Laboratory Standard Operating Procedure

SOP for Calibrated Color Measurements of RTB Foods Using Image Analysis

Montpellier, France, January 2020

Denis CORNET, Centre de cooperation Internationale en Recherche Agronomique pour le Développement (CIRAD), Montpellier, France

This report has been written in the framework of RTBfoods project.

To be cited as:

Denis CORNET (2020). *SOP for Calibrated Color Measurements of RTB Foods Using Image Analysis*. Montpellier, France: RTBfoods Project Report, 17 p.

Ethics: The activities, which led to the production of this manual, 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.

Acknowledgments: This work was supported by the RTBfoods project https://rtbfoods.cirad.fr, through a grant OPP1178942: Breeding RTB products for end user preferences (RTBfoods), to the French Agricultural Research Centre for International Development (CIRAD), Montpellier, France, by the Bill & Melinda Gates Foundation (BMGF).

RTBfoods

WP3: High-throughput phenotyping protocols

SOP: Calibrated color measurements of RTBfoods using image analysis

Date: 31/01/2020	Release: 1

Written by: Denis CORNET

Collaborators:

- Erick MALEDON
- David LANGE
- Lucienne DESFONTAINES

For information on this SOP please contact:

- Denis CORNET, denis.cornet@cirad.fr
- Emmanuel ALAMU, o.alamu@cgiar.org

This document has been approved by:

Partner	Name of the person who approved	Date
IITA	Emmanuel Oladeji ALAMU	24/03/2020

CONTENTS

Table of Contents

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 determination of color can be carried out by visual (human) inspection, but in this case, the result could be subjective and immensely varies from observer to observer. For this reason, it is recommendable to determine color using color measuring instrumentation.
Color can be rapidly analyzed by computerized image analysis techniques, also known as computer vision systems (CVS). These systems not only offer a methodology for measurement of uneven coloration, but it can also be applied to the measurement of other attributes of total appearance (Hutchings, 1999).
This protocol describes the use of a camera and a calibration target to realize a standardized characterization of tuber, root or banana color. The fresh yam tuber is taken as an example to illustrate our point,

2 REFERENCES

2.1 Colour correction

- Gong H, Finlayson GD (2017) Root Polynomial Color Homography Color Correction. Proceedings of 13th AIC Congress.
- Mendoza F, Djemek P, Aguilera JM. 2006. Calibrated color measurements of agricultural foods using image analysis. Postharvest Biology and Technology 41, 285–295.

2.2 Perception of colour difference

- Luo MR, Cui G, Rigg B. 2001. The development of the CIE 2000 colour-difference formula: CIEDE2000. Color Res Appl 26:340 –350.
- Mokrzycki W, Tatol M. 2011. Color difference Delta E - A survey. Machine Graphics and Vision 20(4):383-411.

2.3 Colour space transformation

- Leon K, Mery D, Pedreschi F, Leon J (2006) Color measurement in L*a*b* units from RGB digital images. Food Research International 39: 1084-1091
- Rec. ITU-R BT.709-5. 2002. Parameter values for the HDTV standards for production and international programme exchange (1990, revised 2002). International Telecommunication Union, 1211 Geneva 20, Switzerland.

2.4 Object recognition

- Ng HP, Huang S, Ong Sh, Foong KC, Goh PS, Nowinski WL (2008) Medical image segmentation using watershed segmentation with texture-based region merging. Conference proceeding 78: 559-578

