

# Work Package 3 High-Throughput Phenotyping Protocols (HTPP)

State Of Knowledge

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#### **G**LOSSARY

ABR = Acid-Brix Ratio,	PHC = Phosphorus Content		
AC = Amylose Content	PKV = Peak Viscosity		
AGD = Average Granule Diameter	PLS = Partial Least Square		
AIS = Alcohol Insoluble Solids	PLSDA = PLS Discriminant Analysis		
ANN = Artificial Neural Network	PRC = Protein Content		
ANOVA = Analyse Of Variance	R2p = Coefficient of determination of prediction		
AP = Amylose Percent	RBF-PLS = Radial Basis Function		
BGP = Big Granule Percent	RMSEP: Root Mean Square of Error of		
CRY = Crystallinity	Prediction		
DM = Dry Matter	RPD = Ration of Performance to Deviation		
F-IR = Fourier	SB = Setback		
HCN = Acid Cyanogenic	SEC = Standard Error of Calibration		
HPV = Hot Paste Viscosity	SECV = Standard Error of Cross Validation		
HSA = Hierarchical Clustering Analysis	SEP = Standard Error of Prediction		
HSI = Hyperspectral Imaging	SGP = Small Granule Percent		
ICC = Individual Carotenoids	SOL = Solubility		
KNN = K-Nearest Neighbors	TAA = Total Antioxidant Activity		
LDA = Linear Discrimination Analysis	TBC = β-carotene		
LF-NMR = Low Field pulsed 1H Nuclear Magnetic Resonance	TCC = Total Carotenoids		
LS-SVM = Least-Squares-Support Vector	TMA = Total Monomeric Anthocyanins		
Machines Sapport Vester	TSC = Total Starch Content		
MCDM = Multiple Criteria decision Making	TSP = Total Soluble Phenolics		
MLR = Multiple Linear Regression	TSS = Total Soluble Solids		
MPLS = modified partial least square	VC = Vitamin C		
NIRS = Near Infrared Spectroscopy	VIS = Visible Spectroscopy		
OCPLS = One Class PLS			
PCA = Principal Component Analysis			



#### INTRODUCTION

The objective of RTBfoods project is to pinpoint the quality traits that determine the adoption of root, tuber and banana (RTB) varieties developed by breeders according to consumer and farmer preferences.

The aim of work package 3 (WP3) of RTBfoods project is developing high throughput phenotyping protocols mainly Near Infrared Spectroscopy (NIRS) that could be applied in national and international breeding programs, postharvest processing and quality control procedures. This paper reviews research progress on high throughput methods (mainly NIRS) applied for RTB products characterization. This characterization may concern the quantification of different biochemical constituents, the measurement of physical properties and/or the sensorial profiling.

This literature review is based on a selection of papers found through Scopus, Science Direct, Web of Science and Google Scholar. The formula research was NIRS OR HTTP OR non-destructive OR Spectroscopy OR Near Infrared Spectroscopy and Cassava (And Yam) (And Banana) (And Potato) (And Sweet Potato) (And Root) OR tuber.

According to these requests, 148 references were found (Annex1), these papers were published in different scientific journals between 1988 and 2018. Until 2009, the number of published articles was quite low (1 to 4) and stable (Figure 1) *Figure 1*. The number of references increased after 2009 with a maximum of 24 publications in 2016. After 2016, the number of publication fell to 7 and only 5 articles were published in 2018 for the 5 crops.

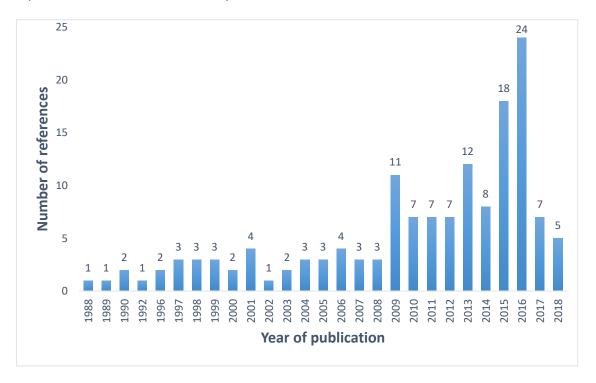


Figure 1: Number of references per year related to characterization of cassava, yam, banana, sweet potato and potato using high throughput techniques (mainly NIRS).

More than the half of the articles (58%) concerns potato and potato products (**Erreur! Source du renvoi introuvable.**). Over this period of 30 years, 24% of the scientific researches on high-throughput technics focused on banana and only 5, 6 and 7% were related to yam, cassava and sweet potato, respectively.



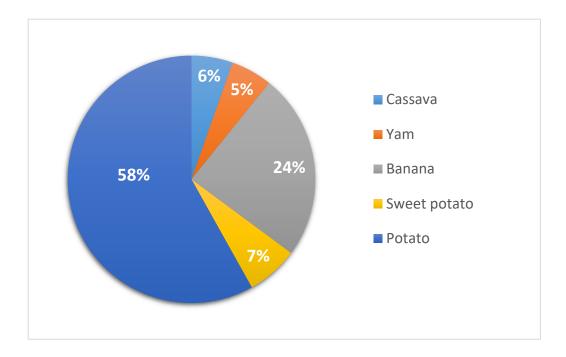


Figure 2: Percentage of publications related to cassava, yam, banana, sweet potato and potato (period: 1988 – 2018).

#### 1. METHODOLOGY AND CONSTITUENTS

These publications report developments of rapid, reagentless, and non-invasive techniques: spectroscopic techniques, such as visible-infrared spectroscopy, Raman spectroscopy, nuclear magnetic resonance spectroscopy, X-ray imaging, ultrasonic systems and hyperspectral imaging, applied for the quality evaluation of RTB crops and products. In their review, "Non-Destructive and rapid evaluation of staple foods quality by using spectroscopic techniques: A review", which include a large part of the present list of publications, (Su et al., 2017) highlighted the advantages of these technics for characterization of common quality attributes of staple foods. They also pointed out the challenges linked to their large diffusion for routine analysis. Such as VIS-IR spectroscopy which focuses only on a small portion of sample and does not provide information on spatial distributions of whole specimen. Or Raman spectroscopy which requires high-stability laser sources and sensitive amplification equipment and NMR spectroscopy which is expensive and takes more time to interpret the complex spectra. The spectral imaging, especially full spectral imaging and HSI, needs lots of energy to extract characteristic information from lengthy and detailed spectral data. There is an inevitable trend for multispectral imaging with only a few important bands instead of full wavelengths in the non-destructive and rapid evaluation of food quality. While the development of X-ray imaging is restricted (Rady and Guyer, 2015a) by the limitation of detection to density-changing tissues and not to chemical composition or mechanical damage forms.

The authors recommended "the development of innovative calibration and prediction models with higher accuracy by eliminating data redundancy more conveniently for determining food quality in the future". Furthermore, they recommended to evaluate the models by using the same quality attributes with different varieties of one specific staple food species. This will improve model robustness and extend application boundary.

In that way the work done by **(Lebot and Malapa, 2013)** showed that NIRS can be used to produce a rapid prediction of total N, starch and sugar concentrations with a single calibration applied to five different root crop species (cassava, yam, sweet potatoes, taro and cocoyam) and across a wide range of varieties.



#### 1.1 Spectroscopic Techniques

Spectroscopic techniques are based on interactions between electromagnetic radiations and vibrational properties of chemical bounds. The visible (VIS) spectra covers the spectral range of 380 nm to 780 nm which returns mainly information based on color due to pigments. NIRS refers to the 780-2500 nm part of the electromagnetic spectrum and is more useful for quantitative analysis of complex mixtures (Sun, 2009). NIR spectra of foods comprise broad bands arising from overlapping absorptions corresponding mainly to overtones and combinations of vibrational modes involving C-H, O-H and N-H chemical bonds, NIR range is usually divided into short-wave NIR (SW-NIR) spectral region (780–1100 nm) and long-wave NIR (LW-NIR) spectral region (1100–2500 nm). The mid-infrared (MIR), approximately 2500-25000 nm (4000–400 cm<sup>-1</sup>) may be used to study the fundamental vibrations and associated rotational-vibrational structure. MIR is used to determine the chemical functional groups of a sample in both qualitative and quantitative ways (Sun, 2009).

Spectral imaging is imaging that uses multiple bands across the electromagnetic spectrum. The spectral imaging technique is a combination of spectroscopy and imaging where some spectral information is located at every spot in a scene, which means that the spectral imaging technique can be used for the simultaneous acquisition of spatial images and spectral information (Sun, 2010). Spectral imaging can be divided into two main subcategories: Multispectral imaging which captures a small number of spectral bands, typically three to fifteen, through the use of varying filters and illuminations and Hyperspectral imaging (HSI) which combines spectroscopy and digital photography. A hyperspectral camera captures hundreds of wavelength bands, for each pixel, which can be interpreted as a complete spectrum. It is this notion of continuous spectrum, therefore of narrow and contiguous spectral bands, which is essential and makes it possible to make the best use of the information. Thereby, the spectral imaging can be applied for the quantitative prediction of the chemical and physical properties as well as their spatial distribution simultaneously (Sun, 2010).

