPFD ~ 1000  $\mu\text{molm}^{-2}\text{s}^{-1}$ , assumed non-saturating), 2 lenses for laser beam expansion, a CCD camera (Ophir BeamStar FX33) with appropriate lenses, an optical neutral density filter (ONDF) and an optical band pass filter (OBF) centred at 690 nm. Images were stored through the camera software and processed in MATLAB® (2008). Several images were averaged in order to eliminate the fluctuation in the intensity of the LEDs due to the mains 50 Hz.

### ***Data analysis***

The effects of time and storage conditions on each of the scald parameters were tested by a two-way ANOVA with SigmaStat 2.0 (SPSS Science, USA).

Two types of images were obtained: blue reflectance images (R), taken with the ONDF, and fluorescence images (F), taken with the OBF. Both F and R images suggested a non-uniform field illumination effect. In order to eliminate this effect, images were treated according to the following procedure. Let  $I(r)$  be light intensity incident on the fruit surface. Through the imaging lens each point,  $r$ , on the fruit surface, is imaged on a



**Figure 1:** CFI system set up consisting of six commercial blue light LED lamps (1W @ 475 nm), two Ar-ion lasers emitting at 50 mW @ 477 nm, 2 lenses for laser beam expansion, a CCD camera with appropriate lenses, an optical neutral density filter (ONDF) and an optical band pass filter (OBF) centred at 690 nm

pixel  $p$  of the CCD sensor and from now on we will identify  $r$  by its imaging pixel,  $p$ . The counts of each pixel at 477 nm are given by  $C_{477}(p) = A_{477}BR_{477}I(p) + N(p)$ , where  $A_{477}$  is a wavelength dependent factor including the detector quantum efficiency at 477 nm and the filter transmittance,  $B$  is an wavelength independent factor including a geometrical factor related with the camera's acceptance angle and the camera gain,  $R_{477}$  is the pear reflectance at 477 nm and  $N(p)$  is a noise factor, which we have determined by taking a photo with lights off (dark or background photo). By the same token, the counts at 680 nm are given by  $C_{680}(p) = A_{680}B\Phi_{680}I(p) + N(p)$ , where now  $\Phi_{680}$  gives the proportionality between fluorescent intensity and incident light intensity (assumed non-saturating). The variable presented in the following pictures is  $S(p) = (C_{680}(p) - N(p)) / (C_{477}(p) - N(p)) = (A_{680}/A_{477})\Phi_{680}(p)/\Phi_{477}(p)$ . This variable is expected to become independent of the illumination profile, as required.

### 3 Results and discussion

This is the first report on the use of a non-invasive method, namely a CFI system to detect superficial scald in the valuable 'Rocha' pear (*Pyrus communis* L.).

All scald parameters were determined for each fruit in the beginning of shelf-life (0 d) and periodically up to the 6<sup>th</sup> d and remained constant for each pear along that period (**Table 1**). Scald intensity (%) was higher in the NA 'Rocha' pears group, as previously found (ISIDORO & ALMEIDA 2006) (**Table 1**). Although the limits observed for any of the parameters were different between NA and CA fruits, in what concerns the average values obtained for both groups, they were statistically ( $P < 0.05$ ) equivalent.

**Table 1:** Scald parameters of 'Rocha' pears determined in the beginning of shelf-life (0 d) and periodically up to the 6<sup>th</sup> d. Results presented are those registered on 6<sup>th</sup> d for both NA and CA 'Rocha' pears used along this study (NA set: n= 11; CA set: n= 8). Scald Intensity stands for the % of scalded fruits among the whole fruit sets, which in this case were about 300 for NA and CA.

Scald Parameters of pears on the 6 <sup>th</sup> shelf-life day				
Samples	SI (0-5)	Scald Intensity (%)	SSD (%)	SS (0-3)
NA	0-4	48	0-100	0-3
CA	0-3	8	0-75	0-1
<b>NA (Mean±SE)</b>	1.64±0.41	-	36.36±9.74	0.86±0.21
<b>CA (Mean±SE)</b>	1.00±0.33	-	34.38±10.49	0.70±0.16