2.5 Colour Indices

- Croes AW. 1961. Measurement of flour whiteness. Cereal Chem.38:8.
- Judd DB, Wyszecki G. 1975. Color In Business, Science And Industry. 3rd ed. NewYork, NY. John Wiley & Sons, pp.420-438.
- Hunter RS. 1979. The Measurement of Appearance. New York, John Wiley and Sons, pp.18-57.
- Francis FJ, Clydesdale FM. 1975. Food Colorimetry: Theory and Applications. Van Nostrand Reinhold/AVI, New York.
- Buera M, Lozano R, Petriella C. 1986. Definition of colour in the non-enzymatic browning Process. Die Farbe, 32. 318-322.
- Pathare PB, Opara UL, Al-Said FAJ. 2013. Colour Measurement and Analysis in Fresh and Processed Foods: A Review. Food Bioprocess Technol, 6:36–60
- Akissoe N, Hounhouigan J, Mestres C, Nago M. 2003. How blanching and drying affect the colour and functional characteristics of yam (Dioscorea cayenensis-rotundata) flour. Food Chemistry 82, 257–264
- Rhim J, Wu Y, Weller C, Schnepf M. 1999. Physical characteristics of a composite film of soy protein isolate and propyleneglycol alginate. Journal of Food Science, 64(1), 149–152.
- O'Leary, E., Gormley, T., Butler, F., & Shilton, N. (2000). The effect of freeze-chilling on the quality of ready-meal components. Lebensmittel-Wissenschaft und Technologie, 33(3), 217–224.

3 PRINCIPLE

Image analysis allows estimating the standardized colour value of plant organ. First, the acquisition of images of plant organ and calibration target is required under a stable light environment. Once acquired, images will be standardized using colour correction algorithm relying on the difference between the true colour values of the calibration target patches and their values measured on the image. After standardization, sRGB colour values will be translated into the CIE L*a*b* colour space to perform the calculation of colour indices (e.g. brown index, whiteness index, yellow index). Images allow for the estimation of colour variation and gradient analysis of heterogeneous surfaces.
The image analysis steps (i.e. thresholding, segmentation, object detection) are performed using open-source software (i.e. OpenCV, R and python). Depending on the products and the light environment, threshold values are susceptible to vary and need to be manually adjusted based on colour distribution. For batch analysis, all necessary algorithm and functions could be gathered in an R package or script.
Half of the calibration target patches can be set aside for the estimation of colour measurement error.

4 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.	
Colour calibration target Commercial colour target 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 target using product-specific colours more able to improve colour corrections. We recommend printing the target on a matt quality paper. Of course, after printing the target, it is necessary to measure the colour of each patch with a chromameter and record values. At least one patch of the target 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.	
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.	

5 PROCEDURE

5.1 Sample preparation

Sample preparation may differ between product and product profile. As an example, we describe the sample preparation for crude yam tuber characterization.
Three healthy tubers of large, medium and small sizes are harvested at maturity and cleaned for clod of earth. A delay of a maximum of 2 weeks after full senescence is acceptable before taking images.
Tubers are then cut longitudinally (Figure 1) with a ceramic knife. Afterwards, cut tuber needs to be placed straight into the image acquisition design before oxidation start.

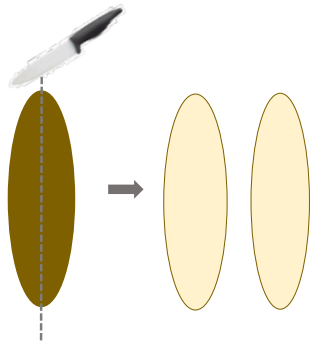


Figure 1: Longitudinal cut.

To hasten acquisition and to facilitate further analysis, three tubers of the same genotype are placed at the same time in the field of view of the camera beside the calibration target. Objects (tubers and target) mustn't be overlapping with each other. If other traits have to be estimated (e.g. color gradient), it could be useful always to dispose of the tubers in the same position (e.g. horizontally with the distal part on the left).

5.2 Photo studio preparation

To generate a stable light environment, it is preferable to work in a room without windows or at least with dark blinds in front of it. The light should be produced with a lightning kit while avoiding direct light (e.g. diffuse radiation). This can be provided by softboxes or with reflective umbrella placed such as the primary light source reach the sample with a 45° angle (Figure 2).

Figure 2: Image acquisition design (left) and samples disposals (right).

The samples should be placed upon a highly contrasting background with a matte and homogenous surface. The camera is positioned vertically above the sample using a tripod with an extension pole.