#### 1.2 Constituents and physical properties

The work that has been done in the selected articles on each product is summarized according to the analytical techniques used, the sample preparation procedure and the chemometric methods applied (



Product	Technique	Quality trait	Process	Multivariate analysis	Reference	
	NIRS	TBC, DM	Fresh	Local PLS	(Belalcazar et al., 2016)	
	NIRS	TCC, DM	Fresh, Ground	MPLS, Local regression	(Davrieux et al., 2016)	
	NIRS	TCC, TBC, DM	Fresh, Mashed, Intact	MPLS, PCA, K- means	(Ikeogu et al., 2017)	
Cassava	NIRS	TCC, HCN	Fresh, Ground	MPLS, PCA,	(Sánchez et al., 2014)	
	UV-VIS	TCC, TBC	Fresh	PCA, ANOVA	(Moresco et al., 2015)	
	UV-VIS	Cyanide	Processed	Not specified	(Phambu et al., 2007)	
	NIRS	Maleic acid	Starch	OCPLS, LS- SVM	(Fu et al., 2017)	
	HSI-NIRS	Adulterations	Flour	PLS, PCA	(Su and Sun, 2017)	
	NIRS	Quality	Bean	Not specified	(Agaba et al., 2016)	
Yam	NIRS	Starch, Amylose, Sugars, Proteins, Minerals, Cellulose	Fresh	PLS, PCA	(Lebot and Malapa, 2013)	
	FT-IR	Dioscin	Freeze-dried, ground	PLSDA, PCA, HCA	(Kwon et al., 2015)	
	FT-Raman	Proteins	Fresh	Not specified	(Liao et al., 2004)	
	NIRS	Pesticide residue	Ripe and Unripe	PCA	(Misal, 2013)	
	FT-NIRS	Brix, PH	Fresh, five maturity stages	PLS	(Ali et al., 2018)	
	VIS-NIRS	TCC	Fresh, Frozen, crush to fine powder	PLS, Cross validation	(Davey et al., 2009)	
	NIRS	Cellulose crystallinity	Residues	PLS	(Rambo and Ferreira, 2015)	
	VIS-NIRS	Chlorophyll, Glucose, Sucrose, Fructose	Fresh	PLS	(Zude, 2003)	
	VIS-NIRS	TSS, DM, Starch, Sugar	Fresh	PLS	(Subedi and Walsh, 2011)	
Banana	NIRS	Classification	Rachis	PCA	(Tamburini and Larenas Uria, 2016)	
	NIRS	TSS, Firmness	Fresh	MLR	(Liew and Lau, 2012)	
	HSI-UV- NIRS	TSS, Firmness, Moisture	Fresh	PCA, PLS, MLR	(Rajkumar et al., 2012)	
	VIS	Chlorophyll	Fresh, Peel	Regression model	(Li et al., 1997)	
	UV-VIS- NIRS	PH, Sweetness, TSS, ABR	Fresh	PLS, MLR	(Jaiswal et al., 2012)	
	Image- processing	Maturity	Fresh	ANOVA	(Surya Prabha and Satheesh Kumar, 2015)	
	VIS-NIR	Chlorophyll, Elasticity, TSS	Fresh	ANN	(Adebayo et al., 2016)	
	HSI-VIS- NIRS	Color, Firmness	Dried, Stored	PLS, PCA, PLS-DA	(Xie et al., 2018)	

Table

1).

Regarding fresh or processes cassava and yam, most of the NIRS investigations reports quantification of chemicals constituents. These constituents are TCC, TBC, DM, hydrogen cyanide, starch, sugars, proteins, minerals and cellulose. Some of the papers concern adulteration such as adulteration of



cassava flour. The main scanning mode is diffuse reflectance and the principal chemometric methods selected and applied are PLS and PCA.

As for cassava and yam, NIRS was applied to banana pulp and banana skin mainly for quantification of chemical constituents: TCC, DM, sugars, cellulose and TSS. The majority of the applications for banana are based on VIS-NIR spectroscopy in diffuse reflectance or transmittance. Some physical criteria like firmness, chlorophyll and maturity on banana peel were investigated. Some studies refer to classification of banana according to their maturity stages. Hyperspectral imaging was used for predicting chemical constituents and physical characteristics in fresh and intact samples (TSS, firmness and moisture).

As said before, a lot of researches using non-destructive techniques such as spectroscopic techniques concerns fresh and processed (chips and French fries) potato. A large part of the researches concerns chemical constituents (carbohydrate, protein, vitamins, minerals, carotenoids, moisture, starch and fat) for quality control or high throughput phenotyping. Another part concerns physical properties such as specific gravity, skin color and texture. Some researches focused on contaminant quantification such as acrylamide in processed products. Other studies concern different quality aspects and potential use: external or internal defects, greening, bruises, enzymatic browning, non-enzymatic browning and physiological disorders. A large part of those applications is based on VIS or VIS-NIR spectroscopy together with quantitative or qualitative approaches using linear methods PLS, PLSDA and PCA.



Product	Technique	Quality trait	Process	Multivariate analysis	Reference
	NIRS	TBC, DM	Fresh	Local PLS	(Belalcazar et al., 2016)
	NIRS	TCC, DM	Fresh, Ground	MPLS, Local regression	(Davrieux et al., 2016)
	NIRS	TCC, TBC, DM	Fresh, Mashed, Intact	MPLS, PCA, K- means	(Ikeogu et al., 2017)
Cassava	NIRS	TCC, HCN	Fresh, Ground	MPLS, PCA,	(Sánchez et al., 2014)
	UV-VIS	TCC, TBC	Fresh	PCA, ANOVA	(Moresco et al., 2015)
	UV-VIS	Cyanide	Processed	Not specified	(Phambu et al., 2007)
	NIRS	Maleic acid	Starch	OCPLS, LS- SVM	(Fu et al., 2017)
	HSI-NIRS	Adulterations	Flour	PLS, PCA	(Su and Sun, 2017)
	NIRS	Quality	Bean	Not specified	(Agaba et al., 2016)
Yam	NIRS	Starch, Amylose, Sugars, Proteins, Minerals, Cellulose	Fresh	PLS, PCA	(Lebot and Malapa, 2013)
	FT-IR	Dioscin	Freeze-dried, ground	PLSDA, PCA, HCA	(Kwon et al., 2015)
	FT-Raman	Proteins	Fresh	Not specified	(Liao et al., 2004)
	NIRS	Pesticide residue	Ripe and Unripe	PCA	(Misal, 2013)
	FT-NIRS	Brix, PH	Fresh, five maturity stages	PLS	(Ali et al., 2018)
	VIS-NIRS	TCC	Fresh, Frozen, crush to fine powder	PLS, Cross validation	(Davey et al., 2009)
	NIRS	Cellulose crystallinity	Residues	PLS	(Rambo and Ferreira, 2015)
	VIS-NIRS	Chlorophyll, Glucose, Sucrose, Fructose	Fresh	PLS	(Zude, 2003)
	VIS-NIRS	TSS, DM, Starch, Sugar	Fresh	PLS	(Subedi and Walsh, 2011)
Banana	NIRS	Classification	Rachis	PCA	(Tamburini and Larenas Uria, 2016)
	NIRS	TSS, Firmness	Fresh	MLR	(Liew and Lau, 2012)
	HSI-UV- NIRS	TSS, Firmness, Moisture	Fresh	PCA, PLS, MLR	(Rajkumar et al., 2012)
	VIS	Chlorophyll	Fresh, Peel	Regression model	(Li et al., 1997)
	UV-VIS- NIRS	PH, Sweetness, TSS, ABR	Fresh	PLS, MLR	(Jaiswal et al., 2012)
	Image- processing	Maturity	Fresh	ANOVA	(Surya Prabha and Satheesh Kumar, 2015)
	VIS-NIR	Chlorophyll, Elasticity, TSS	Fresh	ANN	(Adebayo et al., 2016)
	HSI-VIS- NIRS	Color, Firmness	Dried, Stored	PLS, PCA, PLS-DA	(Xie et al., 2018)

Table 1: Summary of spectroscopic techniques for the quality evaluation of RTB



Product	Technique	Quality trait	Process	Multivariate analysis	Reference	
	NIRS	AC, AP, TSC, PRC, PHC, SOL, SP, AGD,BGP,SGP, CRY,PKV,HKV,SB	Starch	PLS	(Lu et al., 2006a)	
	NIRS	Starch, Sugars, Cellulose, Protein, Minerals	Fresh, Peel, Cut	PLS, PCA	(Lebot et al., 2011)	
	NIRS	DM, TBC, PRC, Starch, Sucrose, Fe, Zn, Mg, Ca	Peel, Cut, Freeze- dried, Milled	ANOVA	(Tumwegamire et al., 2011)	
	NIRS	Starch thermal	Cut, Crushed, Sieved, Dried	MPLS	(Lu et al., 2006b)	
Sweet	NIRS	Starch, Moisture, Sugar	Fresh, Sliced	MLR	(Katayama et al., 1996)	
potato	NIRS	TBC, Starch, Fe, Zn	Freeze-dried, Milled	MPLS	(zum Felde et al., 2009)	
	NIRS	Proteins content	Peeled, Cuted, Freeze-dried, Milled	PLS	(Magwaza et al., 2016)	
	NIRS	Moisture, Protein, Fiber, AIS, Starch	Stored, Peeled, Cut, Freeze-dried, milled	MLR	(Diaz et al., 2014)	
	NIRS	Classification	Powder	RBS-PLS, PCA, SPA, KNN, LDA	(Ding et al., 2015)	
	NIRS, LF- NMR,	Sensory Texture	Cut, Cooked, Sliced	PLS, PCA	(Thybo et al., 2000)	
	NIRS	Texture, DM, Starch	Fresh, Stored Steam-Cooked	PLS	(van Dijk et al., 2002)	
	NIRS	TCC, ICC	Fresh, Freeze- dried and milled	ANOVA, PCA	(Bonierbale et al., 2009)	
	NIRS	DM, Starch	Fresh, Stored, Cut, and Crushed	PLS	(Haase, 2003)	
	VIS-NIRS	DM, Acrylamide, Fat	Chips	PLS	(Pedreschi et al., 2010)	
	NIRS	Texture, DM, Starch	Stored, Steam- Cooked	PLS, PCA	(van Dijk et al., 2002)	
	NIRS	Starch, Protein, Phosphorus	Fresh, stored, Cut, Crushed, Starch	MPLS	(Haase, 2006)	
	NIRS	Fat, Moisture, Acid, Peroxide	Crisps, crushed, Stored	PLS, KPLS, LS-SVM, SPXY, MCDM	(Ni et al., 2011)	
	SWNIRS	DM	Fresh, Dried, Sliced	PLS	(Subedi and Walsh, 2009)	
Potato <sup>1</sup>	HSI-VIS- NIRS	Glucose, Sucrose, Primordial leaf count, TSS	Fresh, Sliced	PLS	(Rady et al., 2014)	
	MIR-NIRS	Acrylamide	Chips, Blended	PLS	(Ayvaz and Rodriguez- Saona, 2015)	
	NIRS	Sprouting Capacity	Fresh, Intact	MPLS	(Jeong et al., 2008)	
	NIRS	TSP, TMA, TC, VC	Fresh, Intact	PLS-DA, ANOVA	(Tierno et al., 2016)	
	NIRS	DM	Fresh, Intact	PLS	(Helgerud et al., 2015)	
	VIS-NIRS	Moisture	Crisps	PLS	(Yee, 2001)	
	MIR-NIRS	Moisture, Fat	Chips	PLS	(Shiroma and Rodriguez-Saona, 2009)	
	NIRS	DM	Slices, Intact	Linear regression	(Dull et al., 1989)	
	NIRS	DM, Specific gravity, Fructose, Glucose	Fresh, Cut, Stored	Not specified	(Scanlon et al., 1999)	