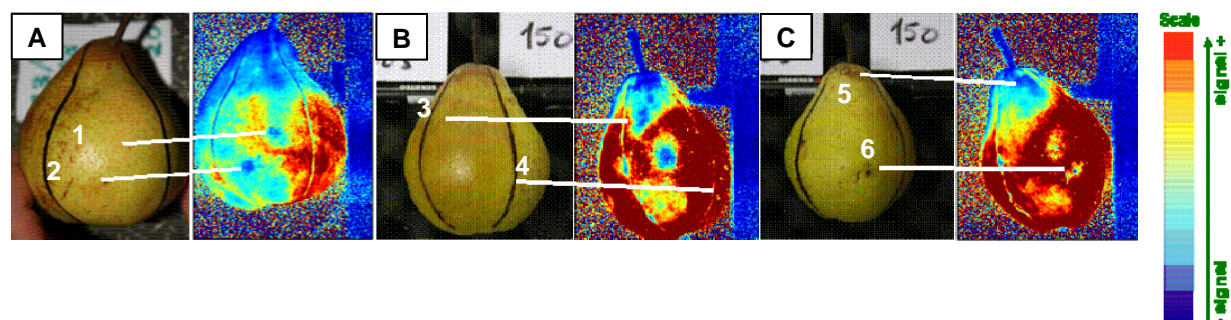
Figures depicted in the following section were chosen among the sub-sets of NA and CA pears used in the study, in order to show the detection potential of this system set up.

All scald typical patches were easily detected by this CFI system, as low fluorescence intensity areas (**Figures 2B.3, C.5 and 3B.5, 6**). Sometimes however, these fluorescence results did not reproduce the apparent homogeneity of the scalded area detected visually, as seen in **Figure 2B** in comparison to **Figure 3C**. The latter might reflect not only the scalded patches, but also the loss of chlorophyll associated to an over-ripening stage for pear 348.

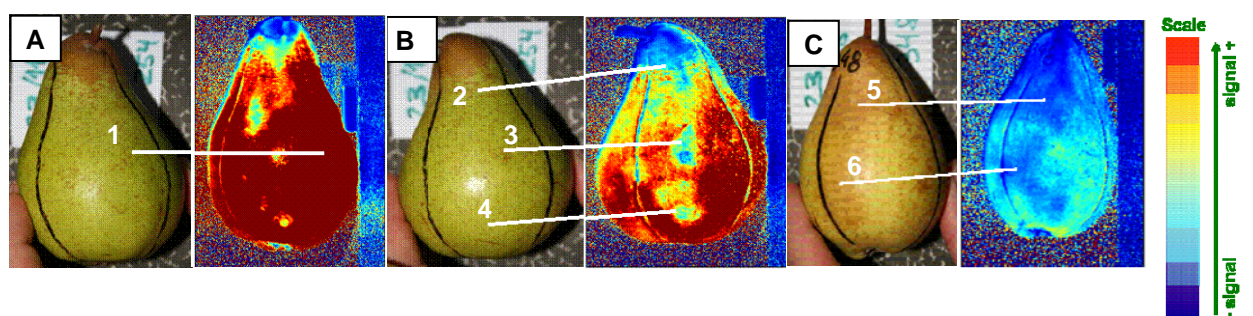
Additionally, the CFI system used was able to distinguish low fluorescence intensity areas on the fruit surface, apparently normal to the naked eye (**Figures 2A.1 and 3B.2, 4**). It is not yet known if these areas would be further expressed as a future scald patch with a longer cold storage.

Yet, spots of other origin, such as peel scars were also easily detected by this system, leading to false positives, if one are only concerned with scalded areas (**Figures 2A.2, C.6 and 3B.3**).

Although in a preliminary stage, the CFI system set up used in this experiment seems quite promising to be further developed as a fast and low cost device to be included as a machine vision system, to be used in on-line grading and sorting, once it shows quite accurately, several kind of surface defects. Additionally, these results also suggest that more studies are needed to explore and hopefully, confirm this method to be used as a simple, friendly-user and non-invasive prediction method to make an early diagnosis of superficial scald of 'Rocha' pear, at the sorting stage and along storage.



**Figure 2:** NA 'Rocha' pears 6 d after shifting to shelf-life. A. Non-scalded fruit (1. Starting scald patch not seen by the naked eye?). B. Scalded fruit (3. Scald patch; 4. Non-scalded peel). C. Scalded fruit (5. Scald patch; 6. Peel scar)



**Figure 3:** CA 'Rocha' pears 6 d after shifting to shelf-life. A. Non-scalded fruit (1. Normal, uniform peel to the naked eye). B. Non-scalded fruit (2. Starting scald patch not seen by the naked eye? 3. Peel scar; 4. Starting scald patch not seen by the naked eye? C. Scalded surface (5. and 6. Scald patches).

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