Erreur ! Signet non défini. The dimension of the tripod depends on the scene to capture. Even if there is some small variation (e.g. FX or DX-format), as a rule of thumb the following equation can be used to calculate the distance between the sample and the camera lens (d, mm):

$$d = f\left(1 + \frac{F_w}{S_w}\right)$$

Where f is the focal length (mm), Fw is the width of the field of view (mm), and Sw is the sensor width (mm). For instance to capture a scene of 2m width with a camera characterized by a focal length of 18mm and a sensor width of 23.5mm, the camera need to be placed at least at 1.55m above the sample.
To preserve lengths and avoid perspective distortion, the camera should be levelled.

5.3 Image acquisition

Pictures should be taken using Flash-Off mode and stored in JPEG format using sRGB color space. The sRGB stands for standard Red Green Blue. It is a colour space that defines a range of colors that can be displayed on-screen or in print. It is the most widely used color space and is supported by most operating systems. Other parameters (e.g. white balance, focus, etc.) can be set automatically.
It is important to keep raw pictures to have access to metadata (e.g. focus mode, white balance, etc.). Indeed, most cameras provide EXIF data associated with the picture. EXIF Stands for "Exchangeable Image File Format". It is a standard means of tagging image files with metadata, or additional information about the image. It is supported by both the TIFF and JPEG formats but is most commonly seen in JPEG images captured with digital cameras.
If available, the use of a remote shutter is advised to minimize the vibration when taking the image and getting a sharper result. If not possible, we can use the built-in timer of the camera.

5.4 Target detection and colour value measurements

To detect colour patches of the target, we use OpenCV (i.e. Simple Blob Detector with minimum and maximum color thresholds, step size and minimum and maximum patch area) and geometric rules. Figure 3 present original images before and after patches detection for various plant organs.