	NIRS	DM, starch, Polarimeric, Protein, Sugars.	Fresh, Peeled and Cut	MPLS, PCA	(Hartmann and Büning-Pfaue, 1998)
	NIRS	Texture	Stored, Steam- cooked	PLS, PCA	(Boeriu et al., 1998)
	NIRS	DM, Starch, Protein	Pulped	PLS	(Brunt and Drost, 2010)
Potato <sup>1</sup> HS	NIRS	Specific gravity	Intact	PLS	(Chen et al., 2005)
	HSI-NIRS	Blackspot	Intact	PCA,PLSDA	(López-Maestresalas et al., 2016)
	NIRS	Sucrose and glucose	Fresh, Stored, Sliced	IPLS, GA, PLSR, LDA, KNN, ANN, PLSDA	(Rady and Guyer, 2015a)
	NIRS	Acrylamide	Crisps	PLS	(Segtnan et al., 2006)
	NIRS	Nitrogen	Dried, Ground	PCA	(Young et al., 1997)
	NIRS	Protein	Cut, Freeze-dried	PLS, PCA	(López et al., 2013a)

<sup>&</sup>lt;sup>1</sup>Only the most cited publications are listed in this table.

#### 2. RESULTS AND APPLICATIONS

In this part we discuss the main results found in the literature about application of non-destructive methods for high throughput phenotyping of RTB and RTB products.

#### 2.1 Cassava

Phambu et al., investigated in 2007 different techniques (fluorescence, infrared spectroscopy, scanning electron microscopy, UV-visible spectroscopy, and X-ray diffraction) to monitor the effect of processing mode on the residual cyanogens in cassava roots. The infrared spectra of the samples were obtained using a Spectrum One spectrometer from Perkin-Elmer. For transmission spectra, samples were prepared as KBr discs with 10% w/w of each sample. For ATR-IR spectra, an ATR DuraVision accessory was used to record the spectra of pure samples, without any preparation. The authors demonstrated that the processing modes (boiling, soaking, and sun-drying) do not significantly affect the initial chemical composition of cassava. The infrared technique (4000-600 cm<sup>-1</sup>) was able to detect residual cyanide at relatively very low concentrations. Other researches concern biochemical components quantification (TCC, TBC and DM), Ikeogu et al., 2017, developed models for DM, TBC, TCC quantification, with an accuracy expressed as SECV = 0,9, 1,6 and 2,1% respectively. These models based on PLS regression were developed on crushed fresh cassava by using a portable VIS-NIR spectrometer (QualitySpec Trek: S-10016, ASD, Longmont, USA) and were more accurate than those based on spectra of intact fresh cassava samples. This study was complementary of the works conducted by Sánchez et al., 2014; Belalcazar et al., 2016; Davrieux et al., 2016 and Moresco et al., 2015. These different papers are related to quantification of TCC and TBC in fresh cassava samples. The accuracy of the models developed by Davrieux et al., 2016 enables the use of NIRS for high throughput phenotyping of cassava genotypes for high TB and TC contents. The models are based on a large database (6026 samples) together with a Local PLS regression approach, doing this way the SEP values were 1,38% and 1,03% respectively for TBC and TCC. For the first time, Fu et al., 2017 quantified maleic acid content (RMSEP=0.026%) in order to detect unexpected frauds in cassava starch. They used OCPLS model and Nicolet 6770 FTIR spectrometer FTIR with a spectral range of 1000-2500 nm.

#### **2.2** Yam

Very few studies have been done on yam characterization using non-destructives analysis. The studies done are related to the quantification of biochemical constituents. The feasibility of starch and sugars quantification by NIRS has been studied by **Lebot and Malapa in 2013**. For this study, they developed PLS regression models based on 1096 samples from five different species: cassava, cocoyam, sweet



potato, taro and yams. The spectra (1000-2500 nm) were collected using an ASD LabSpecPro (ASD, Longmont, USA) spectrometer on dried ground samples. The authors demonstrate that NIRS can be used to produce a rapid prediction of total N, starch and sugar concentrations with a single calibration applied to five different root crop species and across a wide range of varieties. In **2015**, **Kwon et al.** have studied whether or not FT-IR spectroscopy could be used for taxonomic and metabolic discrimination of African yam lines. The work was conducted on whole-cell extracts using a FT-IR spectrometer (Bruker Optics GmbH, Ettlingen, Germany). Each spectrum was recorded from 4000 to 400 cm<sup>-1</sup>. They demonstrated that a hierarchical dendrogram based on partial least square-discriminant analysis (PLS-DA) of FT-IR data from 7 African yam species shows phylogenetic relationship. In addition, the content of dioscin was predicted using a PLS regression model with regression coefficient R<sup>2</sup> = 0,72. The authors consider based on their results that FT-IR combined with multivariate analysis could be applied as a novel tool for metabolic evaluation and high-throughput screening of African yam lines with higher content of dioscin.

#### 2.3 Banana

The applications of rapid non-destructive techniques on banana are numerous and varied in their purpose and in terms of the techniques used. They can be classified into two types: quality evaluation through the quantification of main constituents and evaluation of physical properties linked to maturity and sensorial quality. The techniques applied refer to spectroscopy technics (VIS and NIR), Imaging, HSI, Electric Impedance Measurement (or dielectric properties), electronic noise and <sup>1</sup>H-NMR.

The main constituents, linked to quality, studied are carotenoids (TCC and TBC), total soluble solids (TSS), Dry matter (DM), acid–Brix ratio, chlorophyll and starch. Some physical properties were also investigated such as firmness and elasticity.

Carotenoids quantification was investigated by Davey et al., 2009 on powdered, lyophilized Musa fruit pulp samples using a Labspec Pro Vis-NIR spectrophotometer (Analytical Spectral Devices Inc., Boulder, CO). They conclude that VIS-NIRS could be used for high throughout phenotyping based of their TCC and TBC contents using PLS regression models obtained. Models were developed on a limited number of samples (47) the SEP estimated on an independent set were 28,70 nmol/gdw for TCC and 17,07 nmol/gdw for TBC. In **2011, Pereira** et al. have established a pre-screening of banana according to Provitamin A content determination using pulp color characterization by colorimetry. Potential of spectroscopy in the wavelength range of 299-1100 nm for nondestructive prediction of DM, pH, TSS and acid–Brix ratio (ABR) for banana was studied (Jaiswal et al., 2012). The purpose was to have proper estimation of fruits maturity and ripening stage. They developed a PLS regression models to predict DM, ABR, TSS and pH, with  $R_p^2=0.87$ ,  $R_p^2=0.78$ ,  $R_p^2=0.81$  and  $R_p^2=0.83$ , respectively. The spectra of 95 intact bananas were obtained by transmittance in wavelength range of 299 nm -1100 nm using a portable NIR spectrometer (model AvaSpec-2048, 0.04–20 nm resolution Avantes, USA) connected to 30 W halogen lamp and sample holder. Prediction of total soluble solids and pH in banana was also investigated by Ali et al., 2018. Thirty banana samples were measured at five different maturity stages. Each banana sample was scanned at three different locations (top, middle and bottom) using a Spectrum 100N FT-NIR spectrometer (Model: PerkinElmer, USA) to acquire absorbance spectra in the range of wavelength from 1000 to 2500 nm. The prediction model, based on PLS regression, for the Brix values obtained a coefficient of determination (R<sup>2</sup><sub>p</sub>) of 0,81 and root means square error of predictions (RMSEP) of 3,91 Brix. The prediction model for pH values had an R<sup>2</sup> of 0,69 and RMSEP of 0,36 pH.

