Laboratory Standard Operating Procedure

Characterization of RTB Product Colour Change During Time

High-Throughput Phenotyping Protocols (HTPP), WP3

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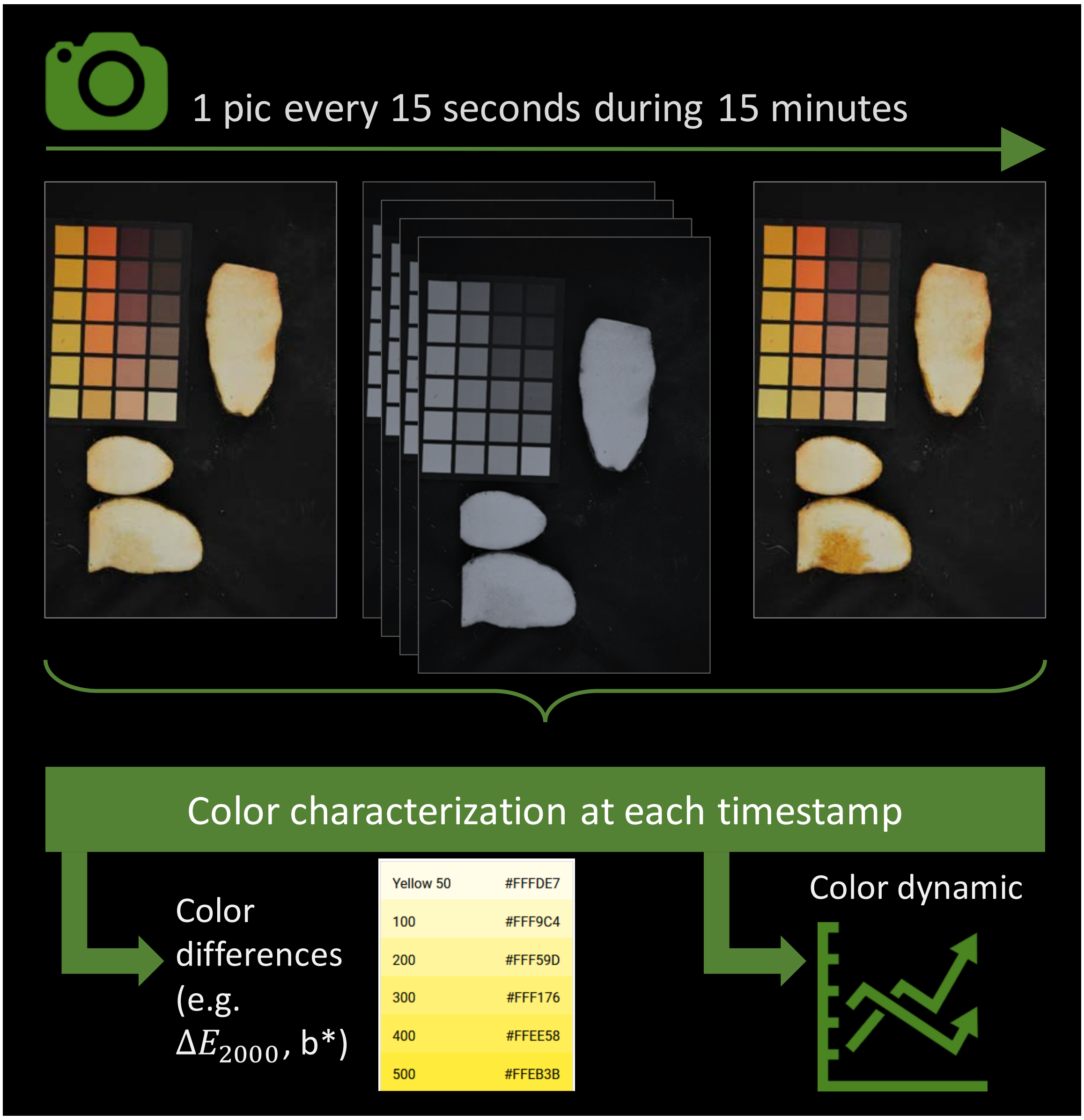

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Ethics: The activities, which led to the production of this document, were assessed and approved by the CIRAD Ethics Committee (H2020 ethics self-assessment procedure). When relevant, samples were prepared according to good hygiene and manufacturing practices. When external participants were involved in an activity, they were priorly informed about the objective of the activity and explained that their participation was entirely voluntary, that they could stop the interview at any point and that their responses would be anonymous and securely stored by the research team for research purposes. Written consent (signature) was systematically sought from sensory panelists and from consumers participating in activities.