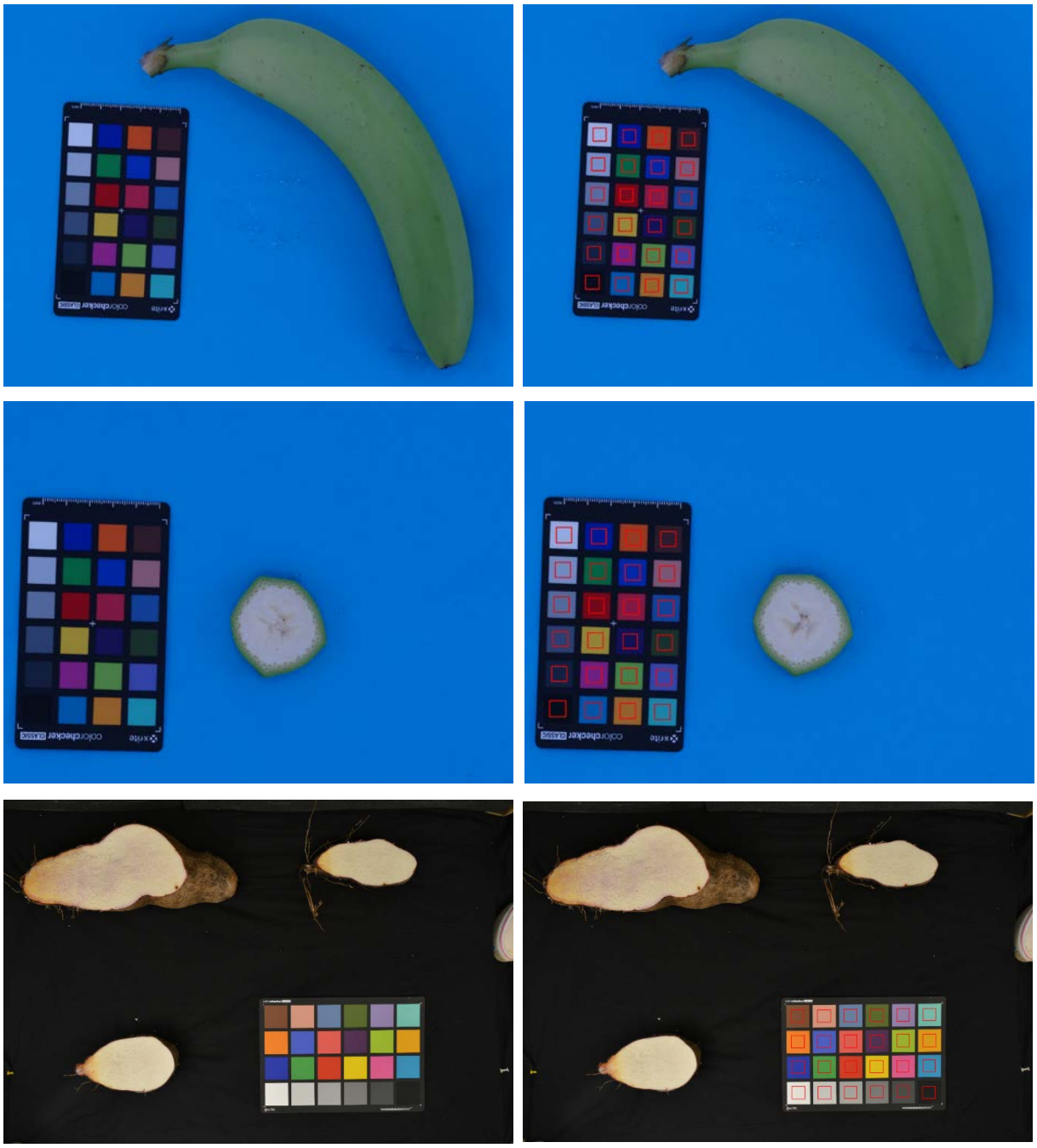


Figure 3: Example of calibration target detection with different plant organs. Left: original image. Right: image with detected patches (analysis zone highlighted with red rectangles).

5.5 Colour correction

In food research, color is frequently represented using the L*a*b* color space. This color model is considered approximately uniform, i.e. distance between two colors in a linear color space corresponds to the perceived differences between them. L* is the luminance or lightness component that goes from 0 (black) to 100 (white), and parameters a* (from green to red) and b* (from blue to yellow) are the two chromatic components, varying from −120 to +120 (Figure 4).

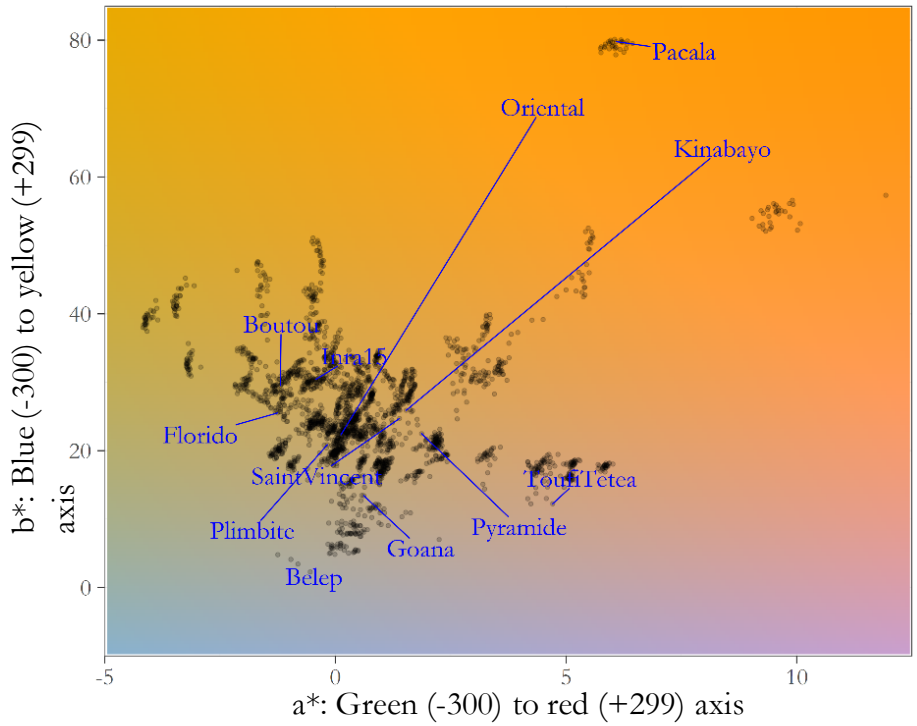


*Figure 4: Example of tuber mean color of different yam genotypes projected into the CIE L*a*b* color space.*