Physical properties such as Firmness and elasticity was studied using hyperspectral imaging for determining banana color (L, a\* and b\*) and firmness by **Rajkumar** *et al.*, **2012**. The visible/near infrared hyperspectral imaging system, covering the wavelengths of 380-1023 nm, was used to capture hyperspectral images of intact fruits at different ripening stages. Partial least squares (PLS) together with two-wavelength combination method was used to predict the color (L\*, a\* and b\*) and firmness values. Firmness was taken using the texture analyzer (TA-XT2i, Stable Microsystems Texture Technologies Inc., UK) fitted with a 5 mm diameter flat probe. The overall results proved that spectral reflectance information extracted form hyperspectral images can be applied in determining color and firmness values. The corresponding RPD were 2,23, 6,10, 2,12 and 2,06 for L, a, b and firmness respectively. This study was also carried out to classify successfully unripe and ripe bananas using the



spectral reflectance information. Sanaeifar et al., 2014, studied different banana quality indices (TSS, TA, pH and firmness) from color features using support vector regression (SVM) and artificial neural networks (ANN). For this study a machine vision system was designed that includes two main parts: an image acquisition system to take images from banana samples at any stage of shelf-life and an image analysis algorithm written in MATLAB V8.1 (The Mathworks, Natick, MA, USA). To measure the pulp firmness of samples, an Instron universal testing machine Model SANTAMST-5, with an 8mm cylindrical probe, was used. The respective RMSE were 6,17 for firmness, 1,36 for TSS, 0,21 for pH and 0,48 for TA. The capability of ANN and SVR models was also applied successfully for classifying the banana fruit according to their degree of shelf-life. Considering the same purpose, Liew and Lau, 2012 demonstrated that NIR spectra in the wavelength region of 680-2500 nm, can be applied to develop calibration models for prediction of firmness and soluble solid content of Cavendish banana at different ripeness indices. The models using multiple linear regression enable predictions with RMSEP ranged from 0,01 to 0,26 kgf for firmness and 0,039 to 0,788 Brix for SSC. Banana pulp firmness measurements were conducted with a penetrometer mounted on a test stand using a 7 mm diameter cylindrical probe (Wagner, USA). Spectra were collected over the wavelength range of 680-2500 nm using a NIR reflectance spectrometer (Unity Scientific, NSW). The NIR spectra were taken from the middle part of the fruit at equal distant from proximal and distal ends by averaging 15 scans.

Prediction of quality attributes (SSC, chlorophyll and elasticity) and ripeness classification of bananas using optical properties was also investigated by **Adebayo** *et al.*, **2016**. The intact bananas were measured non-destructively using the multi-spectral system (DA-Meter, Turoni srl, Italy) to capture the backscattered images of the samples. The elasticity test was performed on the banana fingers using a universal testing machine (TA·XT Plus Texture Analyser, StableMicro Systems, Godalming Surrey UK) with a 12 mm diameter spherical probe and pressure force of 2 N. The authors applied ANN to develop predictive models and classification model, the visible wave-length region of 532, 660 and 785 nm gave the highest correlation coefficients (r): for chlorophyll the r coefficient range between 0,977-0,981 and between 0,955-0,976 for elasticity. While the near infrared region of 830 and 1060 nm gave r coefficients between 0,964 and 0,980 for prediction of SSC. The classification of banana into ripening stages was assessed by using the visible wavelength region with a classification accuracy of 97,53%.

**Zude, 2003** studied internal and external characterization of fresh banana using VIS-NIRS (350-1700 nm). The PLS regression was applied on spectra acquired by OMEGA 20 spectrometer in order to predict chlorophyll content in banana skin and fructose and sucrose contents in banana pulp. The R<sup>2</sup><sub>p</sub> obtained were 0,96, 0,94 and 0,96 respectively. Others studies concern various aspect such as chilling injury appearance using backscattering imaging (**Hashim, 2012**); detection of pesticides residues in banana skin using NIRS (**Misal, 2013**); the browning degree using Image analysis (**Cho et al., 2016**) and ripening stages (**Chowdhury et al., 2017**; **Gomes et al., 2013**; **Juncai et al.**; **Raffo, 2005**; **Soltani et al., 2011**; **Surya Prabha and Satheesh Kumar, 2015**) using respectively electrical impedance, colorimetry, classification, <sup>1</sup>H-NMR, dielectric properties and image processing.

#### 2.4 Sweet potato

There are few publications on non-destructive characterization of sweet potato and sweet potato products compare to potato. The publications refer to composition determination and physical properties of the raw product or processed products. One of the first publication was published by Katayama et al., 1996, and report quantification of starch, moisture, and sugar in sweet potato by near infrared transmittance. The spectra were acquired on slices (5 mm Thick) of dried roots, and a NIR spectrophotometer (model 6250; Pacific Scientific, Silver Spring, Md.) was used to measure the transmittance spectra of the slices from 680 to 1235 nm. A slice was put on a sample holder provided with a glass disk 2 cm in diameter. Monochromatic light was used to illuminate the bottom of the slice on the holder, then the amount of radiation transmitted through the slice was measured by a detector located just above the slice. Calibration equations were derived by multiple regression analysis, the best calibration for starch, moisture and sugar were obtained from combination of different harvests samples (1989 to 1991) with SEP= 1,91 and 2,00, 1,21 % respectively. Lebot et al., 2011; evaluated the potential of NIRS (1200-2400 nm) and VIS-NIRS (400-2500 nm) to predict major constituents of fresh and processed sweet potato. To do that, 240 samples of ground dried chips were analyzed in reflectance mode to develop PLS models of starch, sugars, cellulose, proteins, minerals, moisture, AIS and fiber, the SEP (%) were 3,13, 2,31, 1,26, 0,87 and 0,56 respectively. In 2016 Magwaza et al.,



related the development of NIRS models for rapid quantification of protein content in sweet potato. To do this, they sampled a pool of 104 sweet potato varieties and scanned the freeze dried and ground roots using NIR spectrometer (Model XDS, Foss Inc., Silver Spring, MD, USA). The calibration model using PLS regression is accurate with R<sup>2</sup> = 0,98 and RMSEP = 0,29%. Total and individual carotenoid profiles in Solanum phureja cultivated potatoes using NIRS was studied by Bonierbale et al., 2009. The cross, external and independent validation of the calibrations indicate that total carotenoids and zeaxanthin concentrations can be estimated by NIRS with high accuracy and that NIRS can be used to differentiate accessions with low, medium and high concentrations of violaxanthin, antheraxanthin, lutein or β-carotene. Zum Felde et al., report a study done on freeze dried and milled sweet potato samples scanned by NIRS within the range of 400 to 2500 nm using a NIRS monochromator (model FOSS 6500; NIRSystems Inc., Silver Spring, MD, USA) for protein, β-carotene, iron, zinc, starch and individual sugars quantification. NIRS calibration equations developed on the basis of 216-422 selected samples showed high coefficients of determination for the calibrations (R2c) (0,81 to 0,98) with slightly lower coefficients of determination for cross validations (R<sup>2</sup>cv) (0,80 to 0,97). The highest R<sup>2</sup>c and R<sup>2</sup>cv were found for β-carotene (0,98 and 0,97, respectively), starch (0,97 and 0,96, respectively), and for protein (0,97 and 0,95). The iron and zinc calibrations for freeze dried and milled sweet potato material have medium precision compared to those for protein, β-carotene, starch and individual sugars. The calibrations are used for screening for high β-carotene, iron and zinc in sweet potato germplasm in a global NIRS network.

The thermal properties and quality of sweet potato starches were studied by **Lu et al., 2006b**. They developed MPLS models established on 93 genotypes samples analyzed in diffuse reflectance on a FOSS NIR 5000 Systems spectrometer (1100-2500 nm). The performances of models obtained for gelatinization onset temperature, gelatinization peak temperature (Tp), gelatinization temperature range (Tr) and cooling resistance (CR) were (SEP=2,014 °C, R²<sub>p</sub> =0,85), (SEP=1,371 °C, R²<sub>p</sub>=0,89), (SEP=2,234 °C, R²<sub>p</sub> =0,86) and (SEP=0,528, R²<sub>p</sub> =0,89). Other predictive models using NIRS were also developed by **Lu et al., 2006a** in order to measure the physicochemical quality and pasting properties of sweet potato starch. The PLS model were developed with 93 samples from different varieties, results indicate that NIRS is reasonably accurate for predicting amylose content (AC), amyloid percent (AP), total starch content (TSC), protein content (PRC), phosphorus content (PHC), solubility (SOL), swelling power (SP), average granule diameter (AGD), large granule percent (BGP), small granule percent (SGP), crystallinity (CRY), peak viscosity (PKV), viscosity hot paste (HPV), setback (SB), and pasting temperature (Ptemp) with high  $R^2_p$  range between 0,85 and 0,92.

#### 2.5 Potato

As presented in introduction, 58% of the publications found are related to potato and potato products characterization. Among these publications two excellent review articles are particularly informative and comment for a large part the publications listed in this report. The first one "A Review of the application of Near-Infrared Spectroscopy for the analysis of Potatoes", written in 2013 (López *et al.*, 2013b) reports a detailed and critical overview of the main applications of NIR for potatoes and potatoes product characterization. The second one, "Rapid and/or nondestructive quality evaluation methods for potatoes: A review", written in 2015 by Rady and Guyer, includes spectroscopic methods but extends the review to other rapid and/or non-destructive methods.

We agreed with the fact, that some chemical constituents and physical properties in potato tubers determine their quality and use for either the processed industry or as fresh, or prevent the use of tubers if the levels of these parameters are beyond the suggested thresholds (**Rady and Guyer, 2015b**). Furthermore, the preparation method employed affects potato composition (reduce fiber and protein content, increase of fat content, oxidation...). So we can distinguish applications that focus:

- On constituents quantification in relation with intrinsic quality linked to genotype and/or growing conditions
- On constituents quantification linked to the process.

These constituents are, but not limited to, dry matter, carbohydrate, protein, vitamins, glycoalkaloids, minerals, carotenoids, fat and anthocyanins. Additional physical parameters refer to quality such as: specific gravity, flesh and skin color, texture. Finally, other aspects which determine quality and potential



use are the external or internal defects such as greening, bruises, enzymatic browning, non-enzymatic browning, and other physiological disorders.