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RTBfoods ***WP3: High-Throughput Phenotyping Protocols (HTPP)***	

SOP: Characterization of RTB Product Colour Change During Time

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ABSTRACT

The superficial appearance and color of foods are the first parameters of quality evaluated by consumers and are thus critical factors for acceptance of the food item by the consumers. The colour of these products is likely to change when exposed to air (e.g. oxidation, greying). The characterization of this colour change over time is a necessity in order to evaluate the acceptability of a genotype by the consumer. The present document aims at describing step by step a standardized procedure to characterize this color change over time.

The principle relies on repeated image acquisition of the same RTB product over time followed by image analysis to characterize RTB product colour evolution at each timestamp. After image acquisition, the first step aims at identifying the pixel coordinates of the RTB product using segmentation based on RGB values of the first image. This procedure allows to subtract the background from all images of a series. The RTB product colour is then characterized at each timestamp allowing to estimate colour difference indices and colour change dynamic.

Key Words: Food quality; Browning; Computer vision; Non-destructive measurement; Colour index; Color difference metrics

1 Scope and Application

The superficial appearance and color of foods are the first parameters of quality evaluated by consumers and are thus critical factors for acceptance of the food item by the consumers. The SOPXXXX explain how to use a computer vision system to realize a standardized characterization of tuber, root or banana colour products.

However, the colour of these products is likely to change when exposed to air (e.g. oxidation, greying). The characterization of this colour change over time is a necessity in order to evaluate the acceptability of a genotype by the consumer. Starting from the SOPXXX, the present SOP describe the additional steps necessary to be able to screen RTB product colour change.

2 References

2.1 Perception of colour difference

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3 DEFINITIONS

A **colour chart** is a flat, physical object that has many different color samples present. Typically there are two different types of colour charts:

- Colour reference charts are intended for color comparisons and measurements. Typical tasks for such charts are checking the color reproduction of an imaging system, aiding in colour management or visually determining the hue of color. Example, the ColorChecker chart.
- Colour selection charts present a palette of available colors to aid the selection of spot colors, process colors, paints, pens, crayons, and so on.

A **colour space** is mostly a system for describing color numerically. It rely on a "color model", i.e. an abstract mathematical model describing the way colors can be represented as tuples of numbers (e.g. triples in RGB or quadruples in CMYK).

4 PRINCIPLE

The principle rely on repeated image acquisition of the same RTB product over time followed by image analysis to characterize RTB product colour evolution at each timestamp. After image acquisition, the first step aim at identifying the pixel coordinates of the RTB product using segmentation based on RGB values of the first image. This procedure allow to subtract the background from all images of a series. The RTB product colour is then characterized at each timestamp allowing to estimate colour difference indices and colour change dynamic.

5 APPARATUS

Material	Image
Ceramic (or coated metal) knife. Ceramic knives are known for being chemically inert, which means they are perfect for cutting fruits and vegetables that are prone to browning. Compare to metal knife it reduces the risk of catalytic reactions with metal ions.	

Material	Image
Photo studio lighting kit *i.e.* a couple of monolights with stands and softboxes or umbrella. If available, choose LED light. The significant advantage of *LEDs* is that they tend to stay relatively cool, they last a long time, and use a fraction of the energy of fluorescent, incandescent *or* tungsten bulbs.	
Reference color chart Commercial colour chart is readily available at low cost, but they also cover a wide range of colour, including additional colours. We advise building a custom colour calibration chart using product-specific colours more able to improve colour corrections (See SOPXXXX). We recommend printing the chart on a matt quality paper. Of course, after printing the chart, it is necessary to measure the colour of each patch with a chromameter and record values. At least one patch of the chart should be pure white.	
Camera Numeric camera with manual mode and, if possible, a remote shutter to minimize the vibration when taking the image and getting a sharper result. If not available, we can use the built-in timer of the camera. A second battery could reveal useful.	
Intervalometer or remote control To avoid vibration, camera movement and the shadow cast by the user when taking the picture, it is necessary to use a remote control or intervalometer.	