The definition of L*a*b* is based on the intermediate system CIE XYZ which simulates the human perception and which may be converted from sRGB as follow (Rec. ITU-R BT.709-5. 2002):

$$\begin{bmatrix} X \\ Y \\ Z \end{bmatrix} = [M] \begin{bmatrix} sR \\ sG \\ sB \end{bmatrix}$$

with M, a color matrix depending on the reference white used (i.e. D50, D65):

$$M = \begin{cases} \begin{bmatrix} 0.4124 & 0.3576 & 0.1805 \\ 0.2126 & 0.7152 & 0.0722 \\ 0.0193 & 0.1192 & 0.9505 \end{bmatrix} & if\ reference\ white\ is\ D65 \\ \begin{bmatrix} 0.4361 & 0.3851 & 0.1431 \\ 0.2225 & 0.7169 & 0.0606 \\ 0.0139 & 0.971 & 0.7142 \end{bmatrix} & if\ reference\ white\ is\ D50 \end{cases}$$

Thus, L*a*b* is defined as

$$L^* = 116 f\left(\frac{Y}{Y_n}\right) - 16$$

$$a^* = 500\left[f\left(\frac{X}{X_n}\right) - f\left(\frac{Y}{Y_n}\right)\right]$$

$$b^* = 200\left[f\left(\frac{Y}{Y_n}\right) - f\left(\frac{Z}{Z_n}\right)\right]$$

with $f(q) = \begin{cases} q^{1/3} & if\ q > 0.08856 \\ 7.787q + {}^{16}/_{116} & otherwise \end{cases}$,

And Xn, Yn, and Zn correspond to the theoretical XYZ values of the white patch of the calibration target.
After this color correction based on reference white, color differences between theoretical and observed calibration target patches can be calculated using ΔE_{2000} formula (Luo et al. 2001) implemented in the color science library. If too large differences are noticed in the range of studied colors, some supplementary color correction algorithms could be implemented (i.e. polynomial color correction matrices).