#### 2.5.1 Raw tubers

The applications of NIR for raw tubers cover different sample presentation: whole tubers, sliced samples, mashed and freeze-dried or any other non-cooked forms. The spectral range regions studied include: VIS, NIRS, VIS-NIRS, MIR.

One of the first publication was published by **Dull et al.**, 1989, and report the quantification of DM in potato by near infrared transmittance. Spectral measurements from 800 nm to 1000 nm on slices and intact tubers were made using two single beam NIR spectrophotometers: a biological spectrometer (the Biospect) and the LT 7000 (LT Industries, Rockville, MD, USA). Potato samples were analysed as thin and thick slices and intact tubers by direct transmittance. The standard errors of prediction were SEP = 1,69% for thick slices samples and SEP = 1,52% for intact tuber. It was shown by **Fraser et al., 2000** and Lammertyn et al., 2000, that NIR radiation intensity inside fruit tissue decreases in an exponential trend with depth. Obviously, this links to lower performance for whole tubers than slices for estimating chemical constituents. Moreover, skin is a factor resulting in dispersing, interfering, and weakening of detected signals, and mostly yields lower correlation between spectra and chemical compounds inside the tissue (Rady and Guyer, 2015b). The works done by Brunt and Drost, 2010; Haase, 2003, 2011; Hartmann and Büning-Pfaue, 1998 on homogenized, mashed, and ground samples, using respectively, NIR or VIS/NIR reflectance (1100-2500 nm, 300-2500 nm, and 850-2500 nm) have led to contrasting prediction performances for DM (RMSEP = 0,50%, 0,19%, 0,56% and 0.42%). Helgerud et al., 2012, have developed an on-line measurements of DM content for whole potatoes. Whole potato tubers were illuminated with two 50W halogen bulbs (Osram, Augsburg, Germany) and backscattered light was collected through a collection tube. The instrument was equipped with a detector recording 30 equally spaced channels from 449 to 1040 nm, thus recording both visible and near infrared light. For the on-line NIR measurements the potatoes were moving along a conveyor belt below the collection tube. The performance of the PLS model were  $R^2 = 0.92$  and RMSECV = 1.06%. The quantification of dry matter content of potato tubers was also assessed by Subedi and Walsh, 2009 using shortwavelength near-infrared spectroscopy (750-950 nm) in a partial transmittance optical geometry. The sampling optics did not involve contact with the sample and could be used on a moving stream of product. The authors developed models based on spectra of whole (dirty and clean) tubers, peeled tubers and sliced tubers. The respective RMSECV were 0,86%, 0,87%, 0,70% and 0,72%. A model which combine the four types of samples led to a RMSCV of 0,98%. The model developed for moving sliced tubers had an RMSEP of 0,42%. The result is encouraging and suggests the technology could be used for in-line assessment of average moisture concentration of sliced tubers, with the assessed moisture level used in process control operations.

The quantification of proteins content of potatoes using NIRS was studied by Hartmann and Büning-Pfaue, 1998; Haase, 2006; López et al., 2013a, Bernhard and al., 2016. Best results were obtained by Hartmann and Büning-Pfaue, 1998 on peeled ground homogenised potato samples. The PLS model developed on the basis of reflectance NIR spectra (1100-2500 nm) led to an SEP equal to 0,06% FW for crude protein. Brunt and Drost, 2010 developed a PLS model for the quantification of coagulating proteins with a SEP= 0.06%. The NIR spectra (1100-2500 nm) of potato pulp were measured by applying an optical fiber NIR probe (EDAPT-1) connected to the NIR spectrophotometer (Technicon Infralyzer IA 500). This result for coagulating proteins is in accordance with results obtained by Haase, 2006; on unpeeled ground homogenised potato samples. Bernhard et al., (2016) studied the quantification of crude protein content (CP) and dry matter content (DM) of potato tuber samples (n = 117) using two different NIRS devices. Samples were analysed as fresh (grated and mashed) using a mobile NIRS system (Zeiss CORONA Plus NIR 1.7 inclusive Zeiss Turn step, Carl Zeiss Microscopy GmbH, Jena, Germany) and dried (flour) using a NIRSystem 6500 (FOSS NIRSystems Inc., Silver Spring, Maryland). Calibrations were developed using MPLS regression, for fresh grated samples the SECV were 1,20% for DM and 0,83% for CP, the SECV for fresh mashed samples were 1,14% for DM and 0,80% for CP and the SECV for dried ground samples was 0,36% for CP. The newly developed NIRS calibration for CPC on fresh potato samples is useful for the selection of potato cultivars with comparatively high or low tuber protein content. In 2006, Fernández-Ahumada et al. have developed a discriminant model by using PLS2 regression which correctly classified 87.5% of mashed potato



samples in groups of low (< 14 mg g<sup>-1</sup>) and high (≥ 14 mg g<sup>-1</sup>) protein content. **Young et al., 1997**, have developed a NIR calibration for quantification of nitrogen (N) in potato leaves, stems and tubers. Samples were grown in the field under specific nitrogen treatments in order to have a wild range in N. Dried ground samples were scanned in duplicate by NIR using a Bran+Luebbe InfraAlyser 260 fitted with nine fixed-wavelength filters selected for analysis of nitrogen, protein, starch, water and oil. The PCR was used to develop the calibration models, the SEP were 0,112%, 0,036% and 0,092 % for leaves, stems and tubers respectively.

Sugars are presented in narrow concentration in potatoes. NIRS analysis of sugars content was studied by (Hartmann and Büning-Pfaue, 1998; Rady and Guyer, 2015; Scanlon *et al.*, 1999). In 1998, Hartmann and Büning-Pfaue have used NIRS to predict individual sugars components such as glucose, fructose, and sucrose along with the estimation of total reduced sugars content of 20 peeled, ground and homogenized potatoes samples. To do this, spectral data were collected by measurement of diffuse reflectance from the sample in the NIR region within 1100-2500 nm using a NIRSystems 5000 (Perstorp Analytical Co.) and PLS regression was applied to calculate the relationships between spectra and sugar contents. The SEP for individual sugars were 0,04% for Glucose, 0,028% for fructose and 0,037% for sucrose while the SEP for TRS was 0,06%. The performance of near infrared method was also examined by Chen *et al.*, 2009 for moisture, carbohydrate, protein and amylase contents determination as well for pasting viscosity properties. The intact potato tubers (n = 150 for calibration and n = 100 for validation) were analysed by NIRS (400-1100 nm) in interactance mode. The standard error of prediction was 0,87% for moisture, 0,95% for carbohydrate, 0,6%. for amylose and 0,15% for protein. SEP was equal to 30 RVU (the default Rapid Viscosity Unit) for peak viscosity, 34 RVU for final viscosity, 24 RVU for breakdown, and 22 RVU for setback.

Whole tubers and 12.54 mm slices of Frito Lay 1879 (chipping) and Russet Norkotah (table) potato cultivars were evaluated (Rady et al., 2014) using NIR transmittance spectroscopy, visible/NIR interactance spectroscopy, and visible/NIR hyperspectral imaging modes. Those spectroscopic techniques were used to estimate glucose, sucrose, specific gravity, primordial leaf count, and soluble solids. Both the whole tuber and the sliced samples were used in the case of visible/NIR interactance and visible/NIR hyperspectral scattering modes, however, just the sliced samples were used in case of the NIR transmittance mode. The system used for VIS/NIR interactance mode experiments contained an Ocean Optics fiber spectrometer (model No. USB 4000, Ocean Optics, Inc., Dunedin, FL, USA) with a 200 µm diameter fiber optic. The InGaAs spectrometer (model No. NIR512L-1.7T1, Control Development, Inc., South Bend, IN, USA) with an optical resolution of 0.4 nm FWHM was used in the transmittance mode with a field of view (FOV) of 10.44°. The calculation of the relative transmittance was done over the NIR wavelength range between 900 and 1685 nm. The hyperspectral system (model No. C4880, Hamamatsu Photonics, Hamamatsu, Japan) was used to capture diffuse scattered light from both the whole and sliced samples in the range of 400–1000 nm. The VIS/NIR interactance system in the range of 446– 1125 nm was shown to yield the best correlation for glucose, sucrose, specific gravity, soluble solids, and primordium leaf counts in the case of sliced samples for Frito Lay 1879. The same trend was achieved for whole tubers except for specific gravity and soluble solids content which showed weaker correlation than other constituents. For sliced samples, VIS/NIR hyperspectral and NIR transmittance systems resulted in relatively consistent correlation performance for glucose, sucrose, and primordium leaf counts. However, the performance was weaker than that obtained using VIS/NIR interactance. In general, this study demonstrated a potential for using VIS/ NIR spectral interactance toward the development of dedicated instrumentation for rapid in-field measurements, storage monitoring, or for online sorting purposes of potato tubers.

The relatively high content of starch in potatoes and the broad distribution inside the tuber (in cortex, vascular ring, and parenchyma) resulted in strong correlation with NIR or VIS/NIR spectroscopic system. As underlined by (**Rady and Guyer, 2015b**), monitoring starch content in potatoes using spectroscopic systems was feasible with most of tests conducted on mashed or ground tubers, and resulting in relatively low RMSEP values: 0,651% by **Haase (2004)** and 0,740% by **Haase (2011)**, compared to limits recommended for tuber processing. **Hartmann and Büning-Pfaue, 1998**, have developed a model for starch quantification with a SEP=0,28%, using peeled ground homogenised samples.