Material	Image
Dark background The background should be a matte surface with high contrast compared to the plant organ to study. For rounded-shape organs (e.g. tuber) a sand bed can be used under the background to prevent it from rolling.	
Tripod with extension pole (giraffe) Tripod should at least reach 2m and extension pole 2.5m.	
Bubble level A levelling base rotule is a must.	

6 PROCEDURE

6.1 Image acquisition

Image acquisition follows the procedure described in the SOPXXX (see section 5.1 to 5.3). However, for each genotype, instead of a single image, a series of images is taken. The frequency and duration of acquisition depends on the product being studied. For example, we perform yam tubers images every 30 seconds for 15 minutes, i.e. 30 images per genotype.

In order to be able to compare the images of the same series, the acquisition device must remain unchanged during the whole series of photos. It is therefore necessary to avoid vibrations, camera movement and the shadow cast by the user when taking the picture. To do this, it is preferable to use a camera with an intervalometer option that allows you to program the frequency and duration of a series of photos (Figure 1). Otherwise it is possible to control the camera's shutter release remotely using a remote control or an external intervalometer device plugged on the camera. In any case, it is necessary not to touch the camera during measurement in order not to make it move. Indeed, the initial (i.e. on the first image) pixel coordinates of the product is used on subsequent timestamp. If the camera or the product move, even slightly, it can introduce a bias into the colour characterization.

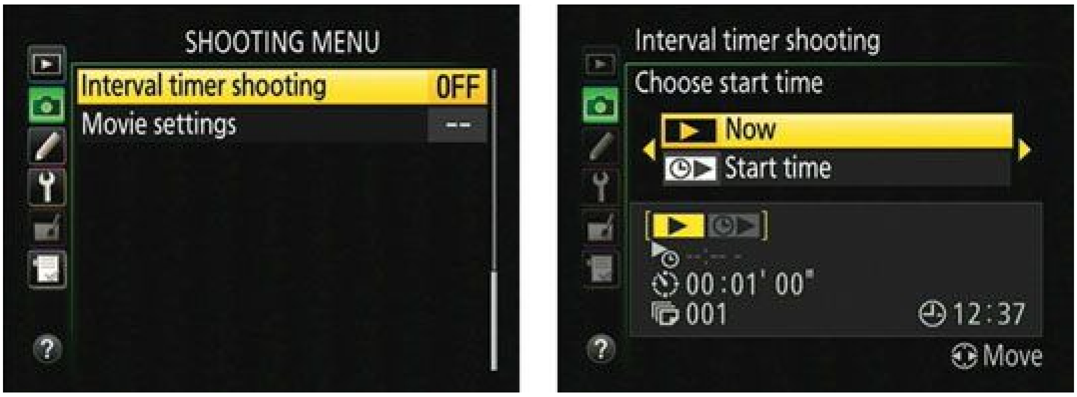

Figure 1 : Example of intervalometer settings.

6.2 Colour correction of all images

Depending on the stability of the acquisition device (i.e. type of lighting environment and camera setting), it may be necessary to perform a colour correction. To do so, the reference chart placed on each image is used according to the procedures described in the SOPXXXX (section 5.4 and 5.5) in order to homogenize the exposure of images of the same series.

6.3 Initial tuber pixel coordinates

After image acquisition and colour correction, the first step aim at identifying the pixel coordinates of the RTB product on the first image of the series. To extract pixel coordinates we process image segmentation based on RGB values to differentiate the object from the background (see SOPXXXX, section 5.6). To remove smaller object (e.g. colour chart patches), an opening is applied (i.e. an erosion followed by dilation).

6.4 Colour quantification at every timestamp

Once the RTB product coordinates of the first image is known, other images of the series can be cropped using these pixels information. This procedure allow subtracting the background from all images of a series. The colour characterization is then done using RGB values of remaining pixels. Depending on the colour index, different colour space could be used (i.e. L*a*b*, RGB, HSV).

7 Expression of Results

7.1 Colour difference

Here we propose 3 colour difference indices. But many others can be used depending on the product specificity and the awaited colour change (i.e. White index or yellow differences).