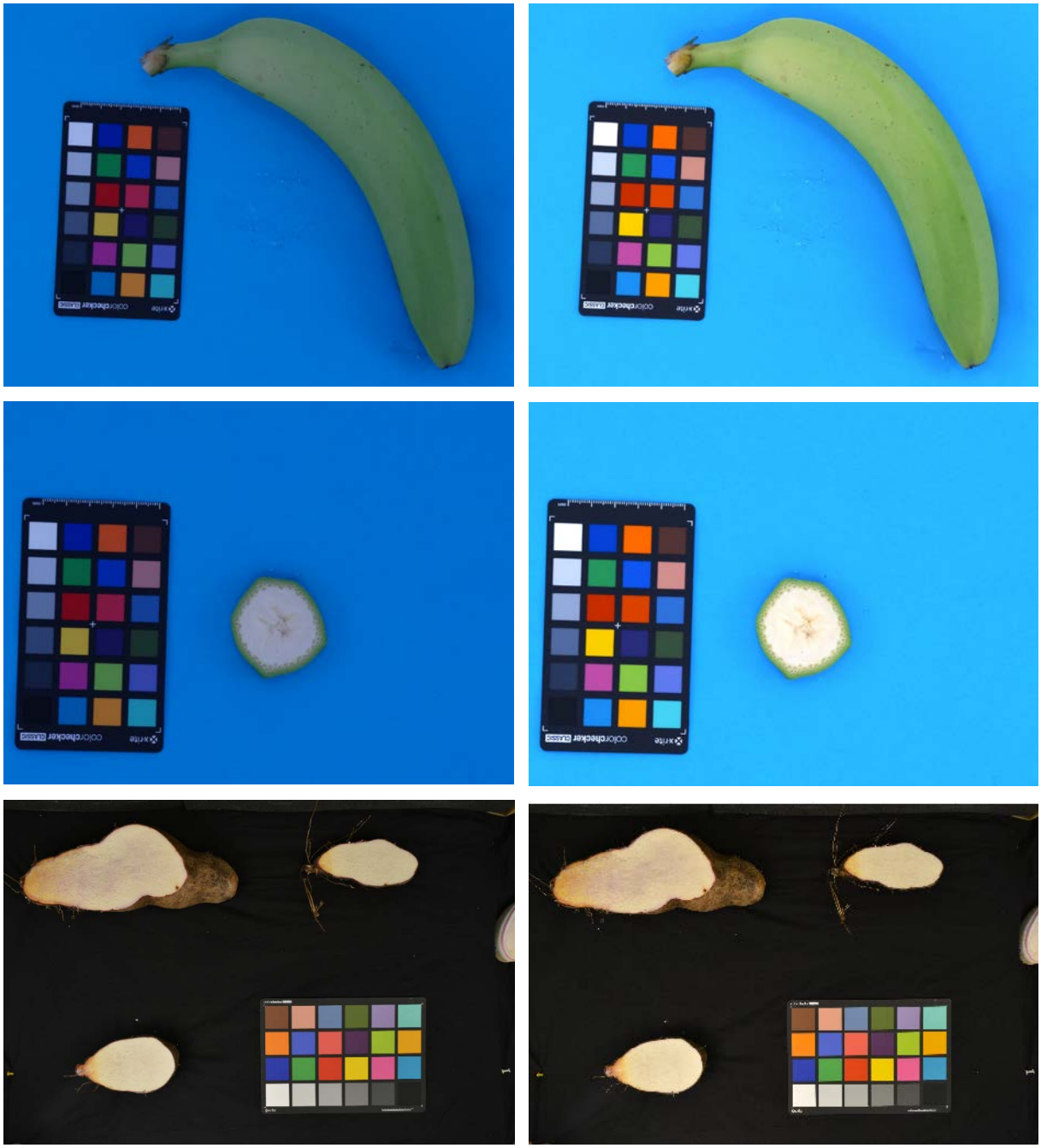


Figure 5: Example of image color standardization based on white correction for banana skin, slice or yam tuber. Left: original image. Right: corrected image.

5.6 Organs detection

The dark background was removed from the preprocessed image using a threshold value of 70. This threshold may differ depending on the background used, and the object studied.