Wen-Hao Su and Da-Wen Sun (2017) investigated the potential of chemical imaging for rapid measurement of dry matter concentration (DMC) and starch concentration (SC) in both potato and



sweet potato tubers. The raw hyperspectral images including 256 bands were captured in the spectral range of 897–1753 nm with the interval of 3.34 nm between contiguous bands. The spectral data of regions of interest (ROI) can be extracted from the whole areas of the tested sample in hyperspectral images using masking and morphological techniques such as image subtraction, erosion or dilation. The mean spectral values of all pixels in the ROI were calculated by averaging all spectral values of each wavelength in 964–1655 nm. Hyperspectral chemical imaging was demonstrated to be great potential for rapidly quantifying time series DMC and SC of tuber samples. This research was carried out to evaluate the potential of hyperspectral imaging system (900–1700 nm) for rapid authentication of organic tubers and grading of tuber moisture levels in both spectral and spatial domains. The MC-PLSDA model, using optimum wavelengths, for identification of organic tuber samples from the other tuber varieties, provided an overall accuracy of 100%. Another simplified PLSDA model was applied for grading tuber moisture levels, resulting in a correct classification of 91.6%.

Ainara López et al., (2014) investigated the quantification of total phenolic content (TPHEN) of 98 different potato varieties by NIRS. Spectral data were collected through a portable NIR spectrophotometer. Each tuber was scanned at four different points along the equatorial area using a Luminar 5030 "Hand held" AOTF-NIR (Acousto-Optic Tunable Filter-Near Infrared) Analyzer (Brimrose) in the reflectance mode. This device covers a spectral range of 1100-2300 nm. Partial least squares regression was applied to the spectral data to develop a calibration model, the RMSE of calibration (RMSEC) and cross validation (RMSECV) were 1,47 mg GAE/g DW and 2,01 mg GAE/g DW, respectively, and the RMSE of prediction (RMSEP) was 3,50 mg GAE/g DW. In 2015, Tierno et al. have developed classification models for purple and red fleshed potato based on phytochemicals and NIR spectra. NIRS measurements were made for whole intact tubers (unpeeled and free of soil) using a Luminar 5030 Miniature 'Hand held' AOTF-NIR Analyzer (Brimrose, Baltimore, MD, USA). Samples were scanned at four different points along the equatorial area and the average spectrum was used for the analysis. Each spectrum was an average of 50 scans. Three PLS-DA models were performed to classify the varieties into three groups according to their phytochemical concentration levels and called: low content (LC), mid content (MC) and high content (HC). The first PLS-DA model corresponded to varieties grouped according to their content of total soluble phenolics (TSP), total monomeric anthocyanins (TMA) and hydrophilic antioxidant capacity (HAC). The second PLS-DA performed comprised groups categorized by their total carotenoids (TC) content. Finally, a third PLS-DA was accomplished covering varieties grouped by means of vitamin C (VC) level. The best classification was obtained using TSP, TMA and HAC based groups; with a correct classification of 97,6% for LC, 83,8% for MC and 76,9% for HC. Classification based on TC groups only led good results for HC (classification rate = 80,4%). No acceptable classification was reached for VC groups. López-Maestresalas et al., 2017 studied the potential of NIRS for quantification of reducing sugars (RS), nitrogen (N), total soluble phenolic (TSP) and hydrophilic antioxidant capacity (HAC). Tubers were lyophilized prior to spectral acquisition (n = 363 tubers) using a Miniature 'Hand held' AOTF-NIR spectrometer (Luminar 5030, Brimrose, Baltimore, MD, USA). Good PLS regression models were obtained for the prediction of nitrogen and TSP with R<sup>2</sup> above 0,83. PLS models obtained for the estimation of HAC could be used for screening. No good model for RS was obtained, probably due to the low concentration of those compounds in potato tubers. The results obtained in this study are similar to those obtained by Mehrubeoglu and Cote (1997) and Haase (2011).

Recently, **Escuredo** *et al.*, **2018**, investigated the potential of NIRS for quantification of phenols, flavonoids, antioxidant activity (IC50), dry matter, total soluble solid content, and apex texture in potatoes flesh. In this study, 73 potato samples corresponding to 35 potato varieties were used. The lyophilized potato flesh was scanned in reflectance mode using a NIR system 5000 (Foss, Hillerød, Denmark) with a standard 1.5m 210/210 bundle fiber-optic probe. The apex and flesh textures of each potato were determined using a penetrometer (BERTUZZI-FT 327) fitted with an 8mm plunger tip and were expressed in units of kg cm<sup>-2</sup>. The modified PLS was used to develop calibrations, the SEP and (R²) were: phenols 0,21 mg.g<sup>-1</sup>(0,82); Flavonoids 9,57  $\mu$ g.g<sup>-1</sup>(0,82), IC<sub>50</sub> 2,69 mg.ml<sup>-1</sup>(0,84); Dry matter1,32%(0,89); TSS 0,29°Brix(0,90); Apex texture 0,44 kg cm<sup>-2</sup>(0,84) and flesh texture 0,48 kg cm<sup>-2</sup>(0,48).

Some physical and others quality traits in potatoes were investigated. In **2005** Chen *et al.* concluded that NIRS was able to accurately measure the specific gravity of intact potatoes. To do this, a total of 250 potatoes of three cultivars, Irish-Cobbler, May-Queen and Kita-akari, were analysed by NIRS (700-1100 nm) in interactance mode. PLS regression was used to develop a predictive model for specific



gravity. An accurate PLS model was obtained with  $R_p^2 = 0.94$  and SEP= 0.0047 g/cm<sup>3</sup>. **Helgerud et al.**, **2012** compared the performance of two different NIR instruments to the performance of the traditional specific gravity measurement method. They used a 1D NIR interactance for stationary analysis and a commercially available 2D NIR interactance system to provide online estimation. Using the 1D NIR interactance the performance were:  $R^2 = 0.95$  and RMSECV = 0.91g/cm<sup>3</sup> for SG.

Damage to potato tubers either by mechanical harvesting or by transport causes a great loss of quality of the final product, and as a result almost two-thirds of the potatoes sold in the market show external or internal damages (López et al., 2013b). Therefore, several investigations were conducted in order to reduce the degree of damages. In 1999, Evans and Muir investigated the feasibility of NIR spectroscopy as a method for determining the discoloration of potatoes associated with bruising. NIR reflectance spectra (700-1700 nm) were collected for both unpeeled and peeled tubers as in bruised and unbruised sites. Linear classification was used to discriminate samples, 75 to 95 % were correctly classified as bruised. Recently, López-Maestresalas et al., 2016 investigated the detection of bruises in potato by hyperspectral imaging (HSI). Two HSI setups were used, Visible-Near Infrared (Vis-NIR) ranging from 400 to 1000 nm (CCD camera, TXG14, Baumer, Germany), and Short Wave Infrared (SWIR) covering the 1000-2500 nm HS SWIR XSM320C4-60 (Headwall Photonics Inc., Fitchburg, MA). For this study, 188 samples were divided into two groups. Bruises were manually induced and samples were analysed 1, 5, 9 and 24 h after bruising. The PLS-DA model allowed an overall correct classification rate above 94% for both hyperspectral setups. Furthermore, more accurate results were obtained with the SWIR setup at the tuber level (98.56 %), allowing the identification of early bruises within 5 h after bruising. Alander et al., 2013, investigated potatoes internal hollow heart detection. To do this, the spectral measurements of both potatoes and peeled potatoes were done with a spectrometer from Ocean Optics (Florida, USA) which wavelength range includes visible wavelengths and short near infrared (195 - 1118 nm). The target was illuminated with a Velleman halogen lamp 1000P64S which input power is 1000W. The spectral transmittance was measured using an integrating sphere. The total number of measurements of potatoes with skin was 100. Both good and defect classes had 50 measurements. In the skinless set, the total number of samples was 99. The good class had 49 samples and defect class counted 50 potatoes. The classes were confirmed after the measurements by slicing the potatoes. For classification a procedure combining principal component analysis and a support vector (kernel) classifier was used. This approach led to a classification rate of 90% for whole tuber and 92,5% for peeled potatoes, the classification was tested on an independent set of 40 sample (20 healthy and 20 defect potatoes). In the same way, Zhou et al. 2015 studied a non-destructive detection of blackheart in potato by vis/nir transmittance spectroscopy. For the experiment, Potato tubers (Kexing Six var.) were purchased from a local market. To induce blackheart development, the samples were enclosed in zip-lock bags (one potato in each bag) and all potatoes were stored in a cold storage at a temperature of 4°C and 85% relative humidity for four months. Potatoes with decay were taken out, and 519 tubers were finally used for experiments. For every sample, morphological properties including weight, maximum length, maximum width, maximum height and volume were measured before spectral acquisition. Transmission spectra of each tuber were collected with an apparatus that consisted of a spectrometer (USB4000, Ocean Optics Inc., USA) having a range from 200 to 900nm, an optic fiber, a collimating lens, a light source, a sample holder, a sponge flexible shield (to maintain the potato) and a computer. Tubers were placed centrally with the maximum height axis vertically. Partial least squares-linear discriminant analysis (PLS-LDA) was used to classify potatoes according to spectra in two classes: the tubers with blackheart and normal tubers. PLS-DA was tested on raw data and corrected data according to 1) weight, 2) height and 3) volume of the tubers, The PLS-DA on height corrected data gave the best classification rate with a classification rate of 97,11%. As claimed by the authors, this might be due to the fact that light transmittance decreases as the pathlength increases, and height correction would reduce the effect of light path variation. A simplified model was also tested with only six wavelengths selected (on PCA loadings), this model based on height correction led to a classification rate of 96,82%. These results are promising for the development of a real-time and portable systems for the classification of blackheart potatoes.