7.1.1 Browning index difference

The browning index (BI) represents the purity of brown colour and is reported as an important parameter in processes where enzymatic or non-enzymatic browning takes place (Buera et al., 1986; Palou et al., 1999). The difference I browning index can be calculated as follow:

$$x = \frac{(a^* + 1.75L^*)}{(5.645L^* + a^* - 3.012b^*)}$$

$$BI = [100(x - 0.31)]/0.172$$

$$\Delta BI = BI_{initial} - BI_{15\,minutes}$$

7.1.2 Colour difference

The ΔE^*_{2000} colour difference formula is the reference colour difference equation approved by the CIE. It includes not only lightness, chroma, and hue weighting functions, but also an interactive term between chroma and hue differences for improving the performance for blue colours and a scaling factor for the CIELAB a* scale for improving the performance for gray colours. ΔE^*_{2000} can be calculated as follow:

$$\Delta E^*_{2000} = \sqrt{\left(\frac{\Delta L'}{k_L S_L}\right)^2 + \left(\frac{\Delta C'}{k_C S_C}\right)^2 + \left(\frac{\Delta H'}{k_H S_H}\right)^2 + R_T \frac{\Delta C'}{k_C S_C} \frac{\Delta H'}{k_H S_H}}$$

$$\Delta L' = L^*_2 - L^*_1$$

$$\bar{L} = \frac{L^*_2 + L^*_1}{2}$$

$$\bar{C} = \frac{C_2^* + C_1^*}{2}$$

$$a_1' = a_1^* + \frac{a_1^*}{2}\left(1 - \sqrt{\frac{\bar{C}^7}{\bar{C}^7 + 25^7}}\right)$$

$$a_2' = a_2^* + \frac{a_2^*}{2}\left(1 - \sqrt{\frac{\bar{C}^7}{\bar{C}^7 + 25^7}}\right)$$

$\bar{C}' = \frac{C_1' + C_2'}{2}$ and $\Delta C' = C_2' - C_1'$ where $C_1' = \sqrt{a_1'^2 + b_1^{*2}}$ and $C_2' = \sqrt{a_2'^2 + b_2^{*2}}$

$$h_1' = atan2(b_1^*, a_1') \; mod \; 360°$$

$$h_2' = atan2(b_2^*, a_2') \; mod \; 360°$$

$$\Delta h' = \begin{cases} h_2' - h_1' & |h_1' - h_2'| \leq 180° \\ h_2' - h_1' + 360° & |h_1' - h_2'| > 180°, h_2' \leq h_1' \\ h_2' - h_1' - 360° & |h_1' - h_2'| > 180°, h_2' > h_1' \end{cases}$$

$$\Delta H' = 2\sqrt{C_1' C_2'} \sin\left(\frac{\Delta h'}{2}\right)$$

$$\overline{H'} = \begin{cases} (h_1' + h_2' + 360°)/2 & |h_1' - h_2'| > 180° \\ (h_1' + h_2')/2 & |h_1' - h_2'| \leq 180° \end{cases}$$

$$T = 1 - 0.17\cos(\overline{H'} - 30°) + 0.24\cos(2\overline{H'}) + 0.32\cos(3\overline{H'} + 6°) - 0.20\cos(4\overline{H'} - 63°)$$

$$S_L = 1 + \frac{0.015(\bar{L} - 50)^2}{\sqrt{20 + (\bar{L} - 50)^2}}$$

$$S_C = 1 + 0.045\overline{C'}$$

$$S_H = 1 + 0.015\overline{C'T}$$

$$R_T = -2\sqrt{\frac{\overline{C'^7}}{\overline{C'^7} + 25^7}} \sin\left[60°.\exp\left(-\left[\frac{\overline{H'} - 275°}{25°}\right]^2\right)\right]$$

Following Mokrzycki and Tatol (2011), a standard observer sees the difference in color as follows:

- 0 < ΔE_{2000}< 1 - observer does not notice the difference,
- 1 < ΔE_{2000}< 2 - only experienced observer can notice the difference,
- 2 < ΔE_{2000}< 3.5 - unexperienced observer also notices the difference,
- 3.5 < ΔE_{2000}< 5 - a clear difference in color is noticed,
- 5 < ΔE_{2000} - observer notices two different colors.

7.1.3 Chroma difference

Exposure variation strongly affect colour differences based on the luminance (L*). Since it is useful to look at colour errors independently of exposure error, the chroma difference omit ΔL^*. The chroma difference can be calculated as follow:

$$\Delta C = (\Delta a^{*2} + \Delta b^{*2})^{1/2}$$

This formula do not entirely remove the effects of exposure variation since a* and b* are affected somewhat by exposure, but they reduce it to a manageable level.