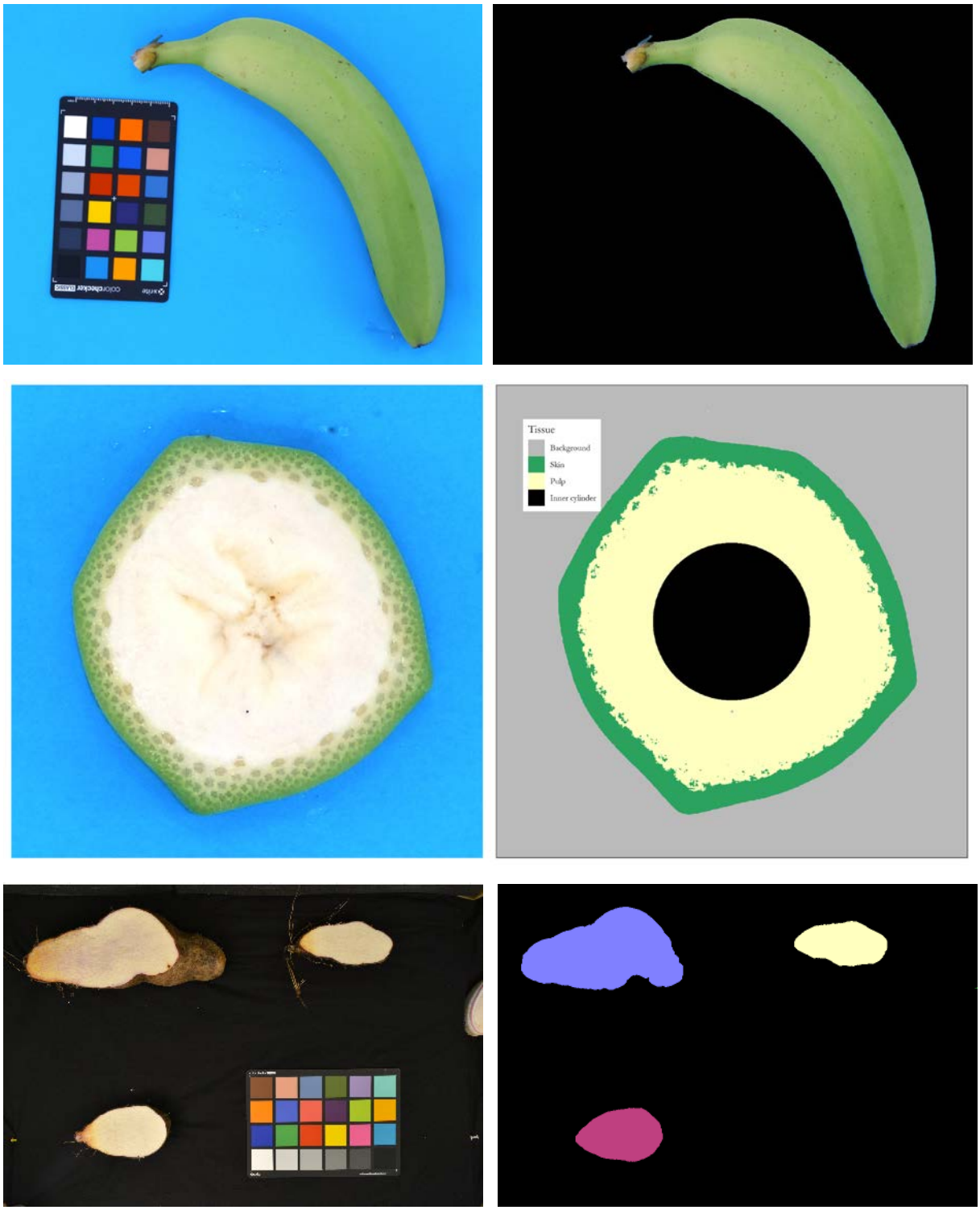


Figure 6: Example of object detection and segmentation for banana skin, slice or yam tuber. Left: original image. Right: segmented objects.

6 EXPRESSION OF RESULTS

Once the object is segmented, it is possible to determine the color value of every pixel it belongs to. From these color value, some useful color indices can be estimated:

- Brown index: $BI = 100 - L^*$ (Akissoe et al. 2003)
- Whiteness index: $WI = \sqrt{(100 - L^{*^2} + a^{*^2} + b^{*^2}}$ (Rhim et al. 1999)
- Yellow index: $YI = \frac{142.86\, b^*}{L^*}$ (Francis and Clydesdale 1975)
- White/yellow ratio: $WY = {}^{L}/_{b}$ (O'Leary et al. 2000)

6.1 Repeatability

Because of yam tuber colour change with time due to oxidation, the repeatability was done using the target at a different time of the day with different tripod height. The mean actual colour difference was $\Delta E_{2000} = 1.35$ indicating differences only experienced observer can notice. Indeed, a standard observer sees the difference in color as follows (Mokrzycki and Tatol 2011):
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 CRITICAL POINTS OR NOTE ON THE PROCEDURE

Avoid direct reflection (photo studio lighting kit, matte paper, no direct light).
Avoid fluctuating light environment (blind room, artificial lightning, and fixed apparatus).
Avoid temperature variation (enzymatic reaction activation).