#### 2.5.2 Processed tubers

Much research has been developed to determine the texture of cooked potatoes by non-destructive methods. **Boeriu** *et al.*, **1998** determined texture profiling of steam cooked potatoes by NIRS. The texture of 20 steam-cooked potatoes samples from three cultivars, classified according to tuber size



and DM content, were sensory evaluated after one, three and six months of storage. NIR spectra were measured using a Bran & Luebbe InfraAlyzer 500 spectrometer (1100-2500 nm). Therefore a quantitative model based on PLS regression was used to relate NIR spectra with sensory perceived texture. Among the sensory parameters, moist, waxy, firm and mealy were best predicted with standard error of prediction (SEP) ranging from 8,64 to 11,28 and coefficients of determination (R<sup>2</sup><sub>p</sub>) between 0,82 and 0,89. A similar study was developed by van Dijk et al., 2002. They studied the relationship between sensory-perceived texture, evaluated on 12 descriptors by a trained panel of 16 panellists, and dry matter and NIRS for steam cooked potatoes. To quantify these relationships, three potato cultivars, respectively representing a firm cooking potato (cv. Nicola), a mealy cooking potato (cv. Irene), and a cultivar (cv. Bintje) with intermediate cooking properties, were used. Spectra of ground homogenised samples were recorded in the reflectance mode using an InfraAlyzer 500 (1100-2500 nm). Regarding the relation between the sensory-perceived texture and NIRS, the results are expressed in terms of correlations between spectra and descriptors: moistness (R = 0,85), mealy (R = 0,79), crumbly (R = 0.79), waxy (R = 0.77), grainy (R = 0.73), mashable (R = 0.70), and firmness (R = 0.68). In the same study the authors have developed a PLS model for DM and starch quantification based on spectra information with respective SEP of 12,9% and 9,88%, these results were quite different and less performant from ones obtained in others studies. However, based on these results the authors conclude that the DM content rather than the cultivar determines the sensory-perceived texture of steam-cooked potatoes and that in the case of potatoes it can furthermore be anticipated that the NIR spectra contain elements related either to the DM content or to cultivar-specific properties. In 2000 Thybo et al., reported a work which evaluated the ability of uniaxial compression, NIR and low field pulsed <sup>1</sup>H nuclear magnetic resonance (LF-NMR) in predicting the sensory texture quality of 24 samples of cooked potato by partial least squares regression (PLSR). They completed the study with the chemical measurement of dry matter content and pectin methylesterase activity. The NIR spectra were collected on raw potatoes samples and on cooked samples using an NIR Systems Inc. spectrophotometer (model 6500, Silver Springs, Maryland, U.S.A.) equipped with a rotating sampling device. The scanning protocol was: two slices of approximately 5 mm were cut transversely 1/4 of the length from the stem end of 10 potato tubers. Ten slices were measured by NIR in the raw stage immediately after being stamped to circular slices of 37 mm in diameter to fit in the standard quartz sample cups closed with a compressible paper disc. Ten slices were cooked for 12 min in 350 mL water, cooled to room temperature for approximately 3 h, covered with plastic film, trimmed to circular slices (d = 37 mm) and measured by NIR. For the sensory texture analysis the potatoes were peeled and boiled in water for 20 to 25 min until they were cooked. All samples were analysed in three replications in a randomized design with six samples per sensory session. The samples were evaluated hot and one tuber per sample was served. A panel of ten trained assessors evaluated texture by quantitative descriptive texture analysis based on eight oral attributes: hardness, firmness, springiness, adhesiveness, graininess, mealiness, moistness and chewiness and one visual attribute: reflection from surface. Uniaxial compression was measured on raw and cooked potatoes. Only 64% of the sensory texture variation was described in a PLSR calibration by uniaxial compression, dry matter and pectin methylesterase activity. The results demonstrate that LF-NMR (CPMG) relaxation on raw potato samples can be applied as an alternative rapid method for detecting sensory texture of cooked potatoes. In another hand, this work point out interesting results, when NIR spectra are used as predictors of sensory texture, which allow to consider the use NIR for sorting fresh potatoes according to their texture properties. The coefficients of correlation between measured and NIRS predicted sensory texture attributes vary between 0,50 (Hardness) and 0,83 (Mealiness) for cooked potatoes and between 0,25 (Adhesiveness) and 0,73 (Mealiness) for raw potatoes. Regarding the raw samples, the results were in some contrast to the results obtained by Boeriu et al., 1998. In 2018, Su and al., have determined textural property of microwave baked tuber using Fourier transform mid-infrared-attenuated total reflectance (FTMIR-ATR) micro spectroscopy. To develop a robust calibration model, fresh tuber samples from five types in terms of Rooster red potato, (origin: UK), Desiree red potato (origin: UK), Evangeline sweet potato (origin: Egypt), Abees sweet potato (origin: Egypt), organic Abees sweet potato were selected. After being peeled and sliced to the thickness of 10 mm, 25 samples of each tuber variety were divided into five equal parts and then respectively baked in a lab-scale microwave oven (800 W) for 0, 10, 20, 30, 35s, resulting in 125 samples in total. The samples were analysed using a LUMOS FT-IR microscope (Bruker Optics, Germany) in ATR mode. This system was equipped with a liquid nitrogen cooled narrow-band photoconductive mercury cadmium telluride (MCT) detector, a deuterated triglycine sulfate (DTGS) detector, a highly resolving digital CCD camera, a germanium (Ge) ATR crystal, a solid state laser, a IR beam splitter, and a permanently aligned RockSolidTM interferometer. The textural property of a tuber sample was assessed by performing double



compression test using a TA.XT.plus texture analyser (Stable Micro Systems Ltd., Godalming, Surrey, England) fitted with a 30 kg load cell. PLSDA was able to discriminate the tuber samples into three separate classes corresponding to their spectral properties. The optimized models were established using LWPLSR for determination of tuber textural property (TTP) in terms of hardness, resilience, springiness, cohesiveness, gumminess and chewiness, with correlation coefficients of prediction (RP) of 0,797, 0,881, 0,584, 0,574, 0,728 and 0,690, respectively. The authors concluded that the FTMIR-ATR spectroscopy provides characteristic information allowing a better understanding of the change of tuber texture under various microwave baking time and that FTMIR-ATR spectroscopy can be considered as an effective technique for non-invasive and rapid measurement of textural property of tuber products.

Acrylamide content was quantified (Ayvaz and Rodriguez-Saona, 2015; Pedreschi et al., 2010; Segtnan et al., 2006, Dutta et al. 2016, Adedipe et al., 2016) in potato chips and crisps. That was successfully conducted by Segtnan et al., 2006 on ground crisps using VIS/NIR diffuse reflectance (400-2500 nm). Results showed high performances for prediction models with  $R^2p = 0.95$  and RMSEP = 246.8 µg/kg. The spectral models are accurate enough to suggest that VIS/NIR spectroscopy can be used for screening of acrylamide contents in processed potato crisps. Pedreschi et al., 2010, studied on-line quantification of acrylamide, moisture and oil content in potato chips using near infrared interactance and visual reflectance imaging. Fat and dry matter of potato chips were predicted with high accuracy, RMSECV = 0,99% and 0,84%. For acrylamide an average prediction error of 266 μg/kg was achieved. Ayvaz and Rodriguez-Saona, 2015 investigated the quantification of acrylamide in commercial potato chips using mid-infrared (MIR) and near-infrared (NIR) spectrometers. Different instruments: Benchtop NIR (Excalibur 3500 FTIR, Varian, Palo Alto, CA, USA), Handheld NIR (MicroPhazir, Thermo Fisher, Wilmington, MA, USA), Benchtop MIR (Excalibur 3500 FTIR) and Portable MIR (Cary 630 FTIR, Agilent technology, Danbury, CT, USA) were tested on ground defatted chips . Both MIR and NIR instruments, portable and handheld systems, performed similarly and led to a quantification of acrylamide analysis in commercial potato chips, with RPD values between 2,0 and 4,8 and predicted error lower than 100 µg/kg. Adedipe et al., in 2016 have obtained a prediction error of 135  $\mu$ g/kg (R<sup>2</sup> = 0,98) for acrylamide quantification on freeze-dried and ground French fried potatoes using NIR (400-2500 nm) reflectance spectra. Dutta et al., 2016 developed an automatic and efficient non-destructive image processing based method for acrylamide identification using KNN classification from potato chips. An image acquisition system was set up with four fluorescent lamps and four CFL of white light. Images were captured using a color digital camera located vertically from the sample at a distance of 25 cm. The digital camera of 8 mega pixels with auto focus is used for image acquisition which provides images in JPEG format. Digital image of potato chip were used for image processing to identify the acrylamide content in potato chip. Input image was transformed in wavelet domain for analysis of image features to explore the discrimination possibility between normal images and contaminated images. The k-nearest neighbour (KNN) classifier algorithm was used for discrimination with 97% accuracy for acrylamide identification.

Prediction of volatility of tuber compositions (VTC) and cooking degree (TCD) using Hyperspectral imaging in combination with multivariate analysis was investigated by **Su and Sun, 2016.** Tuber samples of six cultivars (12 samples for each cultivar) were washed and peeled prior to slicing with an electric slicing machine. One slice was cut from each tuber (n = 12 slices for each variety). Tuber slices were cooked by low temperature baking in a laboratory scale oven with the temperature of  $80 \pm 2^{\circ}$ C for five time periods including 40, 80, 120, 190, and 260 min. Every time tuber samples were first scanned using a line-scan hyperspectral imaging system and then weighed. The acquired hyperspectral image corresponding to each sample is three-dimensional including both spatial and spectral information, with 320 pixels in x-direction, n-pixels in y direction (determined by the sample length) and 256 bands from 897 to 1753 nm in k-direction. The results demonstrated that the multispectral imaging derived from hyperspectral imaging technique was a more efficient and robust instrument for non-destructive and real-time assessment of VTC and TCD. The best model for TCD was based on three layers back propagation artificial neural network (TBPANN) with  $R^2$  = 0,967 and RMSEP of lower than 0,307. For VTC similar results were obtained using PLSR and TBPANN with  $R^2$  higher than 0,9 for almost all varieties and RMSEP lower than 0,017.