7.2 Colour dynamic

Beyond colour difference, colour change dynamic can also help to discriminate between genotypes (Figure 2). Looking at the evolution of colour indices over time allows to identify trends, or even to deduce relevant indicators (e.g. area under the curve, speed of evolution or time needed to reach a stable colour).

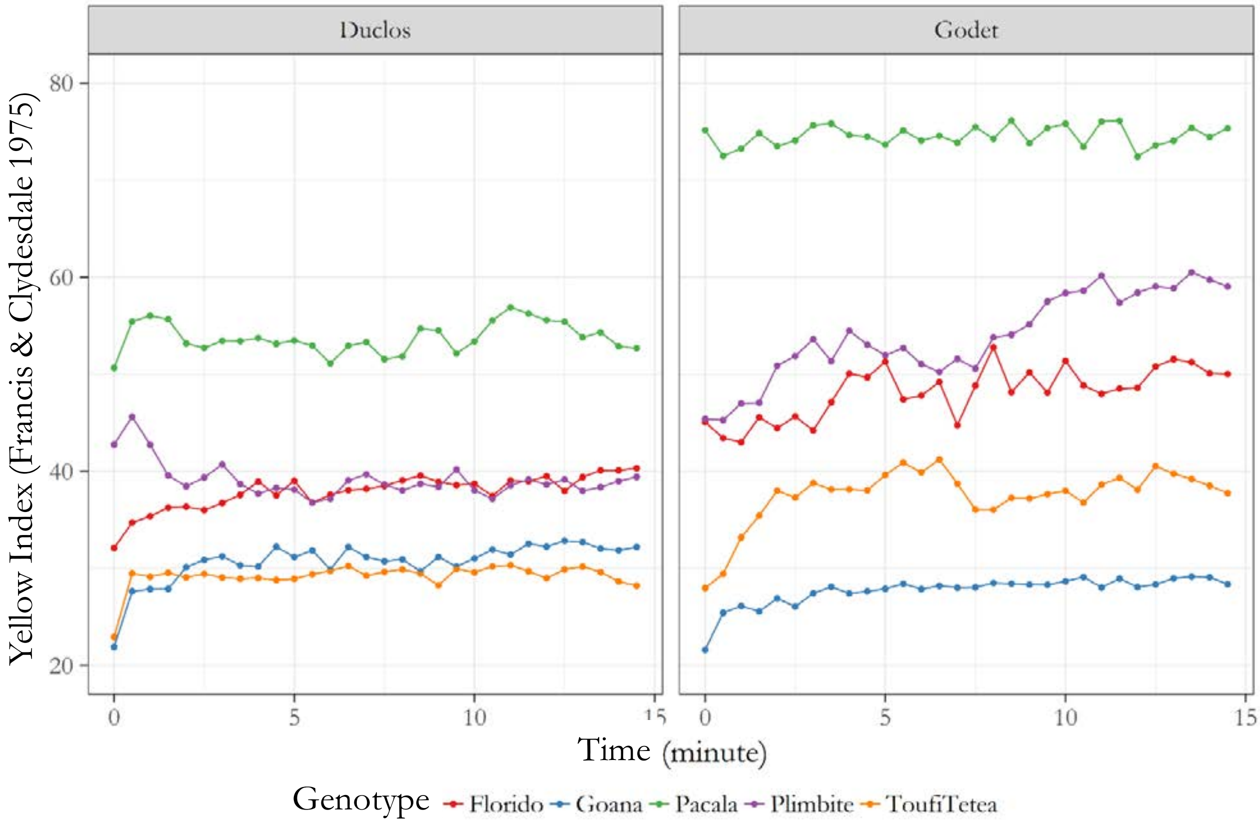

Figure 2 : Example of color index dynamic for five yam genotypes cultivated in two contrasted sites.

7.3 Repeatability

A preliminary experiment using the reference colour chart for yam flesh (Figure 3) at a different time of the day with different tripod height was carried out in order to test the repeatability of the color characterization. The idea was to compare colour values of the same patches of the colour reference chart between the different images. Results showed an average color difference between images of $\Delta E_{2000} = 1.35$ indicating differences only experienced observer can notice.

Figure 3: Reference colour chart for yam tuber flesh with RGB value label.

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