8 PROSPECTS

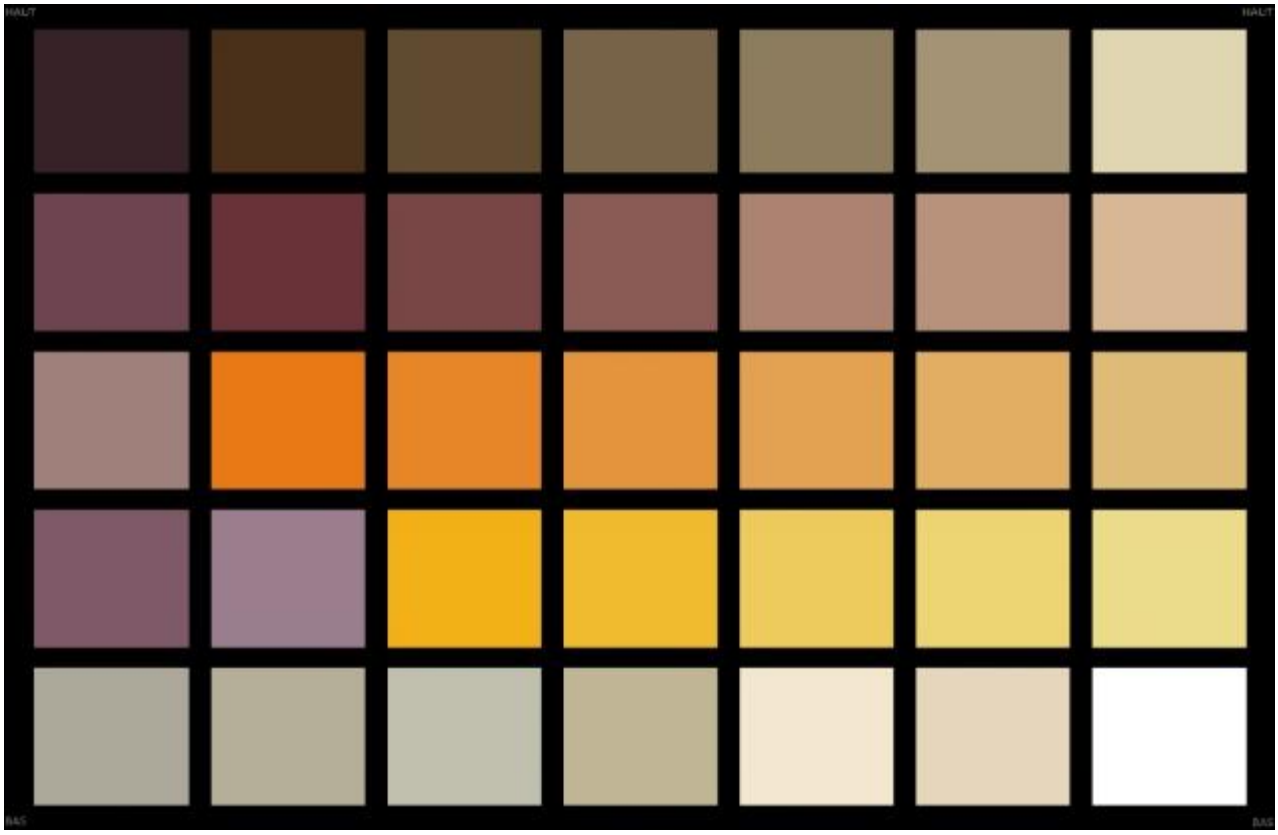
Using the same measuring device, it is possible to gather multiple other traits:
- Spatial color variation (longitudinal, random, radial)
- Oxidation
- Size
- Shape

These prospects will be tackled in coming up SOP.

9 REVISION RECORD

Date	Responsible person	Description of change
DD/MM/YYYY	First Name LAST NAME	

10 APPENDIX: YAM TUBER CUSTOM CALIBRATION TARGET



Institution: Cirad – UMR QualiSud

Address: C/O Cathy Méjean, TA-B95/15 - 73 rue Jean-François Breton - 34398 MONTPELLIER Cedex 5 - France

Contact Tel: +33 4 67 61 44 31

Contact Email: rtbfoodspmu@cirad.fr