Fat and moisture contents using NIR and MIR spectroscopy (1052-2000 nm and 2500-13333 nm, respectively) in potato chips were evaluated by **Shiroma and Rodriguez-Saona, 2009**. To do this, NIR and MIR Spectra of 15 commercial ground potato chips were collected using an AnExcalibur 3500



Fourier-Transform IR spectrometer (Varian Inc., Palo Alto, CA). Then, the spectral data were analysed by PLS regression. Correlation coefficient for moisture was higher than 0,97 with standard error of cross validation (SECV) lower than 0,3% for both NIR and MIR techniques. Prediction models for fat had R equal to 0,96 and SECV lower than 1,60% for both NIR and MIR. As seen before, **Pedreschi et al.**, **2010** were also able to assess fat and dry matter contents with prediction models having R<sup>2</sup><sub>p</sub> (RMSEP) values of 0,99(0,99%) and 0,97(0,84%), respectively. They used a near infrared interactance and visual reflectance imaging.

Best results were obtained by **Ni** *et al.*, **2011** on chips using a Hitachi U-4100 spectrophotometer over the 800–2500 nm. Four similar types of the 'original flavour' potato chips from different manufacturers were analyzed for four quality parameters: fat, moisture, the acid and peroxide values of the extracted oil and for their NIR spectra (ground chips, diffuse reflectance). The best models were based on Least Squares Support Vector Machines (LS-SVM), with high performances for the four parameters in terms of RMSEP: 0,211% (Fat), 0,076% (MC), 0,101 meq.g<sup>-1</sup> (Acid value) and 0,435 mg.Kg<sup>-1</sup> (Peroxide value).

Mazurek et al., 2016 compared the Soxhlet fat extraction method, with infrared, MIR and Raman spectroscopy for homogenized laboratory-prepared chips. Infrared spectra were recorded using a Magna 860 FTIR spectrometer (Thermo Nicolet, Madison, WI, USA). Diffuse reflectance spectra were collected using a Seagull (Harrick Scientific, Pleasantville, NY, USA) optical assembly, working in a DRIFT mode, mounted in the Magna unit. A 45Spec specular reflection accessory (Pike Technologies, Madison, WI, USA) rotated by 180° (upsidedown) was applied to obtain reflectance spectra a DRIFTlike mode (SR). A KBr beam-splitter and a DTGS detector were set up in the MIR range while a CaF2 beam-splitter was used in the NIR region. To register spectra in the MIR region, samples were diluted with a dried KBr (1:99 w/w). Raman spectra were collected using a Thermo Nicolet FT Raman accessory attached to the Magna 860 FTIR spectrometer equipped with CaF2 beam splitter. An indiumgallium- arsenide (InGaAs) detector was used to carry out the measurements. Chips were prepared from a potato variety Verdi. Potatoes were peeled (carborundum peeler, Sirman, Italy), washed and sliced using a rotary slicer (Brown, Germany). Slices between 1.2 and 1.7 mm ± 0.1 mm thick were washed in cold water and dried using paper towelling. Approximately 200 g portions of potato slices were fried in a fryer at three temperatures: 150, 165 and 180 °C. To obtain chips of varying fat content, time of frying was increased by 20 s for succeeding portions. After discharging the oil and cooling chips, samples were homogenized (Retsch GM 200, Germany). Partial least squares (PLS) calibration models were constructed and characterized by the values of relative standard errors of prediction (RSEP), the results show that SR/MIR presents lower error and that the 3 others techniques led to quite similar results with RSEP = 1,23, 1,25%, 0,67% and 1,12% for Raman, Drift/MIR, SR/MIR and Drift/NIR respectively. Yee et al., 2006 tested NIR spectroscopy for separating crisps from different potato cultivars in order to insure constant quality of the batches. Tubers of 'Fianna' and 'Whitu', whose time of availability for processing overlaps, were investigated (n = 192). Potatoes were cut into flat slices of 1.5 mm and fried in Canola oil in a fryer. Frying time was set at 180 s and oil temperature was 180°C. The samples were scanned using a NIRSystems 6500 spectrometer (400 - 2500 nm) in reflectance mode (no details are given on sample preparation prior to NIRS). Identification of cultivar in a potato crisp sample was achieved using linear discriminant analysis and piecewise linear discriminant analysis. Both approaches gave similar results in validation with a classification rate around 94% for the two varieties.

#### 3. CONCLUSION

The literature reviewed hereinabove highlights the potential of non-destructive techniques to qualify, sort and/or characterize roots, tubers or bananas. The techniques used vary in terms of complexity, accuracy, performances, robustness, costs and ease to use. A large part of the techniques involved is based on the interaction between electromagnetic radiations and matter and refers to vibrational properties of the chemical bonds. Because of this, these technologies are commonly known as vibrational spectroscopy and cover the spectral range from visible to mid infrared light. Moreover other noninvasive techniques, such as NMR, Raman spectroscopy, imaging, ultrasound technology and X-ray, have shown the potential for successful applications in quality monitoring of fruits, vegetables and tubers.



Researches using non-destructive techniques concern fresh and processed products. Most of the time, quality control or process monitoring are reached through the quantification of biochemical compounds: carbohydrate, protein, vitamins, minerals, carotenoids, moisture, starch, phenols and fat. Another part of the researches refers to physical properties such as specific gravity, skin color and texture. And some researches focus on contaminant quantification such as acrylamide in processed products or concern different quality aspects and potential use: external or internal defects, greening, bruises, enzymatic browning, non-enzymatic browning, and physiological disorders.

The products were analyzed in different conditions and presentations (intact, peeled, Freeze-dried, mashed, crushed, sliced, cooked, deep frying, chips and crisp). Regarding vision and spectroscopic techniques the measurements were done in, backscattering, diffuse reflectance, transmittance or interactance mode using static or moving sample holding systems. Reflectance mode measurements do not require contact with the sample and light levels are relatively high. However, spectral fingerprint is dependent of the skin properties of the tuber, in case of intact tubers. Transmission mode measurements can be done without contact and spectra are less dependent to skin properties. Transmittance mode is suitable for detecting internal disorders. Interactance mode requires to be in contact with the sample but provides a compromise between reflection and transmission modes and the direct contact between the fibre bundles and the sample eliminates the effect of surface reflection and maximizes the penetration depth. Depending of application different range of electromagnetic spectrum are concerned from Visible to Mid infrared. Hyperspectral imaging covering visible and/or NIR is one of the most recently emerging tools and provides advantages of vision and spectroscopic systems and can be used, after speeding up image acquisition time, in prediction of processing-related constituents as well as defects detection. HIS gives the advantage to provide both quantification and information on spatial distributions of the traits in the whole tuber, root or banana. There is an inevitable trend for multispectral imaging with only a few important bands instead of full wavelengths in the nondestructive and rapid evaluation of food quality.

The chemometrics methods used to achieve calibration are numerous and depend on the product and on the trait to be characterized. The approaches cover linear methods (PCA, PCR, PLSR, LDA, PLSDA, SIMCA...) and non-linear methods (ANN, Local Regression, SVM, KNN, CART...), and are divided into two groups: quantification and classification. In some cases classification (supervised or unsupervised) gives the opportunity to perform HTP screening, when quantification is not relevant. These methods are associated to various signal preprocessing methods which cover and solve a large part of the problems due to the techniques involved and to the mode of measurements.

According to the different publications NIRS (1D or 2D) presents a real potential for high-throughput screening and quality control of a great number of samples of RTB. Applications concerns chemical characterization as well as physical properties. Some studies, mainly on potatoes and potato products, report evaluation of sensorial attributes (hardness, firmness, springiness, adhesiveness, graininess, mealiness, moistness and chewiness) using NIRS with promising results. The instrumentally measured texture of RTB products was also assessed using NIRS.

However, robust models have to be based on large data sets to precisely predict quality attributes for new samples, especially for breeding purpose. The datasets should be obtained from different destinations, growing conditions and post-harvest conditions in order to cover the variability of the trait to be quantified/characterized. Additionally, these HTP techniques are indirect which implies that models accuracy highly depends on the precision of reference methods used to quantify the constituent or trait.

The challenge for RTBfoods will be to translate in measurable variables or in indirect correlated variables the quality traits of interest in order to develop a strategy for calibration. The strategy will cover the choice of the optimum non-destructive HTP technique, the sampling, the sample presentation and preparation, the measurement protocol, and the choice of chemometrics methods. This work, ones the traits identified by WP1, should be done in close collaboration between WP2 and WP3.



#### 4. ANNEXES

#### 4.1 Annex 1: References

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## 4.2 Annex 2: References number per year for each RTB: cassava, yam, banana, sweet potato and potato

Year	Cassava	Yam	Banana	Sweet potato	Potato
1988	0	0	0	0	1
1989	0	0	0	0	1
1990	0	0	0	0	2
1992	0	0	0	0	1
1996	0	0	0	1	1
1997	0	0	1	0	2
1998	0	0	0	0	3
1999	0	1	1	0	1
2000	0	0	1	0	1
2001	0	0	0	0	4
2002	0	0	0	0	1
2003	0	0	1	0	1
2004	0	1	1	0	1
2005	0	0	0	0	3
2006	0	0	0	2	2
2007	1	0	0	0	2
2008	0	1	0	0	2
2009	0	0	2	1	8
2010	0	0	2	0	5
2011	0	0	2	1	4
2012	0	0	5	0	2
2013	0	1	3	1	7
2014	1	0	1	1	5
2015	2	2	5	0	9
2016	2	2	6	2	13
2017	2	0	1	0	4
2018	0	0	4	0	1





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